AGENDA FOR
THE INTERNATIONAL CONFERENCE
ON THE RENOVATION AND RECYCLING OF WASTEWATER
THROUGH AQUATIC AND TERRESTRIAL SYSTEMS

BELLAGIO STUDY AND CONFERENCE CENTER
BELLAGIO, ITALY

JULY 16-21, 1975

WEDNESDAY
JULY 16

CONFERENCE WELCOME

2030-2040	WELCOME OF CONFEREES AND PRESENTATION OF CONFERENCE FORMAT - Dr. Frank M. D'Itri
2040-2050	THE RELATION OF THE CONFERENCE TO THE NATURAL AND ENVIRONMENTAL SCIENCES PROGRAM IN THE ROCKEFELLER FOUNDATION - Dr. Ralph W. Richardson, Jr.

THURSDAY
JULY 17

CONFERENCE SESSION I

WORLDWIDE PROGRAMS PRESENTLY UTILIZING MUNICIPAL WASTEWATER IN AGRICULTURE

MODERATOR

DR. FLORA MAE WELLINGS

0830-0845	WATER RECLAMATION AND WASTEWATER REUSE FOR IRRIGATION OF AGRICULTURAL LANDS IN MEXICO - Dr. Jorge Aguirre
0845-0900	Formal discussion of Dr. Aguirre's paper by Dr. F. M. D'Itri
0900-0930	Open discussion of Dr. Aguirre's paper.
0930-0945	RECYCLING TREATED SEWAGE THROUGH CYPRESS WETLANDS IN FLORIDA - Dr. Howard T. Odum
0945-1000	Formal discussion of Dr. Odum's paper by Dr. T. G. Bahr
1000-1030	Open discussion of Dr. Odum's paper.
1030-1100	Coffee break
1100-1115	THE AGRICULTURAL REUSE OF MUNICIPAL WASTEWATER IN BRAUNSCHWEIG, GERMANY - Dr. Cord Tietjen
1115-1130	Formal discussion of Dr. Tietjen's paper by Dr. J. Aguirre
1130-1200	Open discussion of Dr. Tietjen's paper
1200-1430	Luncheon

CONFERENCE SESSION II

OPERATIONAL WASTEWATER LAND RENOVATION FACILITIES THROUGHOUT THE WORLD - PRESENT AND FUTURE DESIGNS

MODERATOR

PROFESSOR HOWARD T. ODUM

1430-1445	PRELIMINARY RESULTS WITH A PILOT-PLANT WASTE RECYCLING-MARINE AQUACULTURE SYSTEM - Dr. John H. Ryther
1445-1500	Formal discussion of Dr. Ryther's paper by Dr. M. S. Gordon
1500-1530	Open discussion of Dr. Ryther's paper
1530-1545	BIOLOGICAL RECYCLING OF DISSOLVED NUTRIENTS IN TREATED DOMESTIC WASTEWATERS USING HYDROPONIC AND AQUACULTURAL METHODS - Dr. Malcolm S. Gordon
1545-1600	Formal discussion of Dr. Gordon's paper by Dr. F. E. Broadbent
1600-1630	Open discussion of Dr. Gordon's paper
1630-1700	Tea
1700-1715	THE DEBRECEN SEWAGE RESEARCH FARM PROJECT - Dr. Laszlo Vermes
1715-1730	Formal discussion of Dr. Vermes' paper by Dr. C. Tietjen.
1730-1800	Open discussion of Dr. Vermes' paper

FRIDAY
JULY 18

0830-0845	THE MICHIGAN STATE UNIVERSITY WATER QUALITY MANAGEMENT PROJECT - A FACILITY FOR RESEARCH IN RECYCLING TREATED WASTEWATER - Dr. Robert C. Ball
0845-0900	Formal discussion of Dr. Ball's paper by Dr. L. Vermes
0900-0930	Open discussion of Dr. Ball's paper

CONFERENCE SESSION III

EFFECTS OF IRRIGATION WITH MUNICIPAL WASTEWATER ON SOIL AND VEGETATION

MODERATOR

PROFESSOR DAVID PURVES

0930-0945 THE CONTAMINATION OF SOIL AND FOOD CROPS BY TOXIC ELEMENTS NORMALLY FOUND IN MUNICIPAL WASTEWATERS AND THEIR CONSEQUENCES FOR HUMAN HEALTH - Dr. David Purves

0945-1000 Formal discussion of Dr. Purves' paper by Dr. F. A. M. de Haan

1000-1030 Open discussion of Dr. Purves' paper

1030-1100 Coffee break

1100-1115 THE EFFECTS OF LONG TERM ACCUMULATION OF HEAVY METALS AND SELECTED ORGANIC COMPOUNDS IN MUNICIPAL WASTEWATER ON THE SOIL - Dr. Franz A. M. De Haan

1115-1130 Formal discussion of Dr. de Haan's paper by Dr. D. Purves

1130-1200 Open discussion of Dr. de Haan's paper

1200-1430 Luncheon

1430-1445 NITRIFICATION AND DENITRIFICATION IN SOILS RECEIVING MUNICIPAL WASTEWATER - Francis E. Broadbent

1445-1500 Formal discussion of Dr. Broadbent's paper by Dr. H. T. Odum

1500-1530 Open discussion of Dr. Broadbent's paper

CONFERENCE SESSION IV

THE OCCURRENCE, SURVIVAL, AND DETECTION OF VIRUSES AND PATHOGENIC ORGANISMS IN MUNICIPAL WASTEWATER AND THE SOIL ON WHICH IT IS APPLIED

MODERATOR

PROFESSOR HILLEL I. SHUVAL

1530-1545 PUBLIC HEALTH IMPLICATIONS OF MUNICIPAL WASTEWATER REUSE - Hillel I. Shuval

1545-1600 Formal discussion of Dr. Shuval's paper by Dr. G. J. Bonde

1600-1630 Open discussion of Dr. Shuval's paper

1630-1700 Tea

1700-1715 SAMPLING AND ISOLATION METHODS FOR THE DETECTION OF VIRUSES IN MUNICIPAL WASTEWATERS - Ebba Lund

1715-1730 Formal discussion of Dr. Lund's paper by Dr. F. M. Wellings

1730-1800 Open discussion of Dr. Lund's paper

SATURDAY
JULY 19

0830-0845 THE CURRENT STATUS OF BACTERIA AND OTHER PATHOGENIC ORGANISMS IN MUNICIPAL WASTEWATER AND THEIR POTENTIAL HEALTH HAZARDS WITH REGARD TO AGRICULTURAL IRRIGATION - Dr. Gunner J. Bonde

0845-0900 Formal discussion of Dr. Bonde's paper by Dr. H. I. Shuval

0900-0930 Open discussion of Dr. Bonde's paper

0930-0945 THE SURVIVAL OF VIRUSES IN THE SOIL UNDER NATURAL CONDITIONS - Dr. Flora Mae Wellings

0945-1000 Formal discussion of Dr. Wellings' paper by Dr. E. Lund

1000-1030 Open discussion of Dr. Wellings' paper

1030-1100 Coffee break

CONFERENCE SESSION V

PUBLIC ATTITUDES, FOOD PRODUCTION, AND RESEARCH NEEDS RELATED TO MUNICIPAL WASTEWATER RENOVATION AND REUSE

MODERATOR

PROFESSOR GEORGE H. ALLEN

1100-1115 THE CULTIVATION OF FISH IN MUNICIPAL WASTEWATER LAGOONS AS AN AVAILABLE PROTEIN SOURCE FOR HUMAN BEINGS WITH EMPHASIS ON SALMONIDS - Dr. George H. Allen

1115-1130 Formal discussion of Dr. Allen's paper by Dr. B. Hepher

1130-1200 Open discussion of Dr. Allen's paper

1200-1430 Luncheon

SUNDAY
JULY 20

0830-0845 WASTEWATER UTILIZATION IN ISRAEL AQUACULTURE - Dr. Balfour Hepher

0845-0900 Formal discussion of Dr. Hepher's paper by Dr. G. H. Allen

0900-0930 Open discussion of Dr. Hepher's paper

0930-0945 THE RELATIONSHIP BETWEEN ENVIRONMENTAL FACTORS AND VIRUSES IN THE INDUCTION OF FISH TUMORS - Dr. Ron S. Sonstegard

0945-1000	Formal discussion of Dr. Sonstegard's paper by Dr. J. H. Ryther
1000-1030	Open discussion of Dr. Sonstegard's paper
1030-1100	Coffee break
1100-1115	WASTEWATER RECYCLING: A PERSPECTIVE ON RESEARCH NEEDS - Dr. Thomas G. Bahr
1115-1130	Formal discussion of Dr. Bahr's paper by Dr. G. H. Toenniessen
1130-1200	Open discussion of Dr. Bahr's paper
1200-1430	Luncheon
1430-1445	PUBLIC ATTITUDES TOWARD THE RENOVATION AND REUSE OF MUNICIPAL WASTEWATER - Dr. Frank M. D'Itri
1445-1500	Formal discussion of Dr. D'Itri's paper by Dr. R. S. Sonstegard
1500-1530	Open discussion of Dr. D'Itri's paper
1530-1545	THE ROLE OF PRIVATE FOUNDATIONS IN RESEARCH ON WASTEWATER RENOVATION AND REUSE - Dr. Gary H. Toenniessen
1545-1600	Formal discussion of Dr. Toenniessen's paper by Dr. R. C. Ball
1600-1630	Open discussion of Dr. Toenniessen's paper
1630-1700	Tea

CONFERENCE SESSION VI

CLOSING SESSION

MODERATOR

PROFESSOR FRANK M. D'ITRI

1700-1800	THE CLOSING SESSION IS DEVOTED TO A BRIEF REVIEW OF THE CONFERENCE FINDINGS AND THE DEVELOPMENT OF RECOMMENDATIONS FOR THE RENOVATION AND REUSE OF MUNICIPAL WASTEWATER

MONDAY
JULY 21

0830-1800	DEPARTURE FROM THE BELLAGIO STUDY AND CONFERENCE CENTER

DAILY SCHEDULE FOR

THE INTERNATIONAL CONFERENCE ON THE RENOVATION AND RECYCLING OF WASTEWATER THROUGH AQUATIC AND TERRESTRIAL SYSTEMS

BELLAGIO STUDY AND CONFERENCE CENTER
BELLAGIO, ITALY

Time	Thursday July 17, 1975	Friday July 18, 1975	Saturday July 19, 1975	Sunday July 20, 1975
0830-0845	Dr. J. Aguirre (P)	Dr. R. C. Ball (P)	Dr. G. J. Bonde (P)	Dr. B. Hepher (P)
0845-0900	Dr. F. M. D'Itri (D)	Dr. L. Vermes (D)	Dr. H. I. Shuval (D)	Dr. G. H. Allen (D)
0900-0930	Open Discussion	Open Discussion	Open Discussion	Open Discussion
0930-0945	Dr. H. T. Odum (P)	Dr. D. Purves (P)	Dr. F.M. Wellings(P)	Dr. R. S. Sonstegard (P)
0945-1000	Dr. T. G. Bahr (D)	Dr. F. A. M. de Haan (D)	Dr. E. Lund (D)	Dr. J. H. Ryther (D)
1000-1030	Open Discussion	Open Discussion	Open Discussion	Open Discussion
1030-1100	Coffee Break	Coffee Break	Coffee Break	Coffee Break
1100-1115	Dr. C. Tietjen (P)	Dr. F. A. M. de Haan (P)	Dr. G. H. Allen (P)	Dr. T. G. Bahr(P)
1115-1130	Dr. J. Aguirre (D)	Dr. D. Purves (D)	Dr. B. Hepher (D)	Dr. G. H. Toenniessen (D)
1130-1200	Open Discussion	Open Discussion	Open Discussion	Open Discussion
1200-1430	Luncheon	Luncheon	Luncheon	Luncheon
1430-1445	Dr. J. H. Ryther (P)	Dr. F. E. Broadbent (P)	Open	Dr. F. M. D'Itri (P)
1445-1500	Dr. M. S. Gordon (D)	Dr. H. T. Odum (D)	Open	Dr. R. S. Sonstegard (D)
1500-1530	Open Discussion	Open Discussion	Open	Open Discussion
1530-1545	Dr. M. S. Gordon (P)	Dr. H. I. Shuval (P)	Open	Dr. G. H. Toenniessen (P)
1545-1600	Dr. F.E. Broadbent (D)	Dr. G. J. Bonde (D)	Open	Dr. R. C. Ball (D)
1600-1630	Open Discussion	Open Discussion	Open	Open Discussion
1630-1700	Tea	Tea	Open	Tea
1700-1715	Dr. L. Vermes (P)	Dr. E. Lund (P)	Open	Conference Summary
1715-1730	Dr. C. Tietjen (D)	Dr. F. M. Wellings (D)	Open	Conference Summary
1730-1800	Open Discussion	Open Discussion	Open	Conference Summary

P - Presentation of Paper
D - Formal Discussion of the Paper

CONFERENCE PARTICIPANTS

THE INTERNATIONAL CONFERENCE
ON THE RENOVATION AND RECYCLING
OF WASTEWATER THROUGH AQUATIC AND
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JULY 16-21, 1975

1. Dr. Jorge Aguirre, Director for the Center for Research and Training in Water Quality Control, Ministry of Water Resources, Reforma No. 197-ler. piso, Mexico 4, D. F., Mexico.

2. Dr. George H. Allen, Professor and Chairman, Department of Fisheries, Humboldt State University, Arcata, California 95521.

3. Dr. Thomas G. Bahr, Associate Professor and Director, Institute of Water Research, 334 Natural Resources Building, Michigan State University, East Lansing, Michigan 48824.

4. Dr. Robert C. Ball, Professor and Associate Director, Institute of Water Research, 334 Natural Resources Building, Michigan State University, East Lansing, Michigan 48824.

5. Dr. Gunner J. Bonde, Professor and Director, Hygiejnisk Institut, Aarhus Universitet, Universitetsparken, 8000 Aarhus C, Denmark.

6. Dr. Francis E. Broadbent, Professor of Soil Microbiology, Department of Soils and Plant Nutrition, University of California, Davis, California 95616.

7. Dr. Franz A. M. de Haan, Professor, Department of Soil and Fertilizers, Agricultural University, De Dreyen 3, Wageningen, The Netherlands.

8. Dr. Frank M. D'Itri, Associate Professor of Water Chemistry, Institute of Water Research, 334 Natural Resources Building, Michigan State University, East Lansing, Michigan 48824.

9. Dr. Malcolm S. Gordon, Professor of Biology and Director, Institute of Evolutionary and Environmental Biology, 300 Veteran Avenue, University of California, Los Angeles, California 90024.

10. Dr. Balfour Hepher, Director, Fish and Aqua-Culture Research Station, Ministry of Agriculture, Agricultural Research Organization, Dor, D. N. Hof-Hacarmel, Israel.

11. Dr. Howard T. Odum, Professor and Director, Center for Wetlands, Phelps Laboratory, University of Florida, Gainesville, Florida 32611.

12. Dr. David Purves, Professor and Head, Spectrochemistry Department, The East of Scotland College of Agriculture, the Edinburgh School of Agriculture, West Mains Road, Edinburgh, EH9 3JG, Scotland.

13. Dr. Ralph W. Richardson, Jr., Director, Natural and Environmental Sciences, The Rockefeller Foundation, 1133 Avenue of the Americas, New York, New York 10036.

14. Dr. John H. Ryther, Professor, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02541.

15. Dr. Hillel I. Shuval, Associate Professor and Director, Environmental Health Laboratory, Hadassah Medical School, Hebrew University, Jerusalem, Israel.

16. Dr. Ron S. Sonstegard, Research Scholar, Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

17. Dr. Cord Tietjen, Director, Federal Agricultural Experimental Station, D 33 Braunschweig, Bundesallee 50, West Germany.

18. Dr. Gary H. Toenniessen, Assistant Director, Natural and Environmental Sciences, The Rockefeller Foundation, 1133 Avenue of the Americas, New York, New York 10036.

19. Dr. Laszlo Vermes, Senior Research Associate, Research Institute for Water Resources Development/VITUKI/, Kvassay Jeno ut 1, H-1095, Budapest, Hungary.

20. Dr. Flora Mae Wellings, Administrator, Epidemiology Research Center, 4000 West Buffalo Avenue, Tampa, Florida 33614.

THE ROLE OF PRIVATE FOUNDATIONS IN RESEARCH ON WASTEWATER TREATMENT AND REUSE

Gary H. Toenniessen

The last decade has witnessed a change, particularly in the developed nations, in public attitudes toward the environment. People today have a better understanding of the natural life support systems which they are dependent upon. They are more willing to adapt their life styles and technologies in ways which are consistent with maintaining a healthy and balanced ecosystem. This change has been reflected in the increased interest in employing wastewater disposal techniques which utilize wastewater as a nutrient containing resources. Such techniques recycle wastewaters back into natural systems with the potential of leading to an enhancement in water quality and to an increase in beneficial biological productivity. Private foundations, at least in the United States, have played a significant role in encouraging research on alternative approaches to wastewater recycling. It must also be admitted, however, that certain of these foundations also played a significant role in developing, usually for public health reasons, the traditional sanitary systems which treated wastewaters and disposed of effluents in rivers and lakes and led to entrophication and other environmental problems. Our concern for public health, of course, remains and it is essential to ensure that efforts designed to enchance the natural environment do not lead to unacceptable health hazards.

A brief history of wastewater recycling and of the role which one private foundation, The Rockefeller Foundation, has played should be illuminating. It is only in man's recent past, perhaps the last few centuries, that wide-scale community action has been taken to solve problems associated with the disposal of human wastes. In the early 1800's most cities collected and dumped sewage into nearby waterways and depended upon natural purification processes to eliminate health hazards and other deleterious consequences. Often such dependence on natural purification was not satisfactory from a health standpoint. It had for some time also been realized that sewage could be a valuable addition to agricultural lands. Several towns in England, such as Devanshire and Edinburgh, irrigated neighboring farm lands with their sewage. In 1842 Edwin Chadwick advocated the use of untreated sewage as field manure. He thought that urban sewage could be sold to farmers at a price which would pay for the cost of maintaining the sewage system. The concept of sewage farms spread and by 1880 Antwerp, Berlin, Brussels, Paris and Milan all had sewage farms.

By the turn of the century, however, the role of human wastes in the transfer and spread of certain diseases had become well established. There was growing concern that sewage farms could be aiding in the spread of disease. Physician G. W. Hosmer, in an article which appeared in Harpers Weekly XXXIV (1890) entitled, "Sewage and What Shall Be Done With It", noted that the use of raw sewage exposed farm employees to possible infection and that vegetables grown on the farms could be carriers of dangerous microbes or other parasites. At the same time engineers were developing other methods for sewage disposal such as septic tanks, chemical percipitation, and sprinkling filters. As these new systems increased in popularity, sewage farming lost favor.

It was also roughly at this same time that The Rockefeller Foundation was established and its interest in public health inititated. An excellent review of the Foundation's early involvement in this field is presented in Raymond B. Fosdick's "The Story of The Rockefeller Foundation". Even before the formal establishment of the Foundation, the Rockefeller Sanitary Commission was created in 1909. This Commission, which was devoted to the eradication of hookworm in the Southern United States, was directed by Dr. Wickliffe Rose and funded with a $1,000,000 gift from Mr. John D. Rockefeller. At this time the connection between the spread of hookworm and inadequate sanitary procedures had been established and the Commission focused on educating the physicians and the public concerning the causes and cures of the disease. Working with state and local governments, the Commission was able to educate the public, treat those with the disease, and protect against reinfection by vastly improving sanitary procedures including the elimination of soil disposal of human wastes. In 1913 The Rockefeller Foundation was created and the Sanitary Commission was merged with the new organization with Dr. Rose becoming Director of the Foundation's International Health Board. The objective of the Board was to extend to other countries and peoples the work of eradicating hookworm disease as opportunity offers, and as far as practical to follow up the treatment and cure of the disease with the establishment of agencies for the promotion of public sanitation and to spread the knowledge of scientific medicine. In the United States and in other countries the involvement of local government in these Foundation assisted programs led to the creation of local and state public health agencies. The result was that hookworm was no longer a vast public menace but was brought within limits which public health methods can readily control. At this time in the United States and most other nations public health work was relatively undeveloped (the exceptions being Great Britain and Germany which were far ahead of the United States). The attack on hookworm served as a method by which states and nations could be induced to build up permanent machinery to take care of the whole problem of public health. In pursuing these objectives, Dr. Rose soon realized that the major constraint was not so much a lack of either money or of organization as it was a lack of trained people. Special training in the theoretical and practical aspects of public health was necessary if preventive medicine was to keep abreast with conditions. Consequently the Rockefeller Foundation built and endowed the School of Hygiene and Public Health at Johns Hopkins University which opened in 1918. It provided for the first time in the United States a thorough training for full-time public health officers. This pioneering step was followed by a somewhat similar gift to Harvard in 1921, and assistance for related efforts in various nations. Other schools or institutes of public health which The Rockefeller Foundation assisted in these early years were located in Prague, Warsaw, London, Toronto, Copenhagen, Budapest, Oslo, Belgrade, Zagreb, Madred, Cluj, Ankara, Sofia, Rome, Tokyo, Athens, Bucharest, Stockholm, Calcutta, Manila, Sao Paulo and the University of Michigan. Over $25,000,000 was spent in this gigantic undertaking. Thus, The Rockefeller Foundation was one private foundation which was significantly involved in the increased attention given to public health in the early 1900's. This in part led to the reduction in the direct application or recycling of sewage onto agricultural lands.

The interest of The Rockefeller Foundation in public health continues today. Through support of programs seeking to control diseases such as schistosomiasis, the Foundation continues to encourage the development of effective sanitary systems. In addition a significant proportion of the Foundation's support of research on wastewater renovation and reuse (funded under the Foundation's Quality of the Environment Program) has been focused on the public health aspects of such procedures.

Large scale research programs examining the potential of using wastewaters as a nutrient source in agricultural and aquacultural production are now under way in the United States and other countries. At least five private foundations have provided financial assistance to these research efforts. They are: The Rockefeller Foundation, The Ford Foundation, The Kresge Foundation, The Jessie Smith Noyes Foundation and the Mary Flagler Cary Charitable Trust. In several instances it was private foundation support which enabled the research program to be initiated and thereby to attract governmental funding.

It is often difficult to determine exactly why private foundations, particularly the smaller ones, contribute to any given activity. However, with regard to The Rockefeller and Ford Foundations it can be noted that both have continuing programs which are concerned with environmentally sound natural resources management.

The following appendix is an attempt to identify those private foundations which may have an interest in providing support for research and/or demonstration projects which are directly or indirectly related to wastewater renovation and reuse. This list is obviously not complete since information on private foundations in certain nations was not available and when information was available it was probably incomplete. The information may also not be up-to-date since certain of the references and directories used were published four or five years ago. Those United States foundations listed have in the recent past awarded at least one grant related to water resources research or water pollution control. The other foundations listed were selected from directories in which only broad purposes were stated. They were chosen for inclusion if their stated purpose in part included support of research in the natural sciences and/or public health.

APPENDIX

Foundations Which May Have An Interest
In Research On Wastewater Renovation and Reuse.

NORTH AMERICA

United States

Foundations in the United States are considered as non-governmental, nonprofit organizations with funds and programs managed by their own trustees and directors, and established to maintain or aid social, educational, charitable, religious, or other activities serving the common welfare. The Foundation Directory Ed. 5, compiled by The Foundation Center, lists 2,533 foundations with assets of more than $1,000,000 or $500,000 or more in grants reported. In addition, there are more than 22,500 smaller foundations registered with the Internal Revenue Service. The Directory lists 38 foundations with assets greater than $100,000,000.

Allegheny Foundation
615 Oliver Building, Pittsburgh Pennsylvania 15222
Donor - Richard M. Scaife
Purpose - In part supports research in the natural sciences related to conservation and resources management.
1972 Expenditure - $2,585,217

Bush Foundation
W-962 First National Bank Building, St. Paul, Minnesota 55101
Donors - Mr. and Mrs. Archibald Bush
Purpose - In part supports research in the natural and environmental sciences
1973 Expenditure - $6,641,341

Mary Flagler Cary Charitable Trust
P.O. Box 289, Millbrook, New York 12545
Donor - Mrs. Melbert B. Cary, Jr.
Purpose - In part supports research on the conservation of natural resources
1973 Expenditure - $6,316,440

Edna McConnell Clark Foundation
250 Park Avenue, Room 900, New York, New York 10017
Donors - Edna McConnell Clark and W. Van Alan Clark
Purpose - In part supports research in the environmental and marine sciences including research on schistosomiasis
1974 Expenditure - $9,186,429

Charles E. Culpeper Foundation
866 United Nations Plaza, Room 408, New York, New York 10017
Donor - Charles E. Culpeper
Purpose - In part supports research in ecology
1972 Expenditure - $874,910

United States (Continued)

Field Foundation of Illinois, Inc.
135 South LaSalle Street, Chicago, Illinois 60603
Donor - Marshall Field IV
Purpose - In part supports research related to pollution control
1972 Expenditure - $1,117,576

Max C. Fleischmann Foundation
P.O. Box 1871, One East Liberty Street, Reno, Nevada 89505
Donor - Max C. Fleischmann
Purpose - In part supports research in the medical, biological, and environmental sciences.
1973 Expenditure - $4,784,003

Ford Foundation
320 East 43rd Street, New York, New York 10017
Donors - Henry Ford and Edsel Ford
Purpose - In part supports research on resource management and ecology and international environmental problems
1973 Expenditure - $248,715,000

George Gund Foundation
One Erieview Plaza, Cleveland, Ohio 44114
Donor - George Gund
Purpose - In part supports research in ecology and public health
1972 Expenditure - $2,457,936

William Penn Foundation (formerly The Haas Community Fund)
920 Suburban Station Building, 1617 JFK Boulevard, Philadelphia, Pennsylvania 19103
Donors - Otto Haas, Phoebe W. Haas
Purpose - In part supports research related to conservation
1973 Expenditure - $6,610,246

W. K. Kellogg Foundation
400 North Avenue, Battle Creek, Michigan 49016
Donor - W. K. Kellog
Purpose - In part supports research in public health and agriculture
1973 Expenditure - $21,031,779

Kresge Foundation
2401 West Big Beaver Road, Troy, Michigan 48084
Donor - Sebastian S. Kresge
Purpose - Primarily supports building construction including those associated with marine sciences and wastewater reuse
1973 Expenditure - $28,450,875

United States (Continued)

Lilly Endowment, Inc.
2801 North Meridian Street, Indianapolis, Indiana 46208
Donor - J. K. Lilly, Sr., Eli Lilly, J. K. Lilly
Purpose - Has on occasion supported research on water resources development
1973 Expenditure - $33,436,000

Henry Luce Foundation
111 West 50th Street, New York, New York 10020
Donor - Henry R. Luce
Purpose - In part supports research in the environmental sciences
1972 Expenditure - $1,881,090

Andrew W. Mellon Foundation
140 East 62nd Street, New York, New York 10021
Donors - Ailsa Mellon Bruce and Paul Mellon
Purpose - In part supports research in the natural and environmental sciences
1973 Expenditure - $28,867,877

Richard King Mellon Foundation
525 William Penn Place, Pittsburgh, Pennsylvania 15219
Donor - Richard King Mellon
Purpose - In part supports research related to environmental preservation
1973 Expenditure - $11,174,134

Moody Foundation
704 Moody National Bank Building, Galveston, Texas 77550
Donors - William Lewis Moody, Jr., Libbie Shearn Moody
Purpose - In part supports research in the natural and environmental sciences
1973 Expenditure - $7,801,402

Jessie Smith Noyes Foundation
16 East 34th Street, New York, New York 10016
Donor - Charles F. Noyes
Purpose - Primarily provides scholarships and fellowships which in part are in the environmental sciences
1972 Expenditure - $2,064,056

Resources for the Future
1755 Massachusetts Avenue, N.W., Washington, D.C. 20036
Donors - The Ford Foundation and others
Purpose - supports in-house and extramural research on resources management including water resources

United States (Continued)

Rockefeller Foundation
1133 Avenue of the Americas, New York, New York 10036
Donor - John D. Rockefeller
Purpose - In part supports research and training in natural and environmental sciences and in public health
1973 Expenditures - $46,747,537

Rockefeller Brothers Fund
30 Rockefeller Plaza, New York, New York 10020
New York, New York 10020
Donors - Eight members of the Rockefeller Family
Purpose - In part supports research related to conservation and preservation of the environment
1973 Expenditure - $13,629,129

Rockefeller Family Fund
49 West 49th Street, New York, New York 10020
Donors - Members of the Rockefeller Family
Purpose - In part supports research related to conservation and public asthetics
1973 Expenditure - $834,555

Stern Fund
21 East 40th Street, New York, New York 10016
Donors - Members of the Stern Family
Purpose - In part supports efforts to control pollution
1973 Expenditure - $1,179,802

Woods Charitable Fund, Inc.
59 East Van Buren Street, Chicago, Illinois 60605
Donor - Frank H. Woods and Nelle C. Woods
Purpose - In part supports efforts to improve public policy related to water and land resources
1972 Expenditure - $1,532,530

Canada

While there are many Canadian foundations and several with substantial resources, it is difficult to obtain information concerning them because no financial details are published by the Canadian government. Some of the Canadian foundations do publish reports of one form or another but many provide no public reporting at all.

The Atkinson Charitable Foundation
80 King Street West, Toronto, Ontario
Donor -- Joseph E. Atkinson
Purpose -- In part supports research in the natural and medical sciences and in engineering.
1967 Expenditure -- $815,741

Canadian Council on Urban and Regional Research
Suite 511, 151 Stater Street
Donor -- Ford Foundation and Canadian Government
Purpose -- To aid in the understanding of urban and regional problems in Canada
Annual Expenditure -- Approximately $100,000

Donner Canadian Foundation
P. O. Box 122, Toronto-Dominion Center, Toronto 1, Ontario
Donor -- William H. Donner
Purpose -- In part supports inter-disciplinary environmental research
1967 Expenditure -- $598,045

EUROPEAN FOUNDATIONS

Denmark

In Denmark foundations are regulated by the Ministry of Justice but they exist essentially without statutory legislation. There is no general register where information on foundations can be found.

The Carlsberg Foundation
H.C. Andersens Boulevard 35, 1553 Copenhagen V
Donor -- Jacob Christian Jacobsen
Purpose -- In part provides support of research in the natural sciences
1970 Expenditure -- over $2,000,000

The Carlsberg Bequest to the Memory of Brewer J.C. Jacobsen
Vesterfaelledvej 100, 1799 Copenhagen V
Donor -- The Carlsberg Foundation
Purpose -- Supports research in the medical and natural sciences including botany, zoology, forestry, and agriculture
1970 Expenditure -- over $2,000,000

Carlsen-Lange Foundation
Gammelkjøgeggard, 4600 Køge
Donor -- Miss Emmy Henrriette Marie Elisabeth Bertha Hilleburg Carlsen
Purpose -- In part supports research in the natural sciences including agriculture and forestry

Finland

The Foundation Act of 1930 entrusted the Ministry of Justice with authority over Finish foundations. Foundations are obliged to submit to the Ministry an annual report that includes the accounts.

Emil Aaltonen Foundation
Kyllikinkatu 13-15, Tampere
Donor -- Emil Aaltonen
Purpose -- In part supports research in the medical and natural sciences
1970 Expenditure -- over $100,000

The Finish Cultural Foundation
Bolevard: 5A, 00120 Helsinki 12
Donor -- The Finish people
Purpose -- In part supports research in the medical and natural sciences including limnology, forestry, and agriculture
1970 Expenditure -- over $600,000

The Foundation for Research of Natural Resources in Finland
Runeberginkatu 31 A 00100 Helsinki 10
Donor -- Suomen Hiilikauppiaitten Yhdistys
Purpose -- Supports research promoting the technical and economic development of Finish forest and other natural resources
1970 Expenditure -- over $100,000

Saastamoinen Foundation
Box 43 Sasstamoinen Oy 70101 Kuopio
Donors -- Liisa and Osmo P. Karttunen
Purpose -- In part supports research on natural resources development
1970 Expenditure -- over $28,000

France

There is a common tendency to think that in France there are no Foundations, nor can there be any. Foundations do exist in France but most are poorly endowed and the few larger ones are primarily operational as opposed to grant making.

The Pasteur Institute
25/25 rue du Docteur Paris XV
Donor -- the people of France and other nations
Purpose -- Primarily supports in house research on micro-biology, virology and immunology but also awards and scholarships to individuals pursuing research in these fields.
1970 Expenditure -- over $20,000,000

Singer-Polignac Foundation
43, avenue Georges - Mandel
Donor -- Princess Edmond de Polignac
Purpose -- In part supports research in the medical and natural sciences

Germany

After the Second World War the legal basis for foundations was recreated in Germany. It has been estimated that by 1970, in the Federal Republic and West Berlin there were approximately 4,000 independent foundations. Many of these are small and/or operational but several large grant making foundations have also been established.

Chemical Industry's Fund for the Promotion of Research, Science and Teaching
Karlstrasse 21, 6 Franfurt/Main
Donor -- The Confaderation of the Chemical Industry
Purpose -- Supports research and teaching in chemistry at German universities
1970 Expenditure --

Donor's Association for the Promotion of Sciences and Humanities in Germany
Brucker Holt 56-60, 43 Essen-Breduney
Donors -- Industrial and commercial organizations
Purpose -- In part supports research in the medical and natural sciences
1970 Expenditure -- $11,300,500

Fund for Environmental Studies
Adenaurallee 214, 53 Bonn
Donor -- IBM Deutschland
Purpose -- In part supports scientific initiatives related to problems of the environment including conservation of soil, air and water
1970 Expenditure -- Over $10,000

Werner Reimers Foundation
Am Wingertsberg 4, 6380 Bad Homburg
Donor -- Werner Reimers
Purpose -- To promote communication and cooperation in the sciences including interdisciplinary projects focused on the natural sciences
1970 Expenditure -- $700,000

Fritz Thyssen Foundation
Habsburgerring 9, Postfach 27 03 74, 5 Koln 1
Donor -- Amelie Thyssen and Anita Zichy-Thyssen
Purpose -- In part supports research in the medical and natural sciences

1970 Expenditure -- $2,726,000

Volkswagenwerk Foundation
Kastanienallee 35, 3000 Hannover-Dohren
Donor -- Established by the Federal Republic of Germany
Purpose -- To advance the development of science, technology, and the humanities including the medical and natural sciences
1970 Expenditure -- $48,522,500

Greece

In Greece foundations have legal status resulting from the Civil Code of February 23, 1946 which comprises a comprehensive set of rules about foundation's establishment and management. Data on foundations are registered by the Ministry of the Interior and are public.

The National Hellenic Research Foundation
48, Vassileos Constantinou Avenue, Athens
Donor -- Established in 1958 by King Paul with funds from the American Government
Purpose -- In part supports scientific research in the natural sciences and engineering
1970 Expenditure -- $440,435

Italy

Italian law lays down no explicit requirements for a foundation. The deed or formal document by which a foundation acquires juridical personality is a Presidential decree recognizing the existence of the foundation. There is a high degree of uncertainty in interpreting rulings controlling the function of foundations and no easement of tax burden or other forms of encouragement are offered to potential donors. Existing foundations in Italy are primarily concerned with cultural matters and few work in the field of the natural sciences.

Querini Stampalia Scientific Foundation
Castello 477 8, 30122 Venice
Donor -- Count Giovanni Querini
Purpose -- In part supports university research in the natural sciences
1970 Expenditure -- $117,240

The Netherlands

There are approximately 30,000 registered foundations in The Netherlands. Although there is a Foundation Act of 1956 Dutch law requires minimum conditions for the establishment and operation of foundations, without any limitations as to their purpose. Consequently many of those organizations registered as foundations donot function according to the traditional definition of foundations stated previously.

Foundation for the Conservation of Clean Air and Water
21, Presidente Kennedylaan, The Hague
Donor -- A group of petroleum companies
Purpose -- To support the collection and publication of scientific, technical and legal information on the sources, control and effects of pollution, and to promote research on pollution.

Norway

Request to establish private foundations are ratified by the Norwegian government only when they are judged to have public interest with regard to both purpose and size of capital. The capital should be large enough to fulfill the aims of the foundation without having to be touched. Control and auditing of foundations is under the authority of the Ministry for Social Affairs.

Fridtjof Nansen's and Affiliated Funds for the Advancement of Science and the Humanities
Drammensveien 78, Oslo 2
Donor -- Established by private donors in honor of Fridtjof Nansen
Purpose -- In part supports research in the medical and natural sciences including botany, zoology, agriculture, forestry and conservation
1970 Expenditure -- $165,150

The Scientific Research Fund of 1919
Drammensveien 78, Oslo 2
Donor -- Norwegian government
Purpose -- In part supports research in the medical and natural sciences
1970 Expenditure -- $70,750

Spain

In 1963 the Spanish Archives listed 4,425 foundations. Of those only a small group were established with considerable capital and definite aims related to promoting and supporting cultural and scientific progress.

Jann March Foundation
Núñez de Balboa, 70, Madrid 6
Donor -- Jan March Ordinas
Purpose -- In part supports research in the medical and natural sciences
1970 Expenditure -- $6,390,625

Sweden

Following the Second World War there was a rapid increase in the number of Sedish Foundations. This is primarily due to tax laws which make contributions for certain idealistic purposes exempt from both income and gift taxation.

The Magnus Bergvall Foundation
Box 16067, 103 22 Stockholm 16
Donor -- Magnus Bergvall
Purpose -- In part supports research in the medical and natural sciences
1970 Expenditure -- $193,555

The Ekhaga Foundation
Kungsgatan 18, 11135 Stockholm
Donor -- Gösta Videgård
Purpose -- Supports research in the natural sciences focused on the production of food without the use of poisons and artificial means.
1970 Expenditure -- Over $100,000

Knut and Alice Wallenberg Foundation
Box 16067, 103 22 Stockholm 16
Donors -- Knut and Alice Wallenberg
Purpose -- Primarily supports research in the medical and natural sciences
1970 Expenditure -- $2,945,545

Wenner-Gren Foundation
Sturegatan 34, 114 36 Stockholm
Donors -- Axel and Marguerite Wenner-Gren
Purpose -- In part supports research in the medical and natural sciences
1970 Expenditure -- over $100,000

Switzerland

Although there are about 20,000 foundations registered in Switzerland only about 10% pursue social, cultural and scientific aims. Those foundations which are non-profit and in support of general welfare are not subject to tax. Many are associated with Swiss corporations.

CIBA Foundation for Scientific Medical and Technical Research
c/o Ciba-Geigy AG, Klybackstr, 141, 4002 Basel
Donor -- CIBA AG
Purpose -- Supports research in the natural and medical sciences
1970 Expenditure -- $170,000

SANDOZ Foundation for the Advancement of Medical and Biological Sciences
c/o Sandoz S.A., Legal Department, Lichstrasse 35 4000 Basel 13
Donor -- SANDOZ S.A.
Purpose -- Supports research in the medical and biological sciences
1970 Expenditure -- $103,700

The United Kingdom

Foundations in England and Wales have for a long time been a significant element of voluntary activity to promote social progress. It is a clearly stated government policy to regard voluntary action, including foundation activity, as a partnership with government activity in serving the community. The Charities Act of 1960 provided three main developments in the framework within which foundations operate. It required registration of foundations, relaxed conditions under which a change in objectives could be made, and provided a statutory basis for cooperation between foundations and public services.

Commonwealth Foundation
Marlborough House, Pall Mall, London S.W. 1
Donors -- Commonwealth member governments
Purpose -- In part to support research in the medical and natural sciences
1970 Expenditure -- $915,000

The Rayne Foundation
100 George Street, London W.1
Donor -- Sir Max Rayne
Purpose -- In part supports research in the natural and medical sciences
1970 Expenditure -- over $1,000,000

The Royal Society of London for Improving Natural Knowledge
6 Carlton House Terrace, London SW1Y 5AG
Donors -- Private and public sources
Purpose -- Primarily to support research and education in the natural sciences
1970 Expenditure -- over $1,000,000

ASIAN AND AUSTRALIAN FOUNDATIONS

Japan

It is estimated there are approximately 14,000 charitable corporations (foundations) in Japan which engage in grant making activities. They are not regulated by a single agency but are controlled by a variety of agencies depending on the activities they are engaged in. Most were founded by a corporation or group of corporations.

The Chiyoda Mutual Foundation
2-19-18 Kamimeguro, Meguro-ku, Tokyo 153
Donors -- The Chiyoda group of corporations
Purpose -- In part supports research on public health and hygiene in rural areas including water supplies
1973 Expenditure -- over $200,000

The Hatakeyama Cultural Foundation
2-20-12 Shirogan edai, Minato-ku, Tokyo 108
Donors -- A group of corporations including Shinwa Industries
Purpose -- Supports university research in the natural sciences and engineering
1973 Expenditure -- $1,600,000

The Mitsubishi Foundation
15th Floor, Mitsubishi Bldg. 2-5-2 Marunouchi, Chiyoda-ku, Tokyo 100
Donor -- The Mitsubishi Corporation
Purpose -- In part provides financial assistance for research in the natural sciences
1973 Expenditure -- $1,000,000

The Sanwa Midori Fund
Sanwa Bank Bldg. 1-1-1, Otemachi, Chiyoda-ku, Tokyo 100
Donor -- Sanwa Bank
Purpose -- Supports projects to make the environment more livable including research on pollution prevention
1973 Expenditure -- $340,000

Tokyu Foundation for a Better Environment
1-16-14 Shibuya, Shibuya-ku, Tokyo 150
Donor -- A group of corporations including Tokyo Electric Express Railway
Purpose -- Supports research and experiments concerning protection of water purity in the Tama River and its basin
1973 Expenditure -- $380,000

The Toyota Foundation
P. O. Box 236, Shinjuku-Mitsui Bldg., 2-1-1 Nishishinjuku
Shinjuku-ku, Tokyo 160
Donor -- The Toyota Motor Company
Purpose -- In part supports research on the living and natural environments
1973 Expenditure -- $380,000

Australia

The important role which can be played by trusts and foundations in the world of philanthropy has not yet received more than passing attention in Australia. In recent years, however, a few foundations of significance have been established in Australia and recognition of the contribution is growing.

The Raymond E. Purves Foundation
4th Floor, The Raymond Purves Bldg., 140 Arthur St., North Sydney New South Wales 2060
Donor -- Raymond E. Purves
Purpose -- In part supports research in the natural sciences carried out in universities and colleges

R.E. Ross Trust
8th Floor, 492 St. Kilda Road, Melbourne, Victoria 3004
Donor -- R. E. Ross
Purpose -- In part supports research on and conservation of natural areas
Annual Expenditure -- $250,000

Water Research Foundation of Australia
Box 47, PO Kingsford, New South Wales 2032
Donor -- Public and private contributions
Purpose -- Supports research into all aspects of efficient use of water
Annual Expenditure -- $50,000

LATIN AMERICAN FOUNDATIONS

Argentina

The Argentine Civil Code defines foundations as endowed nonprofit institutions dedicated to the public welfare. They may not have commercial purposes, but may carry out transactions designed to increase their endowment. They are regulated by the Federal Ministry of Justice to which they are obliged to provide administrative and accounting information.

Acindar Foundation
- Paseo 357, Buenos Aires, Argentina
- Donors - Numerous individual contributors
- Purpose - In part supports research in the natural sciences
- 1965 Expenditure $50,000

José María Aragón Foundation
- Leandro N. Alem 884, Buenos Aires
- Purpose - In part administers grants and fellowships for agricultural research including water resources research
- Annual Expenditure - approximately $2,000,000

Paul Bardin Foundation
- Córdoba 1513-11° piso, Buenos Aires
- Donor - Paul Bardin family
- Purpose - supports scientific research concerned with the use and conservation of the resources of Argentina
- 1966 Expenditure - $5,000

Bariloche Foundation
- Lavalle 534-2° piso, Buenos Aires
- Donors - National and foreign industries and foundations
- Purpose - In part supports research and training in hydrology, ecology, geology, and biology with emphasis on the postgraduate level
- Annual Expenditures - approximately $800,000

Juan Bautista Sauberán Foundation
- Obligado 2490, Buenos Aires
- Donor - Juan Bautista Sauberán
- Purpose - Supports research in agricultural microbiology and land conservation
- Expenditures - not available

Brazil

In Brazil foundations may be either public or private. A public foundation is an endowed institution funded by a public body for a specific purpose. A private foundation can be funded by a private person(s), and its purposes must be specified. A private foundation is supervised by the Public Attorney of the state in which it is located.

Communitary Action of Brasil - Guanabara
Rua Aureliano Portugal N°. 205, Rio de Janeiro
Donors - Industry and private entities
Purpose - In part concerned with the provision of hygiene including sanitary water supplies
Annual Expenditure - $248,400

Delmiro Gouveia Foundation
Ave. Rio Branco, 257-9° andar, Rio de Janeiro
Donors - Companies
Purpose - In part supports research in the natural sciences and planning
1966 Expenditure - About $100,000

São Domings Foundation
Rua João Brícola, 39-9° andar, São Paulo
Donors - Companies
Purpose - In part supplies research in the natural sciences
Annual Expenditure - About $500,000

The Public Health Special Service Foundation
Caixa Postal 1530, Ave. Rio Branco, 251-12° andar, Rio de Janeiro
Donors - Private gifts and government subsidies
Purpose - In part supports research and projects in the area of public health including water supplies and wastewater treatment
Expenditures - Not available

The Thomas Alberto Wathely Foundation
Al. Franca, 659-2° andar, São Paulo
Donors - Individuals and companies
Purpose - In part supports research on agricultural development including water supplies
Expenditures - Not available

Mexico

In Mexico foundations are considered to be nonprofit institutions formed by the irrevocable transfer of private property to be used for humanitarian purposes. They are controlled by the Private Welfare Institutions Board which screens both income and expenditures

Mexico (Continued)

The Foundation for Engineering
Calzada de Legaria No. 252, Mexico, D.F.
Donors - Various companies
Purpose - In part supports research in water resources engineering
1966 Expenditures - $20,000

The Dolores Sanz de Lavie Foundation
Jalapa 94B, Mexico D.F.
Donor - Not available
Purpose - In part supports research on the public health aspects of water resources
1966 Expenditures - $40,000

The Sears Roebuck of Mexico Foundation
San Luis Potosi No. 214, Mexico, D.F.
Donor - Sears Roebuck de Mexico, Inc.
Purpose - In part supports research in the natural sciences
1966 Expenditure - $20,000

Venezuela

Foundations in Venezuela may be created to serve the public interest in the fields of the arts, sciences, literature, charity or social welfare. The law contains no restrictions on foundations' making commercial investments, provided that the income thereof is used to further the statutory objectives of the foundation.

The La Salle Foundation for the Natural Sciences
Ave. Cota Mil, Tienda Honda a Santa Bárbara, Apartado 8150, Caracas
Donors - La Salle Association for the Natural Sciences
Purposes - Supports research in the natural sciences and operates a station for research in oceanography and marine biology.
1966 Expenditures - $800,000

The Eugenio Mendoza Foundation
Edif. Las Fundaciones Ave. Andrés Bello, Caracas
Donors - Eugenio Mendoza and Luisa Rodriguez de Mendoza
Purpose - In part supports agricultural research and development including water resources research.

The Henrique Otero Vizcarrondo Foundation
Edif. Sudameris, Of. 802 Ave. Urdaneta, Esq., Fuerzas Armadas, Caracas
Donor - Henrique Otero Vizcarrondo
Purpose - In part supports research in the natural sciences

Venezuela (Continued)

The Venezuelan Foundation for Natural Sciences
Ave. Paéz, El Paraiso, Apartado 1521, Caracas
Donor - Venezuelan Society for Natural Sciences
Purpose - To promote the study of the natural sciences and the development of natural resource conservation programs.

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WASTEWATER UTILIZATION IN ISRAEL AQUACULTURE

by

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INTRODUCTION

The use of domestic sewage in fish ponds is almost as old as aquaculture itself. In China and the Far East, where aquaculture originated, the use of domestic wastes in the ponds was quite common. Night soil was added as a manure to the water or laterines were built directly over the pond water (Prowse, 1962). Besides solving the immediate problem of sewage removal, it also brought about an appreciable increase in fish yields. No doubt this may have created hazards to those working in the ponds or to those eating the fish, but awareness of such hazards was small. However, with the expansion of fish culture beyond the Far East region and with the change in its nature from small homestead ponds to large commercial farm ponds, objections on hygienic grounds and the serious oxygen deficiencies caused by the decay of large quantities of organic matter became more important and the use of sewage nearly ceased.

Nevertheless, several attempts have been made to utilize treated municipal wastewater in fish ponds. At times this was initiated by a need to find a solution to an environmental problem where there were no other recipients for the treated effluents; but more often, waste water was used because of its benefits in increasing fish yields.

Because of the high BOD of the waste water and the resulting deoxygenation of the water during the night, fish usually cannot be cultured directly in the waste itself. Means must be taken to reduce the BOD. This usually is brought about by one of the following three methods:

(a) Treating the waste to such a degree that it does not create any hazard to the fish. Mayenne (1933) describes the sewage treatment farm at Lubezetz, Moscow, where a "cascading" series of six ponds receives undiluted sewage. Carp could be stocked only in the last three ponds, and then only for the summer. The dissolved oxygen content in the other ponds was subject to rapid depletion causing fish kill. Carpenter _et al_. (1974) and Coleman _et al_. (1974) describe a newly built lagoon system at Quail Creek, Oklahoma, U.S.A., which consists of six cells operating in series. This system receives a flow of 1 mg (3800 m^3) per day of domestic wastes. The first two cells provide conventional, aerated treatment. The four last cells, six acres each, are stocked with fish. The detention time in each of these cells is about 13 days. Fish survival in these cells was high, except for mortality of _Tilapia_ due to cold. Though no feed was added to the ponds good yields of fish were achieved. Hay (1955) reports experiments at Althone, S. Africa, where sewage was treated by a two stage trickling filter and a final sedimentation. The effluent was introduced into a series of fish ponds with varying flow-through. Oxygen concentrations were low, usually less than 4 mg per liter and this inhibted fish growth. However when the fish ponds were cleaned of the humic residues, filled, and then only regularly topped to make up evaporation and seepage loses, fish, especially Tilapia species, grew well. Wolny (1962) conducted a similar experiment at Kielce, Poland. The sewage was treated by the activated sludge process resulting in a relatively high quality effluent (BOD 10.5 mg per liter). This effluent was introduced into five different ponds. One of the ponds had flow (detention time of two days). The other ponds were filled and then topped to make up evaporation and seepage. The regime of dissolved oxygen was better in the stagnant water ponds than in the flow-through ponds and, as a result, there was better fish growth. Stocking of fish in the ponds increased the dissolved oxygen concentrations.

(b) Diluting the wastewater before its introduction into the pond. This method was adopted by the now classical example of Munich's (Germany) wastewater-fish ponds. A number of works describe this system (c.f. Demoll, 1926; Kisskalt and Ilzhofer, 1937; Scheuring, 1939). The wastewater, having a low BOD (162 mg per liter - Scheuring, 1939) because of dilution by brewary wastes, is passed through a settling tank where it loses about

30% of its BOD. The effluent is then diluted at a ratio of 1:4 with river water before being introduced by spraying into the fish ponds(201 ha). A similar system is described by Vaas for an experiment fish pond of about 840 m^2 in Indonesia. Domestic wastewater coming from a large septic tank was mixed at a ratio of 1:3 with river water. Fish loss in this pond was not greater than non-sewage ponds and a yield of 4000 kg per ha per year was attained, apparently without any supplementary feed.

(c) Diluting the wastewater in the pond by the water contained by the pond itself. The pond water can be freshwater or aged and stabilized wastewater. The best example for this system is that of Calcutta wastewater (Bose, 1944, Basu, 1949, Nair, 1944). The raw sewage is passed through sedimentation tanks where it loses part of its BOD. Some settling occurres also in the long channel leading to the vicinity of the ponds. The final BOD is about 100-115 ppm. The wastewater is then introduced into two fish ponds, 243 and 101 ha, each of about 60-120 cm deep. The sewage is introduced once a month at a rate of about $130\text{-}180\times10^3 m^3$ during a period of 5-10 days. This is diluted by the water in the pond. As the ponds contain $450\text{-}540\times10^3$ cubic meters of water then, the dilution is 3-4 fold. Except for the zone of the wastewater inlet, where the BOD is, at the time of waste inlet, 70-92.5 ppm, and the DO reduced to nil, there was enough oxygen in the pond to sustain the fish (about 6 ppm in the mornings). The water returned to normal 10-12 days in summer, and 15-20 days in winter, after introduction of the wastewater.

The majority of the ponds using wastewater in Israel is of this kind. The area of such ponds in Israel is estimated at about 50 to 100 ha. They usually utilize the wastewater of relatively small rural communities, the Kibbutzim, consisting of about 500-1500 inhabitants, and producing about 100-600 m^3 of wastewater per day. The fish ponds are filled with fresh water. Sewage is then used to replace seepage and evaporation. As this loss amounts to about 1.0 to 1.5 cm per day and as the average depth of water is about 150 cm, the resulting dilution of the wastewater within the pond is about 100-150 fold. Even when the BOD of this inlet water is high (usually it is about 250-300 mg per liter), the high dilution reduces it to a value that is able to be satisfied by the supersaturation of O_2 produced in the pond during the daylight hours. The effect on the early morning dissolved oxygen concentration, the time of critically low DO, is slight. Were more sewage water available in these communities, it could also be treated in the existing fish ponds.

From the foregoing discussion, it is clear that the use of wastewater in fish ponds requires either pre-dilution or a long retention time in the ponds. This limits the amount of wastewater able to be used per unit area of pond. In order to increase the amount of wastewater which can be treated in a pond serving the dual function of waste treatment and fish culture, the retention time optimum for maximum waste treatment and fish yield must be learned. The task of this paper is to discuss the benefits, hazards, and limitations of the integration waste treatment and aquaculture, as experienced in Israel.

BENEFITS IN USING WASTE WATER IN AQUACULTURE

Effects on the Food Chain

The source of the high fish yields in ponds receiving organic wastes appears to lie in the high yield of natural foods produced by these wastes. We have found in many commercial fish ponds that utilization efficiency of supplemental feeds is strongly correlated with the standing crop of fauna in the pond. The organic wastes supply nutrients to the chain of natural foods by two distinct processes. The chemicals carried by the waste, not only nitrogen and phosphorous, but also the trace elements, provide the basic building blocks for phytoplankton. This link in the food chain provides oxygen to the pond via photosynthesis, and foods to herbivorous fish and zooplankton. Photosynthetic production is, however, limited by the reduction of light penetration into the water at high plankton densities. Hepher (1962) has demonstrated that increasing the rate of chemical fertilization does not consistently result in increased primary production per unit area. At a certain level of fertilization, because of reduced light penetration into the deeper layers of the water, primary production in these layers decreased and the rate of primary production per unit area reached a plateau.

It is because of this limit on photosynthetic production, that the second process of nutrient supply by organic wastes is important. The solid fraction of the waste, and especially the suspended "fines", appears to supply a food directly to the second link of the natural food chain, the zooplankton and the benthos. Thus the sun-limited photosynthetic production of phytoplankton is by-passed. In aquatic systems receiving organic wastes, zooplankton can be observed to graze directly on the suspended solids. We have cultured dense populations of Daphnia for 80 days in dark, aerated jars receiving fluid cow manure at a daily rate of 1 part manure per 1,000 to 10,000 parts water. The absence of light eliminated photosynthetic production of plankton as a food source. Only the manure was available. In non-manured control jars, initially containing concentrations of Daphnia equal to the manured jars, the Daphnia concentration fell to near or complete extinction in three days. In the manured jars the Daphnia actively grazed on the suspended waste. Investigation of the guts of the Daphnia showed that they contained bacteria. It may be that the solids, while suspended in the water, provide a plate for bacteria culture. When the solids are ingested by the zooplankton the bacteria are digested providing the zooplankton with a protein and vitamin rich food (Mann, 1972).

In the culture of common carp, a bottom feeder, benthic life is especially important. Wirshubsky and Elchunes (1952) demonstrated that the presence of organic waste resulted in a 30-fold increase in the production of chironomide larvae over ponds without sewage. More eggs were deposited by adult chironomides and the growth of the larvae was more rapid in the waste ponds.

Standing stocks of fauna observed in ponds with or without manure and with and without fish were reported by Schroeder, (1974). Water temperatures during these observations ranged from 9 to 15^{o}C. For ponds not stocked with fish, the standing stock of zooplankton, on a dry weight basis, was 50 to 1000 times greater, and the chironomide concentration was 10 to 200 times greater, in ponds receiving manure than in non-manured ponds. Comparing the data for ponds with and without fish shows the effect of the fish grazing on these feeds and reducing the concentration of the standing stocks by greater than 10 fold. Based on 6.25 times the total Kjeldahl nitrogen analysis, the zooplankton had a 56% protein content.

We observe that fish ponds receiving sewage often have higher dissolved oxygen (DO) concentrations than fish ponds operated without the addition of sewage. The waste by increasing nutrient input to the pond, increases the production of plankton. The grazing of the fish on these plankton keeps the pond in ecological balance with large standing stocks of phytoplankton in the sewage-pond and hence higher DO.

Effects on Fish Yield

The benefits of using wastewater in aquaculture on yield of fish and efficiency of feed utilization are demonstrated by data taken from a commercial fish farm. A Kibbutz of 500 people, located in the interior of Israel, includes among its diverse agriculture operations, a fish farm of 60 hectares. The entire sewage output from the kibbutz, including the wastewater from its laundry, is about 150 m^3 per day. This flow is channeled through a 24 m^3 settling tank, diluted with two to four parts of fresh water and distributed among three fish ponds, having a total area of 2.7 hectares. A fourth pond, two hectares in area, receives the flow of manure and wash-water from the milking room of the kibbutz's 250 cow dairy. All the ponds on the kibbutz, with the exception of the ponds receiving sewage, are fertilized every two weeks with ammonia and phosphate at a rate of 60 kg per hectare, and with sun-dried chicken manure at a rate of 0.5 m^3 per hectare. During the year under study (1974), all of the ponds received a ration composed of about half sorgum grains and half pelletized feed containing 2 to 5% fish meal and 25% total protein. This feed was supplied, six times per week, at a rate of 4% of the carp biomass and 2% of the tilapia biomass present in the pond at the time of feeding. Summer water temperatures ranged from 33°C (day) to 27°C (early morning).

Table I summarizes the production results of six ponds similarly stocked with fish. Four of these ponds received either sewage or manure. The effectiveness of the added organic wastes in increasing both the yield of fish and the efficiency with which the supplied feed was converted into fish flesh is clear. In ponds receiving organic wastes, the yield of fish per hectare is 70% greater than in ponds not receiving organic wastes. Furthermore, 40% less supplemental feed is required to grow a kilogram of fish in the organically fertilized ponds than in the not fertilized ponds. In light of the high market values of edible fish and of supplemental feeds, the economic advantages for using wastewater in aquaculture is clear.

If lower total yields per unit area of pond are acceptable, organic wastes, without supplemental feeds, may be used as the primary nutrient source to the fish pond. In an experiment carried out at the Fish and Aquaculture Research Station, Dor, by Wohlfarth and others (Private communication), two 400 m^2, 80 cm deep earthen ponds were stocked with fish at a rate of 3000 common carp (av. weight 25 gm), 1250 silver carp (100 gm), 1500 Tilapia aurea (2 gm) and 330 grass carp (150 gm), per hectare.

Fluid cowshed manure (containing urine and feces) was broadcast over 1/5 of the pond surface. Initial application was at a rate of 250 liters of manure per hectare five times per week. This rate was doubled when the growth rate of the common carp declined. Every two weeks chicken manure at rate of 0.5 m^3 per hectare, and ammonia and super phosphate at a rate of 60 kg per hectare, were supplied. Water temperatures ranged from 26^{o}C to 31^{o}C. Although the ability to aerate the ponds was present, it was not necessary. Photosynthetic production of oxygen was adequate. Figure I shows the growth rates of the four fish species during the 120 days of the test. Note that during the later part of August and most of September, the common carp did not grow well. Doubling the manuring, at the end of September, resulted in a marked increase in the growth rate. The total yield for the 120 days of this test was 2350 kg per hectare. A 250 day growing season with the polyculture of this test extrapolates to give a net fish crop of 4900 kg per hectare.

Fish grown with wastewater as the source of nutrients have not experienced health problems. They may however be carriers of salmonella and other bacteria without adverse affects to themselves (Shewan, 1962). As a safety precaution for consumers, fish grown in sewage are given a "flush-out" period of several days in fresh water. Fish grown in ponds with no supplemental feeds, and only cow manure as the nutrient to the pond, were of high quality. Fat contents of the common carp was 4% as compared with fat contents in excess of 15% for carp receiving a supplemental ration of grains or pelletized feed (Wohlfarth, 1974). This low fat content likely results from the high protein content of the natural foods which formed the total diet of the fish in these fertilized ponds.

Increased Waste Treatment Capacity

Bacterial and BOD Reduction

The capacity of the pond to purify waste water is greater than the conventional treating methods. Kisskalt and Ilzhöfer (1937), who studied this aspect at the Munich's waste water fish ponds system, state that while reduction of total bacterial count by the trickling filter method was about 89% and by the activated sludge method about 90%, the reduction of bacterial count in fish ponds receiving the wastewater reached 99.6%. Vaas (1948) quotes Schaeffer who found that the outflowing water from a 840 m^3 fish pond receiving a flow of 5.4 m^3 waste water per hour premixed with 16.2 m^3 per hour clean water (total detention time - 38 hrs), has lost all typhoid bacilli, and the coli titer was reduced to 1/100 of its original value. Carpenter et al. (1974) showed that the removal of coliform organisms in the third cell of the Quial Creek Lagoon System containing fish was at least as efficient as in the previous aerated lagoons. Fecal coliforms were reduced in this cell from about 1000 organisms per 100 ml to less than 10 per 100 ml.

We have observed (Hepher and Schroeder, 1974; Schroeder 1975) that for ponds receiving organic wastes, the presence of fish improves the quality of waste treatment and increases the waste treatment capacity of the pond. Table II presents data measured in ponds with and without fish and with and without addition of organic wastes. For three critical parameters listed in this table, the presence of fish improves the treatment potential of the pond. Ponds receiving manure, when stocked with fish, had higher average DO and pH, and lower bacteria counts, than ponds receiving equal amounts of manure but not stocked with fish. These improved conditions result from the fish grazing on the plankton which grow abundantly in the eutrophic conditions of a waste treatment pond. This grazing controls the plankton blooms and eliminates the cycles of plankton bloom and die-off typically observed in waste ponds, with the accompanying anaerobic conditions of the die-off phase. This results in the average DO being higher in the fish stocked ponds.

Wolny (1962) also found that the presence of fish in a pond receiving waste water favors the development of the phytoplankton, especially small forms of green algae. This increases the photosynthesis, and consequently, considerably improves the oxygen regime in the water.

Raising the DO increases the rate at which the BOD of the waste is satisfied. Oswald (1972) reports that increasing DO and pH in an aquatic system, increases the rate of disinfection from coliform. This appears to be reflected in the lower general bacteria count which we observed in waste ponds stocked with fish.

In many agricultural communities, disposal of manure is a significant problem. In the intense and integrated agricultural activities of Israeli kibbutzim, more manure is often produced than can be used for fertilizing fields. The problem is more severe in the vicinity of cattle feed-lots such as the Texas panhandle region (A.D. Little, 1972). Here the integration of steer culture with fish culture could turn a presently polluting waste into the basis of a valuable resource.

Nutrient Removal

The high concentration of nutrients, especially phosphorus and nitrogen, in waste water affect the ecology of natural water bodies into which the waste is discharged. The fate of these two minerals in fish ponds has been studied from the view point of beneficial chemical fertilization of the water for increasing the ponds' productivity. Most of the phosphorus and nitrogen added to the water is lost to the mud or to the atmosphere (Matida, 1956; Hepher, 1958). For phosphorus this is due to an equilibrium between its soluble state and insoluble phosphates in the mud (Hayes et al., 1952; Olsen, 1958, 1964; Hepher, 1966) in which the amount of the insoluble phosphate is far greater than the soluble phase. The addition of soluble phosphate to the water affects this equilibrium, but it tends to be restored quickly by precipitation as iron phosphate and manganese phosphate or absorption of their hydrates (Einsele, 1938, 1941) in water rich in these metals and low in pH. The precipitation of calcium phosphate seems to be much more common as it occurs at a higher pH (Matida, 1956; Hepher, 1958; Golterman, 1967). The removal of phosphate by this process depends on the concentration of Ca in the water, the water alkalinity (Barrett, 1953), and the pH of the water. Nitrogen added to the water is lost partly by denitrification processes when partial anaerobic conditions exist near the bottom; however, most of the nitrogen loss is due to transition of NH_4 to gasous NH_3 and escape of NH_3 to the atmosphere. The equilibrium between NH_4 and NH_3 depends mainly on the pH. At a pH of 9.5 and at a temperature of 25°C, over 60% of the total ammonium compounds will be in the gasous state. These conditions are common in fish ponds in Israel during the summer. Increasing temperature or pH further increases the shift towards the gaseous state. As previously noted (Table II), stocking fish in waste treatment ponds results in higher pH, probably because the grazing of fish on the planktons maintains the pond in a better ecological balance. Oxygen production and respiration are more in balance and excesses of CO_2, which depress pH, do not occur. High pH in ponds is also associated with reasonable detention time, high nutrient concentration and relatively high temperatures, all of which contribute to the photosynthetic activity in the water.

The removal of nutrients added by the wastewater is quite pronounced. Coleman et al. (1974) reports a reduction of 0.39 to 2.68 mg per l total N in each of the ponds in this series. The highest (40% reduction) was at the pond having the highest pH (8.9). The total phosphorus was reduced 0.65 to 2.17 mg P/l in each of these ponds. A higher reduction could probably be achieved at a higher pH.

Improvement of Water Utilization

Several factors impose limitations in the use of waste water in terrestrial agriculture. The most important are:
(a) The seasonability of water demand for irrigation while the seasonal waste water supply is more or less constant.
(b) Limitation of use to certain crops or methods of application because of sanitary consideration and residual pathogenic organisms. (c) Mineral composition unsuited for irrigation, mostly because of high total salt content. (d) Lack of sufficient agricultural areas which can utilize the supply of the wastewater, very often because of unsuitable soil or climatological conditions.

It is obvious that in the last two cases, aquaculture may be the only solution for the utilization of the wastewater for production of food. Aquaculture is much less demanding on the quality of water and the soil than are the common agricultural crops. A variety of fish are euryhyaline in nature and can tolerate a wide range of salt contents. These include valuable food fishes such as trouts, sea basses, mullets, Tilapias, etc. All these fish can be cultured in fresh water as well as in water having a salt content as high as sea water and even in hyperhyaline waters. Soil quality also is less of a limiting factor in aquaculture than it is in terrestrial agriculture. Sand soils, swamps, and peat which usually can not be utilized for agriculture can still be used profitably for aquaculture. Munich's wastewater fish ponds were dug in moorland of a little agricultural value (Kisskalt and Ilzhöfer, 1937). The basic demands from a fish pond are that it will contain the water and that it will be drainable. Organic matter produced in the pond water can clog the intestices even of pure sand and reduce seepage to rates normal for humic soil. Demoll (1926) states that it has been experienced that high seepage from ponds receiving wastewater disappear quickly. Water loss from ponds built on sand along Israel's Mediterranean Coast reduces to about 1 cm per day by the third or foutth year. Rocky subsoil is unsuitable for fish pond because the water will be lost through fissures. Draining of the pond enables to control the fish population. Without this, the management of the fish pond is nearly impossible.

The severity of the limitations on use of wastewater for irrigation because of the seasonality of the demand for irrigation water can be demonstrated by the following figures. TAHAL-water planning for Israel (1972) forcasts the amounts of wastewater in Israel for the year of 1980 to be 281 x 10^6 m^3 per year. Of this 137.4 x 10^6 m^3 will be supplied during the irrigation season and 143.6 x 10^6 m^3 (i.e., over 50%) will be available in winter when there is no demand for irrigation waters.

This seasonal problem, while extreme in Israel, is by no means unique to Israel and is experienced in many regions. This means that in order to utilize the wastewater more fully it must be impounded and stored for long periods. This is usually expensive. Using this water for fish culture can not only justify expenses but also produce valuable food for human consumption. Analysis of extra income achieved by using waste water in the fish farm in Israel mentioned above shows that the increase in yield was 3.52 tons/ha. As the market value (less the extra costs of handling) is about US$ 1000 per ton, the extra income amounts to US$ 3530 or about 26 ¢ per cubic meter of wastewater used in the ponds. Coleman et al. (1974) estimates that the income of the experimental Quail Creek Lagoon System was about 0.6 ¢ per m^3 of treated water after covering the treatment cost. He points out that costs of 1-1.2 ¢ per m^3 have been reported for advanced mechanical waste treatment.

LIMITING FACTORS FOR USE OF WASTE WATER IN AQUACULTURE

Dissolved Oxygen

A key factor in the use of organic wastes in aquaculture is the maintenance of adequate dissolved oxygen (DO) for survival and growth of the fish. The affect of the wastewater on the DO depends on two factors: added BOD, and, pond temperature. With a rise in temperature, the rate of oxygen uptake increases and the wastes are stabilized in a shorter time. At a lower temperatures, there is often an accumulation of the sludge on the bottom of the pond with subsequent rapid decomposition and O_2 uptake when temperature rises. Demoll (1926) reports on the early, large scale experiments of using waste water in fish ponds in Strassburg. The ponds became filled with fermenting sludge which caused much trouble. Kaufmann (1958), found such an accumulation of sludge to exist in the wastewater fish ponds in Munich. In warmer climates, the decomposition of the wastewater in the ponds is more complete and no serious problems are caused by accumulation of sludge. As was already mentioned above for the Calcutta waste water fish ponds, which received a very high rate of wastewater in a short period, stabilization was achieved during winter in 15-20 days after introducing the waste water but in 10-12 days during summer, when temperatures were higher. Naturally when the amounts of waste water introduced into the fish ponds are smaller, as in the case in the fish ponds in Israel, stabilization is achieved after much shorter time. In our case in 2-3 days.

We find that we are able to predict the DO depletion in the pond water due to the added waste from laboratory measurements of the BOD of the waste at the pond temperature (Schroeder, 1974). Knowing the BOD of the waste and the minimum naturally occurring DO in the pond, usually occurring just before sunrise, allows one to calculate the maximum amount of waste able to be added to the pond without hazard of fish kill due to anoxia.

Usually BOD is reported as BOD_5 (20^oC). From an operational stand-point, the overnight DO depletion must be known. This we determined by measuring daily, for five days, the reduction of DO in a standard BOD jar containing a known amount of waste. The maximum 24 hour DO reduction during this five day period was taken to be BOD_1 at the experimental temperature. In practise we found that BOD_1 approximately equalled $\frac{1}{4}$ BOD_5, and that, as with many biological systems, for each 10^oC rise in temperature between 10^oC and 30^oC, the BOD doubled. Since during the daylight hours the BOD_1 is amply satisfied by the DO in the water, it is only half of this BOD which contributes to the depletion of the DO at night and the reduction in the critical DO concentration just before sunrise. Calculating the added load of $BOD_{\frac{1}{2}}$ per liter of pond water volume served than allowed prediction of the 12 hour, overnight, depletion of the pond DO due to addition of organic matter. Interestingly, the observed, in-pond, DO decrease due to organic matter was consistently less than the laboratory predicted DO decrease. This may be due to more rapid satisfaction of BOD in supersaturated DO conditions usually found in fish ponds during day-light hours. The overestimation of DO decrease provides some measure of safety against fish kill by anoxia when using wastes in fish ponds.

Poisonous Material

Wastewater may contain a range of materials poisonous to fish or to their natural food in the pond. Municipal wastewater may contain industrial wastes to which poisonous materials such as heavy metals, oils, etc., were added. It may also contain high concentrations of detergents harmful to fish. Rural wastewater, on the other hand, may include insecticides and herbicides used in farming, the residues of which are at times dumped or washed into the sewer. When planning the use of wastewater in aquaculture special care must be taken to avoid the flow of these materials to the wastewater and to the ponds, if possible, by removing these materials at their sources. One should remember that sub-lethal concentrations of these materials, while not affecting the actual survival of the fish, may affect their growth either directly or indirectly by affecting the natural food in the pond. Some materials, such as phenols, even when in very low concentrations in the pond water, impart an unpleasant taste to fish.

While it may not be difficult to control the input of hazardeous materials originating from industrial wastes, it is not simple to deal with detergents which originate from dispersed, multiple sources in domestic wastewater. Hard detergents are especially troublesome. This problem and its effect on fish culture was studied in an experiment using municipal wastewater from Haifa in fish ponds. Due to the water saving habits of the population (120-150 liter wastewater per capita per day) and the high amounts of detergents used, the concentration of the detergents in the sewage was high. The sewage was treated in a two stage trickling filter system followed by sedimentation. This reduced the BOD by over 90% (from an input of about 450 ppm to an effluent of about 40 ppm). However, the concentration of the detergents in the effluent remained high and reached 10-18 mg ABS per liter. The effluent was introduced into 4 fish ponds of 0.1 ha each. Four days after the introduction the DO concentration at the critical hour, just before sunrise, was high enough (0.6-15.6 mg/l) to sustain the fish. It became much higher in the following days, reaching in the afternoons a concentration of over 300% saturation and before sunrise 2.2 to 11.25 (!) mg/l. No doubt the DO concentration was not a limiting factor for the survival of the fish. Still, all fish introduced into the pond died. Bio-assay analyses have shown that the lethal dose (LD_{50}) to fish of Dodecyl-benzen-sulfonate is 10 mg ABS/l. It is obvious that the fish died because of the high concentration of detergents in the water. There was a natural degredation of the detergents in the pond at a rate of about 1 mg ABS per week. After a period of about 10 weeks the detergents had decreased to a level which did not kill the fish. Wastewater was then added at a rate just to make up evaporation and seepage, about 100 m^3 per ha per day. However, since sub-lethal concentration of detergents were still in the water, the growth of fish in these ponds was not good and much below that of regular fish ponds. The effect of soft detergent may be different due to its degradation at the treatment plant. Nevertheless, its effects on survival and growth of fish in ponds must be studied.

Sanitary and Social Considerations

One can hardly overemphasize the importance of the sanitary and social factors as these may be crucial to the applicability of wastewater utilization in fish ponds. Janssen (1970) reviews a considerable amount of literature dealing with this subject. He concludes that there is a need of much more research on fish as a possible vector of human infectious diseases. All available evidence shows that, unlike warm blooded animals, fish normally do not suffer from infections of Salmonella, Shigella or other entrobacteriacae. They may, however, carry these infections if caught in polluted waters (Shewan, 1962). There seems to be a controversy over whether the entrobacteria can survive and multiply in the gut, mucus and tissues of fish and thus render the fish a potential vector for human disease for long periods or whether the fish serve merely as passive vectors. Janssen (1970) quotes some cases where pathogens such as Vibrio parahemolyticus are thought to maintain themselves in fish for a long period. However, others (Shewan, 1962; Guelin, 1962; Buttiaux, 1962) conclude that these pathogens are carried passively by the fish and that the infected surface of the gut of the fish are cleared of the bacteria within a few days. Guelin (1962) contaminated fish (Ctenolabrus rupestris) by E. Coli. Five hours later he could isolate the bacteria from the viscera directly, but 24 hrs later, the isolation of the germs already required an incubation and on the seventh day no fish contained E.Coli. Very little work has been carried out on the contamination of fish by human pathogenic viruses, but no doubt this creates a no less of a hazard.

Because of these sanitary considerations, it is clear that if the wastewater is to be used in fish ponds in appreciable amounts it should first undergo some treatment to decrease the number of pathogenic bacteria and viruses. Three factors will facilitate further reduction or total elimination of the residual pathogens: (a) dilution of the wastewater in the pond water; (b) the rapid die-off of bacteria and viruses in the pond due to the high DO supersaturation and high pH of the water, both usually much higher than in natural water bodies; (c) a flush-out period of the fish in clean water before marketing. More careful study is required however to establish the effect of these factors.

No less serious to the application of wastewater into fish ponds is the public attitude. There is no point in culturing fish in this water when one cannot sell them. Basu (1949) reports that due to local oposition to the use of wastewater in fish ponds, he had to abandon his experiment on this subject. This attitude is known also from other places. It is obvious that it is connected with the sanitary and aesthetic problem. If the public cannot be assured the fish cultured in wastewater ponds are safe for use, the fish will be unacceptable. This safety does not insure acceptability, which will depend much on public education.

It has been our experience that the taste of fish cultured in wastewater fish ponds is good if kept in clean water for some time. This was also noted by Scheuring (1939) for the fish cultured in Munich's wastewater fish ponds.

It appears that,from the sanitary and social point of view, crude sewage cannot be used directly in fish ponds without its dilution or prior treatment. The degree of this treatment and the amounts of wastewater which can be utilized in a fish pond depend on the many factors discussed in this paper.

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TABLE I

FISH PRODUCTION AND FEED CONVERSION FACTOR FOR FISH PONDS WITH OR WITHOUT WASTEWATER (1974).

Pond type:	no wastewater; chemically fertilized with ammonia and phosphate		wastewater; no chemical fertilization			fluid cow manure; chemically fertilized with ammonia and phosphate
pond area (hectares)	1.4	2.2	0.7	1.0	1.0	2.0
fish yield (Kg/ha per 8 months)	4700	4700	8000	8600	8100	7500
conversion coefficient (Kg food/Kg fish yield)	1.8	1.6	0.9	1.0	0.8	1.1

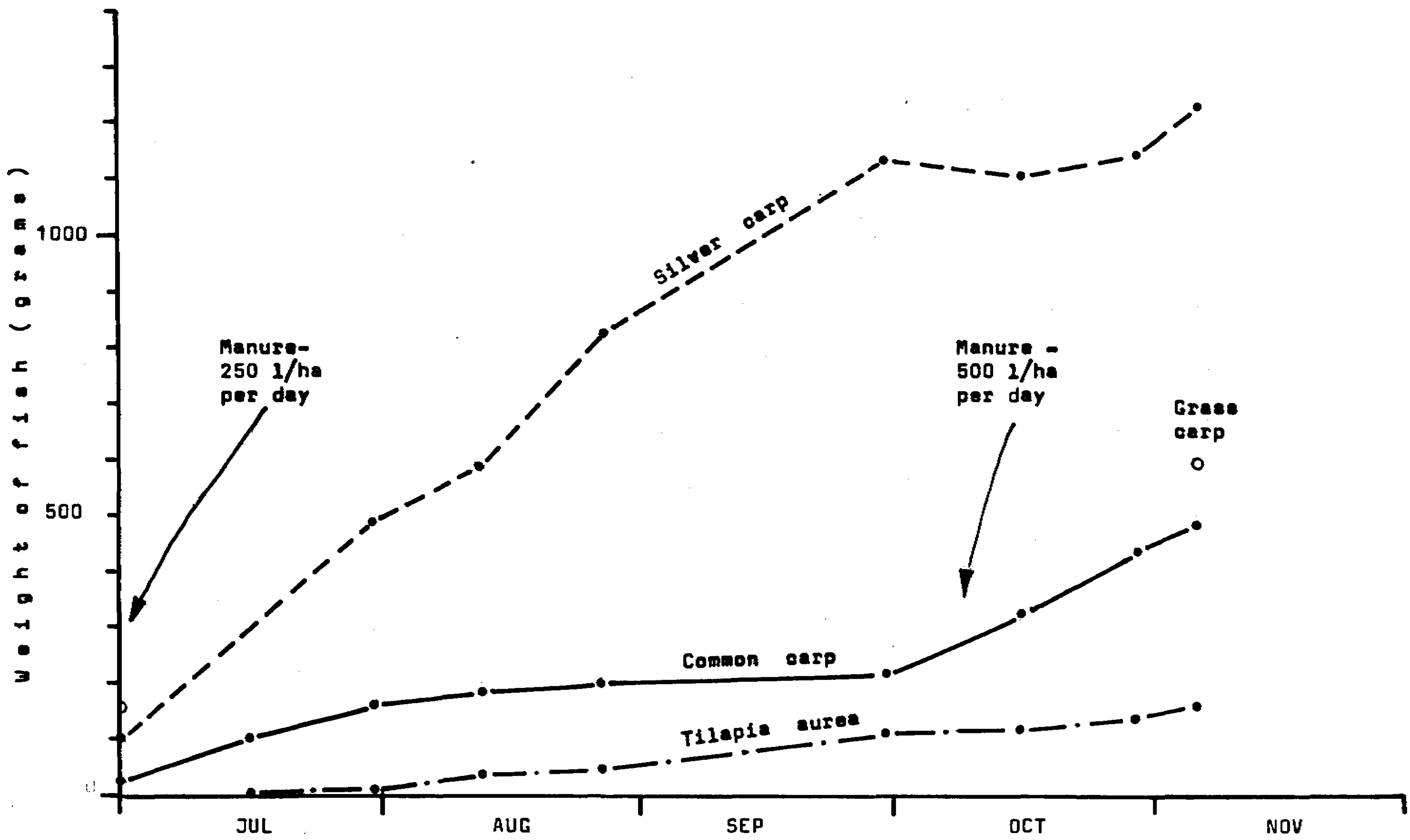
TABLE II

SOME EFFECTS OF STOCKING FISH IN WASTE TREATMENT PONDS.

Pond type	No fish; manured**	fish*; manured	fish; no manure
bacteria (1000/ml)	17-27	1.6-6.7	0.7-4.3
pH (0900 hrs)	7.9-8.3	8.3-8.9	8.6-8.7
DO (ppm)	0.7-9.5	9.0-15.9	10.0-13.8
temperature (^{o}C, 0900 hrs)	9-15	9-15	9-15

* fish stocked: common carp (a bottom feeder), silver carp (a filter feeder).

** fluid cow manure 10-13% dry matter; added at rates up to 800 kg BOD_5 ($20^{o}C$) and 5600 kg solids per hectare, every two weeks.



Weight of fish (grams)
1000
500
0
Silver carp
Common carp
Tilapia aurea
Grass carp
Manure-
250 l/ha
per day
Manure -
500 l/ha
per day
JUL
AUG
SEP
OCT
NOV

FIGURE I. Growth of fish in ponds receiving fluid cow manure as the primary nutrient to the pond (data taken from unpublished work by G.W. Wolfarth, *et al.*, Private communication).

ON THE ACCEPTABILITY OF SEWAGE SLUDGE APPLICATION TO SOIL

by

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(Formal discussion of Dr. D. Purves' paper: The contamination of soil and food crops by toxic elements normally found in municipal wastewaters and their consequences for human health)

The contribution of municipal wastewater and sewage sludge to the dispersion of heavy metals throughout the biosphere via applications to soils has become increasingly important during the last decades and will undoubtedly still increase for a number of years to come. Such enhancement is inevitable in view of the growing number of wastewater treatment plants and the amounts of water that will be subject to some type of purification.

The main objectives of wastewater treatment are, in general, of dual origine, viz. the creation of the opportunity for reuse of water and the prevention of (surface) water pollution. It is, however, necessary, as was indicated by Purves' contribution, that all precautions are taken in order to prevent environmental pollution and especially pollution of soil as a result or consequence of wastewater treatment. A distinction should be made, however, between pollution and contamination.

A definition of soil pollution, which may satisfactorily be used for our purposes here, may read as follows: Soil pollution involves the presence of compounds in soil which according to their nature, concentration or relative amounts impose unfavourable conditions with respect to growth or composition of vegetation or with respect to the composition of ground-water or surface water. Whereas contamination refers to the addition of compounds which may induce, either on short or on long term, undesirable effects as indicated above, pollution refers to the actual prevalence of such conditions. The last then may result from one excessive application of a hazardous compound, in this case heavy metals, as well as from a gradual accumulation following repeated additions in small quantities.

Whereas pollution should be prevented, contamination might be looked upon in many cases as inevitable even when considering the digestion of refuse products like wastewaters and sewage sludges. With respect to heavy metal dispersion, this discussion is limited to sewage sludges but most considerations can equally be applied to contamination following waste-water renovation by land disposal. It should be realized, however, that the distribution in the soil profile may be different in the latter case, i.e. the metals are found to greater depths or are leached to larger extent. This is the result of the huge quantities of water that are applied to the soil in most wastewater renovation systems. Transport in the liquid phase is the main distributing factor, and conditions for leaching are much more favored by wastewater disposal than in the case where the downward movement of water is limited to the excess of natural precipitation over evaporation, as is usually the situation with dried sludge disposal. Disposal of wet sludges provides sort of an in-between situation. This effect of moisture content is easily demonstrated by the following simplified equation which describes the penetration of a pollutant as compared to the movement of the carrier solution (Bolt, 1974)

$$X_p = \frac{V}{R_d + 1} \qquad (1)$$

X_p represents the mean depth of penetration of the pollutant in soil, in cm, and V the depth of penetration of the carrier solution, in cm. R_d stands for the distribution ratio of a compound i under consideration over the soil solid and liquid phases. If adsorption is the only interaction with the solid phase, R_d may be found as:

$$R_d = q_i/\varepsilon.C_i \qquad (2)$$

where q_i stands for the amount of compound i adsorbed per cm^3 of soil and $\varepsilon.C_i$ represents the amount in solution, ε being the moisture content in cm^3 per cm^3 of soil and C_i the concentration of i in solution. The large influence of moisture on the displacement of the polluting zone is exerted via V in equation (1).

The inevitability of heavy metal contamination of soil (aside from sewage (sludge) applications) is also confirmed by Purves' findings. The enhancement of B, Pb and Zn contents of soil after experimental sewage sludge treatments was, although large, not statistically significant due to the high and variable contamination of the control plots. This contamination was ascribed to industrial activities in the environment where these experiments were situated. It is interesting in this respect to mention that the increased Zn content of sewage sludges in areas of high industrial activity is not necessarily caused by direct industrial Zn discharges on the sewer system. The increased acidity of the natural precipitation in such areas as a result of industrial exhausts leads to corrosion of Zn-containing building materials like gutters. In rural areas the excrements of birds, especially of peaceful animals like doves, may induce a comparable corrosion effect at lower level.

Large-scale prevention of environmental heavy metal contamination would not only require a prohibition of heavy metal emissions in industrial and traffic exhausts but also of the contribution by domestic coal and oil burning. And even then, the natural weathering of heavy metal containing rocks constitutes a source for dispersion which is entirely unavoidable.

But, of course, as is the case with all contamination-, accumulation-, and finally pollution-related problems, the question arises which contributions can relatively easily be avoided in order to achieve postponement of the situation of pollution. And in this respect it was suggested by Purves that the contribution from municipal wastewaters and sewage sludges should be diminished by a "treatment at the source", i.e. the favoring of industrial process designs which allow maximum heavy metal use and recycling and minimum discharge with wastewater. The introduction of separate sewer systems for domestic and industrial wastewater was mentioned as another possibility. Such approaches will undoubtedly decrease heavy metal contamination of soil and thus provide the only way to prevent the sewage sludge contribution to undesirable heavy metal effects on the long term, except for the unavoidable contribution as already mentioned for Zn.

It seems realistic, however, to assume that it will take at least several decades before such cleaning-up of industrial discharges is common practice. Let alone that the technical possibilities for effective screening of wastewaters on heavy metals have not yet sufficiently been developed, it also remains questionable whether the sudden realization of potential environmental hazards justifies the introduction of, possibly insufficiently considered, requirements especially since such regulations may induce considerable economic and social disturbance.

Thus the present author would favor a more gradual introduction of measures to prevent heavy metal discharges by industries. In the mean time a way out has to be found for the storage or digestion of heavy metal containing sludges produced during this transitional period, and industrialists, politicians as well as scientists must share equal responsibility in finding solutions that are optimal for the human society as a whole. While industrial engineers are developing process designs which prevent future heavy metal waste and dispersion, agronomists and especially soil scientists must provide ways for a temporary solution of the problem. This is the more so because many soils offer, as a result of their intrinsic properties, an excellent opportunity for safe storage or digestion of sewage sludges.

The first measure to be taken refers to the prevention of further dispersion of heavy metal containing sludges as a result of uncontrolled application in agriculture. Since the interests of sludge producers usually run not parallel with those of farmers and of environmentalists, protection of sound soil conditions can best be gained by legislation, regulating the application of sludges to soil. This requires, besides monitoring and characterization of sewage sludges, standards on acceptable heavy metal contents of soil.

In characterizing sewage sludges the so-called "Zn-equivalent" factor as originally introduced by Chumbley (1971) may be employed as a useful tool. This Zn-equivalent factor is defined as:

$$\text{Zn equivalent (ppm)} = 1 \times \text{ppm Zn} + 2 \times \text{ppm Cu} + 8 \times \text{ppm Ni}$$

and is based on the observation that (although toxicities may differ for different plants and in different soils) in a very general way Cu is about two times as toxic as Zn and Ni is about eight times as toxic as Zn.

Purves' criticism of the use of this factor in the evaluation of acceptable sewage sludge application rates refers to the possible prevalence of other hazardous heavy metals in sludges, especially Cd. Cd accumulation being indeed one of the most serious health hazards with respect to heavy metals, this would mean, as was also suggested by Purves, that heavy metal characterization of sludges should be extended with Cd monitoring in addition to the determination of the Zn-equivalent factor. In this respect it seems warranted for practical purposes to study the possibility for extending the Zn-equivalent factor to a form in which the possible presence of Cd is taken care of.

Moreover it is of importance that the wastewater treatment authorities make an inventory of heavy metal sources discharging on treatment plants in order to assess the possible contaminants of specific sewage sludges.

The question about permissible application rates is hard to answer in a general way because of the great number of factors that exert their influence in this respect. Although this problem must be reconsidered for each specific situation (with sludge composition and soil properties as main variables) attempts have been made to establish very general guidelines.

Chumbley (1971) suggested that total sludge application to soil, if used for agricultural production, would not exceed 250 ppm Zn equivalent (at pH $\geq$ 6.5), thus applying the relative toxicity of the major heavy metals in sludges viz. Zn, Cu and Ni. As stipulated above, the occurrence of other heavy metals can require that this value be decreased considerably and that the more hazardous compound be taken as the criterion.

Chaney (1973) stated that the sludge application should not exceed Zn-equivalent levels equal to five percent of the cation exchange capacity of the original soil. Original soil would than mean in this case: not sewage sludge treated and not considerably contaminated by other impacts. Although this criterion will safeguard the soil, it has the practical disadvantage that only very minor amounts can be applied, thus requiring large surface areas for sludge digestion. Moreover, soils without much CEC are considered as unsuitable. However, a number of sandy soils with low CEC are very capable for heavy metal fixation, especially when they contain oxides of e.g. iron, aluminum and manganese (Jenne, 1968).

In the Netherlands preliminary standards are maintained for sewage sludge applications. These standards are not yet legally valid but have an advisory character. They are based on the following considerations.

Sewage sludges of domestic origin contain roughly 2000 ppm Zn and 500 ppm Cu on dry matter basis (De Haan, 1972). Levels for Zn and Cu are found to be toxic for growth of most plant species (i.e. inhibiting growth and yield in excess of about 10 - 15%) when exceeding:
125 - 150 ppm Zn in soil, extractable with 2½% acetic acid, this value corresponds to roughly 250 - 300 ppm total Zn and is slightly lower than the preliminary one given by Purves;
50 ppm HNO_3 extractable Cu for arable land and
80 ppm HNO_3 extractable Cu for grassland soils.
From a large number of analysis data it was found (Henkens, 1975) that

Cu-EDTA extractable = 0.8 x Cu-HNO_3 extractable.

Thus the above value for Cu is somewhat higher than the one suggested by Purves.

Using these very general data and considering that most Dutch soils meet the minimum Cu level required for plant growth (i.e. 5 ppm Cu-HNO_3 extractable, both for arable land and grassland) one may calculate the amounts of sludge and the corresponding time period of application in order to reach the maximum permissible accumulation. On the basis of the above data this maximum accumulation amounts to 45 ppm on arable land and

75 ppm on grassland. Since the mobility of Cu in soil is very low, the layer thickness available for Cu storage is assumed to equal the plow layer for arable land (with a total weight of 2.5×10^6 kg/ha) and a 5 cm toplayer for grassland (with a total weight of 0.5×10^6 kg/ha). Results of such calculations as performed by Henkens (1975), are presented in table 1.

Table 1: Number of years available in order to increase the Cu content of soil to the maximum permissible level, as a function of land use and the yearly sludge application rate (Henkens, 1975).

amount of sludge metric tons/ha/year dry matter percentage		number of years	
100	4	arable land	grassland
1	25	225	75
2	50	112	38
3	75	75	25
4	100	56	19
5	125	45	15

At the time that the Cu-content of soil has increased with 45 ppm also the maximum permissible level of Zn has approximately been arrived at. For several reasons (a.o. the possible interaction between Cu and Zn) Henkens (1974) proposed that the application rate be not higher than 2 metric tons per ha per year on dry matter basis for arable land and 1 metric ton per ha per year for grassland. The sludge application on a monastery farm near Tilburg, The Netherlands, greatly exceeds this standard (De Haan, 1975). Here the application rate on grassland amounted recently to about 1.7 metric ton dry matter of a sludge containing roughly 1,300 ppm Cu (thus being about 2.5 times higher in Cu than average domestic sewage sludge).

It is realized that the above considerations are entirely based on the occurrence of Zn and Cu as the predominant heavy metals in domestic sewage sludges. As stated before, one should certainly be alert on the presence of other elements, especially in case of industrial sewage sludges.

It is a fortunate situation that the sensitivity of many plant species for high levels of a number of elements is larger than the sensitivity of human beings and animals. As such the elements As, B and Zn may be mentioned. This puts limits to the accumulation of such elements via the food chain.

Finally it should be mentioned that an adaptation of proper cropping systems on sewage sludge amended soils may considerably contribute to the prevention of health hazards. Certain crops accumulate specific metals in their root system whereas other species exert accumulation in foliage. So e.g. it was described by Baumhardt and Welch (1972) that corn grown on soils high in lead exhibited Pb-accumulation in stems and leaves but not in the grain. In this specific case it was suggested that this corn would be used for grain harvesting and not as silage corn.

These and other measures may be taken in order to prevent pollution with heavy metals as a result of sewage sludge disposal on land. At the other side, soil may play a significant role in the temporary solution of this typical environmental problem. It must be kept in mind, however, that the capacity of soil for safe storage of heavy metals is limited, and the work described by Purves constitutes a clear contribution to the delineation of soils limitations.

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PUBLIC ATTITUDES TOWARD THE RENOVATION AND REUSE OF MUNICIPAL WASTEWATER

Prepared for

The International Conference on the
Renovation and Recycling of Wastewater
Through Aquatic and Terrestrial Systems

Bellagio Study and Conference Center
Bellagio, Italy

July 16-21, 1975

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In a sense, the issue of public attitudes toward wastewater recycling is so complicated even for parts of one country that it is difficult to demonstrate clear perspectives on the topic. To consider how cross cultural variations affect these attitudes is far beyond the scope of this particular endeavor. However, even in indicating public attitudes in the United States, we confront a number of socio-economic perspectives that are often affected by cross cultural training. In a number of studies that extrapolated data from other sociological and psychological studies of the American population to predict reactions to wastewater recycling, one point has immediately been made very clear. Among public attitudes, some general patterns have crystallized in various situations, but we must deal with multi-faceted individual perspectives in each instance.

In the United States we assume that the public has a right to know about issues like wastewater recycling; and by "public" I mean the general voting populace of an area in contrast to private interests that may profit from the venture in one way or another. Special groups also have a right to have input to the decisions about these issues, but we are obligated legally as well as morally to educate the general public.

Previously, the influential citizens, often the mayor, a senator, or a local dignitary voiced opinions about new issues on behalf of the public. However, in light of the 1964 Economic Opportunity Act (P.L. 88-253) and the numerous protests from the earlier Black Movement to the Welfare Mothers and Anti-Vietnam movement of the late 1960's, at least lip service has been given "Maximum feasible participation" or what has also be called "plebiscetary democracy." Of course this has generated more talk than action in many quarters, and in some cases has been looked upon with open fear. For one thing, if an agency such as the Corps of Engineers or the Environmental Protection Agency were successful in taking an issue directly to the people, this could severely limit the power of the Congresspersons who think of themselves as representatives of the people. In actual fact, however, at least one study has indicated that agencies may have staff representatives with attitudes that more nearly parallel those of the public than the duly elected peoples' representatives.[1]

Given this educational commitment, we must recognize the need to deal with individuals who not only have different attitudes at different times but also may express contradictory views in various situations. The person who comes home to a quiet suburb may cherish the natural greenery of his yard all the more because he has driven through miles of concrete slums where the ghetto inhabitants are deprived of the advantages that he cherishes in themselves and as a sign of his ability to rise above the masses.

However, some public attitudes have crystallized sufficiently to form general observations about how they will affect the society. While the atomic and hydrogen bombs reduced the scientists' reputation for being able to solve all problems by continuing technological improvement, the general populace still retains some faith in scientific advancement. Thus, many people are willing to be convinced that chemically treated wastewater can be demonstratively purer than the natural resource. This trust in science may contribute to the acceptability of recycled wastewater at least among the better educated segment of the population who are generally more receptive to such innovations. This is particularly apt to be true as people become more aware of the severe contamination of natural water sources. Thus, an argument can be made for recycling, not only where water is scarce, but because we can produce a better grade than some municipal water supplies may be able to provide from contaminated natural watercourses.

Moreover, people have become sufficiently ecology oriented to favor the improvement of natural waterways, and many vehemently resent letting them become more contaminated. Again, this attitude can be highly individual and selective. People on vacation in Yosemite National Park expect to see a clear and sparkling trout stream, but in their daily lives they may be quite accustomed to waste laden waterways that are accepted as a matter of course. This selected visibility toward contamination, willingness to accept it as part of their daily lives, and hope for a better future are often combined with a present apathy and inertia that favors the status quo.

While Americans are increasingly committed to the belief that the waterways should be protected and improved, many of them also seem to be quite satisfied to assume that some action is being taken somewhere by anonymous experts who will eventually improve the waterways without requiring any specific effort on the citizens' part. Regretably, however, overcoming apathy can generate uncertainty because expert opinion is also divided on the values or dangers associated with using renovated waste as potable water. The same split was encountered in the fluoridation controversy waged by the dental profession and in the dispute over polio vaccines. Wastewater experts are divided on whether or not there is a health hazard from viruses or toxicity from high mineral content.

From a technological and health standpoint the major deterrent to land disposal of municipal wastewater is the concern for associated health hazards such as:

1. The viruses, pathogenic organisms and bacteria and their transmission to higher biological forms including man.

2. Chemicals including carcenogenic organic compounds that are not removed during the renovation process and pose dangers to health if ingested.

3. Crop quality when irrigated with wastewater effluents.

4. The propagation of insects that are vectors in the transmission of disease.

In the past the chief health hazard raised as an objection to the use of renovated wastewater was concern for the transmission of waterborne diseases. However, in recent years the health hazards associated with pesticides, heavy metals and refractory carcenogenic organic compounds have increased.

These public concerns have been reinforced by the lack of scientific data or the reluctance of public health officials to issue guidelines describing the relative potential health hazard when wastewater is applied to the land. The acceptance of renovated wastewater by a community will depend on the ability of the municipal wastewater treatment plants to remove these chemical and microbiological contaminants. Furthermore, while present technology is capable of producing renovated wastewater of a chemical quality equivalent to that of drinking water at a cost cheaper than the desalination of sea water, among those who see a danger there is disagreement over whether or not the health hazards and technological problems are so great that we shouldn't attempt to reclaim the wastewater on a wide scale. One technical group believes that the problems can be overcome and we should use renovated wastewater. Other experts feel that we cannot satisfactorily or at least quickly eliminate the health hazards. Until we arrive at a consensus among the public opinion makers and industrial officials, water reclamation is apt to be a slow process because of public confusion.

However, faith that a new technique of recycling may be an improvement on nature's old way and the belief that the waterways and the environment as a whole should be restored to the pristine natural wilderness gives credence to support the hard reality that recycling wastewater is necessary in some areas to adequately meet the current needs of the community or to replenish dwindling groundwater supplies. Moreover, wastewater management may prevent emergencies where a scarcity of water could force hasty recycling. Without adequate public preparation, in at least one instance, this led to a decidedly negative public response.

Between 1952 and 1957 a severe drought in Chanute, Kansas, motivated the recycling of treated municipal effluent into the existing river impoundment that supplies water to the city's potable water treatment plant.[2] From December, 1956, until March, 1957, the citizens of Chanute unknowingly used this renovated water from a new secondary sewage treatment plant that had been completed in 1953. This effluent had considerably lower coliform concentrations than were normally found in the Neosha River at the city's water intake, and water was drawn from this source for several months without any known ill effects. According to local authorities, the tap water met the Drinking Water Standards for bacteriological quality during the entire period, and several biological tests did not reveal the presence of any pathogenic organisms. While no known cases of waterborne diseases or other adverse effects upon health apparently resulted from using the renovated water as part of the potable supply, no systematic morbidity studies were carried out either.

However, physicians reported that stomach and intestinal disturbances were much more prevalent and widespread during the fall and winter of 1957-1958 than during the same period the previous year when the reclaimed water was being used. For one thing, much of the river's flow past Chanute during the drought was composed of the sewage plant effluents from nine communities upstream. Thus, although citizen reaction was quite adverse to using the waste effluent from their own treatment facility, it may have been a cleaner source of water than the river with its higher coliform content from less effective treatment facilities upstream. However, the chemical quality of the water did become progressively worse during the recycling period. The water became increasingly unpalatable because of the buildup of salinity (520 mg/l), synthetic detergents (5.8 mg/l), total dissolved solids (1000-1200 mg/l) and sodium (380 mg/l).

Even though the citizens knew that Chanute's normal water supply received diluted sewage from upstream communities, they had poor acceptance of the renovated water principally because of esthetic considerations and serious reservations regarding the safety of the water from a public health point of view. Additionally, the use of reclaimed water was not publicized until after the recycling process was in operation. Furthermore, as stories about the recycled water appeared in the local newspapers, the initially poor public reaction became even more adverse. As a consequence, the sale of bottled water increased, more private wells were drilled, and more water was privately hauled from neighboring towns and farm wells. The incident confirmed that individuals were willing to pay many times the normal cost to obtain a safe and palatable water supply. Unlike the incident at Chanute, at Windhoek, South Africa, wastewater was recognized as a valuable natural resource.

Whereas the Chanute experiment was implemented out of necessity because of a severe drought and was quickly discontinued when the drought ended, in many semi-arid portions of the world drought or near drought conditions are normal. In these communities purified wastewater constitutes a significant proportion of the region's total potable water supply. Such waters now augment fresh supplies for cooling, irrigation, and in industries such as the manufacture of pulp and paper and textiles. At Windhoek, South Africa, recycled municipal effluents augment potable water supplies in a modern water conservation program.

The Windhoek wastewater treatment demonstration plant was commissioned in November, 1968, and by 1970 it surpassed all expectations. The plant supplies approximately 1.2 million gallons per day or one-third of the requirement for the city.[3] The wastewater can be renovated and continuousl recycled on a large scale in the same cost range as treating fresh water.[4] After the success at Windhoek, a one million gallon per day demonstration plant was built at the Pretoria Municipal Sewage Disposal Works to increase the available water and help sustain the city's economic and industrial growth.[5] An additional objective of this project is to educate the public on the purity and wholesomeness of the renovated water and thereby eliminate public prejudice and aesthetic objections to its daily use.

While wastewater has not generally been utilized for drinking in the United States, it has increasingly been recycled for a number of other applications, some of which involve direct body contact. Therefore, if public campaigns are mounted against wastewater recycling in the future, it will be possible to demonstrate long term applications that have been publically accepted. But it is important that careful scientific studies be conducted continually where wastewater is being unobstrusively applied now so that if pros and cons can be fully, accurately, and truthfully documented. Citizen trust and acceptance, in the last analysis, will depend on our ability to provide full and complete information prepared in a manner that can be understood and accepted.

If we assume as a fundamental goal that wastewater can be recycled for all current uses of fresh water supplies, the first step in the United States is to convince the public that this will yield positive benefits. To do this, the experts must first have full and complete information to insure that there are no problems and any hazards have been alleviated. Public acceptance of wastewater must be based on information about documented applications. Should any problems arise, these have to be confronted fully and truthfully to be resolved as soon as possible. In the post Watergate American society, hopefully, the one thing we will have learned is to face our problems head on and not attempt to cover them up. Such a project is in the planning and implementation stages in Denver, Colorado.

The City of Denver, Colorado, has a 25 year master plan that proposes the direct reuse of municipal wastewater as part of the potable water supply. The long range time schedule called for renovating ten million gallons of wastewater per day for industrial use by 1974-1975, and the renovation of 100 million gallons per day for potable uses is anticipated by 1986. By the year 2000, Denver is planning to supply 25 percent of its total water needs with renovated wastewater.[6]

In the meantime, the Denver Board of Water Commissioners have planned an ongoing program to insure public acceptance. A survey is planned for every 3 to 4 years to determine the general level of information about the project and the attitudes towards renovated wastewater as a supplementary source of water.[7-8] The public opinion measurements will provide direction for the information and education program to stress the beneficial aspects of wastewater renovation and reuse.

Not only is public cooperation essential to recycle wastewater as a supplement for fresh water, but it will also protect natural waters from further contamination, and their reclamation is now required to conform to recent legal specifications. The Environmental Protection Agency under the authorization of PL 92-500 has specified that general recreational waters,[9] surface waters for which no specific use designation is made, should not average more than 2000 fecal coliforms per 100 ml with a maximum of 4000 per 100 ml except in specific mixing zones adjacent to outfalls. The water should be of suitable quality to insure the survival of fishes, waterfowl, and other forms of life. All wildlife available for harvest from the water should be fit for human consumption. These general requirements are premised on the assumption that regardless of whether or not a recreational use is encouraged, people are drawn to and tend to make use of the water for a variety of activities.

Secondary recreational waters[9] include such uses as boating, skiing, sailing, and fishing. Therefore, intimate contact with the water may occur incidentally or accidentally but the possibility that the water will be ingested in appreciable quantities is minimal. However, the average fecal coliform concentration should not exceed 1000 per 100 ml with not more than 10 percent of the samples exceeding an average of 2000 per 100 ml.

Finally, primary recreational waters[9] in which there is intimate and extended body contact, especially by children, should be tested at least 5 times over each 30 day period of the recreational season. The samples must have a log mean of less than 200 fecal coliform per 100 ml with not more than 10 percent of the samples exceeding 400 per 100 ml. The pH of these waters should not be less than 6.5 or more than 8.3 except from natural causes. In no case should it be less than 5.0 or more than 9.0.

With both a legal and moral obligation to improve the quality of our natural waterways, recycling wastewater and lessening contamination by land runoff and airborne fallout should help meet the standards. Nor is recycling as dramatic a change in policy as it may appear initially in light of the fact that some natural waterways and even some underground aquifers may already be so contaminated that the water is, in effect, being recycled even if it has not been officially so designated.

If we discover that the groundwater supplies are being contaminated with viruses and coliforms, our first effort must be directed to finding methods of removing whatever may be harmful to human health. We must not place too much trust in natural purification methods or our beloved chemicals until we are confident that we can reassure the public that recycled wastewater will not endanger human health.

Enough recycling projects of one type or another are now in operation or in the planning stages to provide ample design criteria and sampling schedules with which to collect much of the data necessary to reassure ourselves or solve any problems. A related preliminary step now in progress is to determine what the actual public acceptability of wastewater recycling will be. This is likely to vary from one place to another depending on the amount and kind of information and misinformation that is dissiminated and the motivation of the proponents and opposition. Extremes have already been demonstrated. Whereas people living in Illinois south of Chicago rallied to protest the dumping of solid waste on the land, around Monroe, Michigan, one individual has been accused of stealing solid waste products to spread on his land. Both extremes display limited knowledge adopted to their own perception, on one hand that the waste is a detriment to their property value even on adjacent land, and on the other side that all waste is of nutritional benefit without recognizing that too many heavy metals might inhibit the growth of crops. Between these extremes, the great majority of people will not be familiar with either the pros and cons, and most will adapt to the new innovations sometimes out of ignorance or inertia or because they believe the positive benefits of science are being offered for their benefit.

Other people will have to be convinced; and this is where our skills as scientists will have to be combined with those of the humanists, the psychologists and sociologists who can come to grips not so much with the information itself (which we will present in an overwhelmingly convincing form) but with the basic human biases that resist or are less receptive to information presented, as we all know, for the best interests of this and future generations. This resistance will flower in a multitude of forms that reflect all of the human variables. Nonetheless, some of the objections can now be recognized and anticipated, hopefully to also be overcome gracefully.

While I have stressed the need for complete information about wastewater recycling, which means making careful analyses where such projects are in progress now, we can still be assured of "tough sledding" in some instances where we attempt to utilize recycled wastewater just because certain types of people and issues invariable surface in a public controversy. In the first place, we know that citizens often will submit to stringent measures in a crisis that would not have been acceptable previously. Much of the energy and flood legislation has been passed while the crises were still fresh in people's memories. As they stand in the mud, the citizens are quite receptive to federally financed flood control and insurance, for example. And as the water supply in a local community is rationed, the willingness to use recycled wastewater is apt to rise. However, over the long term, the acceptance of recycled water will rest less on scientific documentation of its advantages but on our ability to learn about and respond sensitively to the complex psychological attitudes manifested toward renovated water.

These public attitudes towards the renovation and reuse of wastewater are not well defined at present. However, the proliferation of reclamation projects (See Tables I and II) seems to indicate that renovated wastewater is slowly being accepted. When no crisis is involved, the limited research to date indicates that people are willing to accept recycled wastewater directly in proportion to how far it is from them as individuals. In their public opinion studies of ten California cities, six from

TABLE I

SELECTED EXAMPLES OF IRRIGATIONAL REUSE OF RENOVATED WASTEWATER.

	Crop Irrigated	Location	Year Initiated	Renovated Water Production (MGD)	Type of Treatment	Ref
1.	Alfalfa	Galt, California	----	0.3	PS+P	10
2.	Farm crops	Augusta, Maine[a]	1872	0.007	-----	11
3.	Farm crops	Pullman, Illinois	1880	1.85	-----	11
4.	Citrus grove	Pomona, California	----	6	PS+P	10
5.	Farm crops	Cheyenne, Wyoming	1881	7.0[b]	-----	12
6.	Farm crops	Pasadena, California	1887	----	-----	13
7.	Farm crops	San Antonio, Texas	1895	20[c]	-----	13
8.	Pasture, corn and rice	Woodland, California	1889	8.9	PS+P	10, 14
9.	Farm crops	Salt Lake City, Utah	1896	4	-----	11
0.	Pasture and corn	South Lake Tahoe, California	----	3.5	PS+S+L+AS+SF+AC	10
1.	Farm crops	Bakersfield, California	1912	11.3[b]	-----	12
2.	Cotton and milo	Fresno, California	1891	18	PS+S+P	14
3.	Farm crops	Vineland, New Jersey	1901	1.2	PS+IB	15
4.	Landscape	Pacheco, California	----	0.5	-----	14
5.	Landscape	St. Petersburg, Florida	1972	0.16	PS+S+SI	14
6.	Golden Gate Park	San Francisco, California	1932	1.0	PS+S	16
7.	Golf course	Ventura, California		0.5	PS+S	10
8.	Golf course	Lake Havasu City, Arizona	1971	0.55	PS+S+P	14
9.	General irrigation	Mesa, Arizona	1957	4.3	PS+S	14
0.	Agricultural irrigation	Calabasas, California	----	3.0	PS+S	14
1.	Agricultural irrigation	Dinuba, California	1954	2.4	PS+S+RF	14
2.	Citrus, hay, and grapes	Fontana, California	1971	2.3	PS+SI+OF	14
3.	Oats, cotton and corn	Hanford, California	1900	2.5	PS+S	14
4.	Golf course, Crops and greenbelt	Laguna Hills, California	1964	1.4	PS+S	14

TABLE I (con't.)

25.	Alfalfa and gold course	Livermore, California	1965	----	PS+S	14
26.	Plants and pasture	Lodi, California	1944	5.0	F	14
27.	Agricultural uses	Irvine, California	1968	2.8	-----	14
28.	Farm crops	Oceanside, California	1957	1.5	F+SI	14
29.	Farm crops	Pleasanton, California	1957	1.3	SI	14
30.	Oramental uses	Colorado Springs, Colorado	1953	7.0	PS+S+SF+SI	14
31.	Oramental uses	Disneyworld, Florida	1972	1.5	PS+S+P+IB	14
32.	Forest irrigation	Ft. Walton Beach, Florida	1972	1.0	P+SI	14
33.	Crops and Forest	Tallahassee, Florida	1966	2.0	PS+S+SI	14
34.	Wooded areas	St. Charles, Maryland	1966	0.5	P+IB+SI	14
35.	Golf course	Cranberry, New Jersey	1967	0.4	PS+S+SI	14
36.	Alfalfa, corn, oats and sorghum	Alamogordo, New Mexico	1963	2.5	PS+S+SI	14
37.	Farm crops	Clovis, New Mexico	1927	3.5	PS+P+RF	14
38.	Crop irrigation	Raton, New Mexico	1950	----	-----	14
39.	Farm crops	Roswell, New Mexico	1930's	2.3	-----	14
40.	Farm and golf course	Santa Fe, New Mexico	1937	2.5	SI	14
41.	Cooling water and golf course	Los Vegas, Nevada	1961	----	P+SI	14
42.	Farm crops	Ely, Nevada	1908	1.5	PS+SF+RF	14
43.	Farm crops	Incline Village, Nevada	1971	0.45	PS+S+RF	14
44.	Alfalfa and pasture	Los Vegas, Nevada	1959	6.0	PS+S+SI	14
45.	Pasture land	Duncan, Oklahoma	1964	0.5	PS+S+P+SI	14
46.	Grasslands	Hillsboro, Oregon	1939	2.0	SI	14
47.	Alfalfa and wheat	Milton-Freewater, Oregon	1946	2.7	PS+S+P+F	14
48.	Forest and farm crops	Penn State, Pennsylvania	1963	0.5	PS+S+SI	14
49.	Wheat and corn	Dumas, Texas	1962	1.0	PS+P+F	14
50.	Agricultural uses	Kingsville, Texas	1952	3.0	P+SI	14
51.	City parks and golf courses	La Mesa, Texas	1960	0.6	PS+S+SI	14
52.	Milo, alfalfa and cotton	Midlan, Texas	1950	4.3	PS+P+F+SI	14
53.	Pasture lands	Monohans, Texas	1945	0.8	PS+SI	14

TABLE I (con't.)

54.	Crops and pasture land	San Angelo, Texas	1933	5.0	PS+P+F+SI	14
55.	Crops and pasture land	Uvalde, Texas	1938	0.9	PS+S+SI+F	14
56.	Wheat	Quincy, Washington	1955	0.75	PS+P+SI	14
57.	Onion, pasture and general	Walla-Walla, Washington	----	----	PS+S+SI	14
58.	Hay	Cheyenne, Wyoming	1881	7.5	PS+S+P+F	14
59.	Alfalfa	Rawlins, Wyoming	1880	----	P+SI	14
60.	None	Flushing Meadow, Arizona	1967	0.5	PS+S+IB	14
61.	General farm crops	Phoenix, Arizona	1975	1.5	PS+S+IB+SI	14
62.	Farm lands	Bunzlau, Germany	1559	----	SF	17
63.	Farm lands	Croydon-Beddington, U. K.	1861	4.5	SF	13
64.	Farm lands	South Norwood, U. K.	1864	0.4	SF	13
65.	Farm lands	Berlin, Germany	1869	150[c]	SF	13
66.	Farm lands	Leamington Springs, U. K.	1875	0.8	SF	18
67.	Farm lands	Birmingham, U. K.	1880	22	SF	18
68.	Farm lands	Melbourne, Australia	1893	120[d]	PS+P	19
69.	Farm lands	Mexico City, Mexico	1902	520[d]	PS+S+SI+RF	12
70.	Farm lands	Paris, France	1923	120	RF	13
71.	Farm lands	Cape Town, South Africa	1928	----	-----	20
72.	Farm lands	Debrecen, Hungary	1959		PS+SI+RF	14
73.	Farm lands	Braunschweig, Germany	1939	12	PS+OF+SI	21

a - abandoned about 1900
b - data for 1972
c - data for 1926
d - data for 1971

TABLE II

SELECTED EXAMPLES OF RECREATIONAL, MUNICIPAL, AND INDUSTRIAL REUSE OF RENOVATED WASTEWATER.

Renovated Water Reuse	Location	Year Initiated	Renovate Water Production (MGD)	Type of Treatment	Ref
RECREATIONAL					
1. Swimming pool	Santee, California	1965	---	PS+S+AC	22-24
2. Recreational lakes	Santee, California	1961	0.4	PS+S+IB	22-24
3. Recreational lakes	Camp Pendleton, California	----	0.7	PS+S+P	10
MUNICIPAL					
1. Groundwater recharge	Whittier Narrows, California	1968	10	PS+S+IB	25
2. Potable water	Windhoek, South Africa	1968	1.2	PS+P+FL+SF+AC	3-5
3. Potable water	Pretoria, South Africa	1970	1.0	PS+P+FL+SF+AC	3-5
4. Potable water	Chanute, Kansas	1956	0.75	PS+P+SF	2
INDUSTRIAL					
1. Power plant cooling water	Los Alamos, New Mexico	----	2.0	PS+S	10
2. Steel mill cooling and process	Baltimore, Maryland	----	95	PS+S	10
3. Power plant cooling water	Burbank, California	----	1.0	PS+S	10
4. Chemical plant cooling water	Midland, Michigan	----	6.0	PS+S	10
5. Refinery cooling and boiler feed	Amarillo, Texas	----	1.5	PS+S	10
6. Refinery cooling and boiler feer	Odessa, Texas	----	2.5	PS+S	10

KEY FOR TABLES I and II

PS = Primary sedimentation
S = Activated sludge or trickling filter
P = Ponds or lagoons
FL = Flotation
SF = Sand or multi-media filtration
AC = Activated carbon treatment
L = Lime treatment

AS = Ammonia stripping
RF = Ridge and furrow land application
IB = Percolation or infiltration basins land application
SI = Spray irrigation land application
OF = Overland flow land application
SF = Sewage farm
F = Flooding

northern and four from southern California, Bruvold and Ward compared attitudes of individuals in one community which used some renovated wastewater with the attitudes of a second community, similar in geographic location, climate and socio-economic characteristics, which did not use reclaimed water.[26] In both cases the public attitudes towards reclaimed wastewater depended almost exclusively on its ultimate use and did not differ appreciably in the two regions. The greatest opposition toward renovated water was recorded for drinking (56 percent), food preparation in restaurants (56 percent), cooking in the home (55 percent), and food canning (54 percent). Respondents were most favorable toward reclaimed water for road construction (99 percent). If an opposition rate of less than 10 percent is recorded for a renovated wastewater use, it would most likely receive little or no opposition from Californians. Eleven of the twenty-five uses for reclaimed water listed in Table III were in this category.

When individual responses were evaluated according to socio-economic classes determined by occupation, education and income, the attitudes of the lower classes were less or nearly equally favorable to industrial uses of reclaimed wastewater as the upper socio-groups.[27] All of them responded favorably to water reuse in industrial processes. However, individuals with the lowest educational training were generally less favorably inclined toward the concept of wastewater renovation and reuse. Education and belief in the effectiveness of the water purification process were the major factors that determined the public attitude toward the reuse of reclaimed sewage effluent. Psychological repugnance and concern about purity were the most frequently mentioned reasons for opposition. Interestingly, the cost of treatment did not appear to be

TABLE III

PERCENTAGE OF RESPONDENTS OPPOSED TO USE OF RECLAIMED WATER[26]

Use	California Location Northern (N = 386)*	 Southern (N = 586)*	 Total (N = 972)*
Drinking water	55.0	57.3	56.4
Food preparations in restaurants	53.4	57.7	56.0
Cooking in the home	52.5	55.8	54.5
Preparation of canned vegetables	52.5	55.1	54.1
Bathing in the home	37.8	39.2	38.7
Swimming	24.8	23.0	23.7
Pumping down special wells	26.1	21.4	23.2
Home laundry	21.1	23.9	22.8
Commercial laundry	19.4	23.5	21.9
Irrigation of dairy pasture	15.6	13.1	14.1
Irrigation of vegetable crops	15.6	13.0	14.0
Spreading on sandy areas	13.2	13.3	13.3
Vineyard irrigation	14.0	12.1	12.9
Orchard irrigation	10.7	9.7	10.1
Hay or alfalfa irrigation	8.3	7.0	7.5
Pleasure boating	9.1	6.1	7.3

TABLE III (con't.)

Commercial air conditioning	7.8	5.6	6.5
Electronic plant process water	6.0	4.1	4.9
Home toilet flushing	3.9	3.7	3.8
Golf course hazard lakes	4.4	2.2	3.1
Residential lawn irrigation	2.3	2.9	2.7
Irrigation of recreation parks	2.8	2.4	2.6
Golf course irrigation	1.8	1.5	1.6
Irrigation of freeway greenbelts	1.8	0.9	1.2
Road construction	1.6	0.3	0.8

*N = number of respondents

an important determinant of opposition. The reasons most often given for negative attitudes are presented in Table IV.

Like the intensive personal interviews, a public telephone survey also showed no differences between individual attitudes in the various communities toward the use of reclaimed sewage effluent. The overall response went from unfavorable to neutral toward renovated wastewater for direct human ingestion by drinking, food preparation or industrial food canning. More significantly, the unfavorable sample respondants were strongly negative.[27] The greatest number of these again were among citizens in low socio-economic groups who were not active in community affairs. Therefore, they would not be likely to mount an organized opposition to uses of renovated wastewater either. Knowing the individual attitudes toward the various uses for reclaimed water can help prevent unnecessary public opposition and hostility. By exploring the stated reasons and underlying factors associated with organized opposition primarily, alternate plans or compromises can be offered to decrease the resistance.

While Bruvold and Ward demonstrate some resistance to wastewater recycling, the most amazing fact is that 45 per cent of those interviewed were initially prepared to accept recycled water for drinking and to prepare food while about 99.6 percent were willing to accept it at least to irrigate highway medians. Granted, the interview sample was very small; and the opposition leadership in a dedicated campaign against recycling could convert many tentatively favorable people to the negative camp. In fact, one of the problems faced in attempts to sway public attitudes is that people may be more receptive to waste recycling when they lack information than after they are better informed. Many golfers are not

TABLE IV

REASONS OF OPPOSITION TO USES OF RECLAIMED WATER[26]

Reason	% Stating Reason
1. Psychologically Repugnant	29.2
2. Lack of Purity	21.5
3. Can Cause Disease	9.8
4. Bodily Contact Undesirable	5.1
5. Taste and Odor Problems	3.9
6. Cost of Treatment Unreasonable	0.8

aware that golf greens damp with morning dew may also be sparkling with recycled water. While this concept is accepted out of ignorance, resistance may increase when a campaign is mounted to inform the public. People become more wary when they are acquainted with some of the pitfalls. And the campaigns against recycling wastewater are generally waged, as with fluoridation, with a cornerstone of concern for public health. That is why documentation to substantiate the positive benefits and assure that potential hazards have been overcome is an essential first step.

Opposition to recycled wastewater is strongest for drinking and preparing food with it; and this decision, like fluoridation, does not permit the individual members of the community to abstain. Whereas spraying wastewater on the golf courses does not affect non-golfers, its inclusion in the drinking water supply affects everyone. And sociologists have confirmed that some opposition can be expected in any community enterprise by a small group of people who feel isolated from the mainstream. They are reluctant to accept anything that the more civic minded members perceive as being for the good of the community. Thus, even where substantial evidence can assure a community that the project is beneficial, it is bound to be opposed by a small minority. If a few powerful opponents are included, they can raise enough doubts about public health to at least make the general public wary. Then, the common tendency is to retain the status quo. If a clear improvement cannot be demonstrated, the assumption is that things are good enough as they are.

The strength of the opposition to wastewater recycling can also depend on a number of political and economic factors that affect individual perception. They have not been adequately charted because they depend, finally, on numerous factors in the formation of the individual's makeup. This has been more clearly documented in studies of television viewing than acceptance of wastewater. Because the cost of purchasing television time in specific regions is so expensive, studies have been conducted of where politicians in the United States can expect to win votes. Thus, a staunch Republican will buy advertising time in a Republican neighborhood to reinforce his image as the candidate of choice or in a neighborhood where the vote may go either way. But he will not spend money to advertise in a staunch Democratic neighborhood because he knows that people who have their biases firmly entrenched on the other side will only view his pleas as a reinforcement for the opposition, however poignantly and cogently they are phrased.

Similarly, people in some areas will be more readily conditioned to accept recycling wastewater, perhaps where the country is normally very arid. Public acceptance can be reinforced first with the less controversial uses such as spray irrigation. This can build confidence that wastewater is also safe and acceptable for direct body contact such as swimming. Surely, starting with "Would anybody care for a drink of sewage?" would be the kiss of death, but "Let's have greener golf courses" could be a promise that ends with drinking recycled water that is purer than what comes from nature's not so sparkling streams. Such a process of social acculturation was demonstrated at Santee, California, where wastewater in body contact has become a mark of civic pride symbolized by the local swimming pool.

Since 1962 the Santee, California, project has demonstrated that municipal wastewater can be renovated in beautiful, recreational lakes enjoyed by the public. This experiment is the forerunner of all such wastewater renovation and reuse projects in the United States. Since no local water supplies are available to the Santee area, the motive for the project was essentially to reduce the amount of expensive imported water by reusing the only water supply available -- the wastewater. Instead of discharging it into the nearest dry stream, the Santee County Water District decided to treat the wastewater to such a degree that it met the standards for general recreational waters. As a result, an arid canyon was transformed into a series of lakes surrounded by a park.[22-24] As the cost of water has increased about 400 percent above the 1959 price of $58.33 per million gallons, the success at Santee has stimulated additional uses for recycled water such as: irrigation, industrial water supplies, ground water recharge and potable water.

Even though the need for water was acute during the Chanute, Kansas, experience, there was little public acceptance for recycling renovated wastewater into the city's potable supply. While the public objected primarily to the poor physical quality of the water and the esthetics of drinking water derived from sewage, no attempt was made to inform or educate the citizens concerning the safety of the program. At Santee, public acceptance was not a problem. Contrary to the predictions of many water experts who assumed that the residents would reject the reclaimed wastewater, the 20,000 inhabitants of the area have enthusiastically accepted the lakes and park as a source of recreation and pleasure. Credit for the acceptance of the project has been accorded to leaders who formulated a comprehensive educational program designed to keep the community informed throughout every phase of the project.

To gain public acceptance for the idea of using water reclaimed from sewage for purposes beyond limited irrigation, the negative connotations of sewage had to be overcome by associating the program with pleasant activities that the public enjoys and approves. To accomplish this, the reclaimed water was placed in an attractive setting where the public was invited to look, smell, picnic, boat, fish and swim in it. This approach allowed great numbers of people an opportunity to examine the water and convince themselves that it was acceptable. At the same time, they learned more about the far reaching public benefits to be gained through the reclamation of municipal wastewater.

In addition, local support was developed by presentations before community organizations of all kinds and through the assistance of newspaper editors who recognized the merits of the proposal and published the details. As interest and support were developing within the community, one of the lakes was constructed and landscaped for a proposed recreational area. When the public could only view this enticing sight through a chain link fence, this forbidden vista did much to arouse enthusiam and desire. With intense public interest in the possible benefits of wastewater reclamation, a study and research program was implemented to provide the answers to critical questions about public health.

In 1962 the Santee Lakes were opened to the public on a limited basis. In April, 1963, boating was permitted; and visitors were allowed to picnic along the water's edge. In 1965 reclaimed water was passed through an activated charcoal filter to fill a separate, sandy-bottomed pool. The sign near the pool area proclaims, "This swimming facility has opened a new field of recreational opportunity for the American public. It is supplied with reclaimed water." Attendance increased from 75,000 in 1965 to 125,000 in 1966.

Fishing has also been a very popular attraction in the recreational lakes, especially for the children. This program was initially operated on a "Fish for Fun" basis. Then it progressed to a regular fishing schedule which allows the fishermen to retain and eat their catches. Catch limits have been set for bass and catfish and as well as seasonal limits for all fish during the spring spawning period. Thus, while families picnic on the shoreline, their children can fish, swim, boat or ride the ferry to one of the many islands. They also occasionally do a bit of unauthorized wading. By now the Santee Lakes Project has evolved into a well balanced ecological system where wastewater from the community is socially accepted as a recreational oasis in an arid community.

While the ole' swimmin' hole may not be what it used to be, the Santee Project demonstrates that demographic data are available to predict, at least to some extent, areas and techniques where wastewater recycling may be received favorably by the general public. Resistance is met primarily where one portion of society feels that it is the recipient of wastes from another portion without deriving any benefits therefrom. On the other hand, if some advantages can be realized, such as irrigation for farmers' crops, then more public support can be anticipated.

Henry C. Hart has very comprehensively documented basic problems related to wastewater disposal or recycling on the land, especially in areas where large cities have to go outside their own boundaries to find disposal sites.[28] Since current estimates suggest that cities of 250,000 population need a land resource equivalent for a jet airport to dispose of their wastewater, they normally have nowhere near this amount. Then city officials turn to surrounding areas where land has to be acquired by dislocating the residents. They immediately provide a hard core of hostility because of the dislocation. Hart has indicated that the opposition intensifies where the political structures of city and county or county and sewage disposal area are separate and the people perceive great differences in status in the two areas. At the Muskegon project, merging the sewage board and county governance structure helped integrate the leadership and decrease the opposition whereas downstate Illinois farmers vehemently resented the idea that city residents in Chicago wanted to dump their waste on the farms. In contrast, public acceptance is greater where rural villages have adjoining land readily available for disposal sites or where new cities are planned with waste disposal sites included in the initital structure. In this case, the reward of potentially increased property values can overcome some local opposition to a new city just as improving underground aquifers and helping irrigate crops overcame some of the farmers' opposition to wastewater discharges in areas south of Chicago.

My discussion includes more limited methods of discharge and reuse that preceed the goal of total recycling on the presumption that building social acceptance of wastewater is a gradual and progressive process that will continue for several years. As resistance was encountered where people had to be relocated, public acceptance was markedly greater where advantages could be cited for wastewater recycling. Besides obtaining water of high quality, other advantages are: replenishing groundwater, irrigating crops, and making water supplies available where they would be scarce otherwise. Such powerful incentives can overcome many objections. At Santee a recreational facility with a swimming pool gave an advantage that the scarcity of water would not have permitted otherwise, and public acceptance set an admirable model to be followed in other places.

So far, I have outlined some of the issues, the problems and rewards of wastewater recycling. I have also stated the goal of total recycling of wastewater for all uses that natural waters currently supply. And I have made the assumption that, in the United States at least, for this to be effective, the public has to support the program in local areas. I have suggested that gradual support can be built on the basis of what is already acceptable, such as watering highway medians, and that some people are more receptive than others, particularly where advantages are viewed as overriding the disadvantages.

Opposition is not always mounted by hard core dissidents, however, it is important to note that special interest groups do not always represent the general public, and some of the most well intentioned among them can be damaging. For the rabid environmentalist, any type of structure that signals further encroachment of manmade activities on the natural scene may be regarded as a step backward. Thus, water quality management planners have to look beyond immediate and transitory objections to recognize and meet the needs of future generations. To do this, they must face the possibility that some of the strong and well intentioned but misguided environmental movements may represent a passing public fancy or a limited group and not the majority.[29] The populace as a whole is never represented by a single vocal minority which sometimes may also represent private interest groups that hope to profit and neglect the public well being. Fortunately, water quality management experts do not have to bear the total burden of responsibility for determining the water quality fate of future generations. Nor do they have to stand alone in interpreting the motivations of numerous interest groups that become vocal over issues like wastewater recycling.

In 1970 United States Water Resources Council report expressed a committment to social goals as part of the water quality management task among four primary objectives: national economic development, environmental quality, regional development, and social well-being.[29] While the latter goal was given federal sanction, experts still have to quantify benefits and rank alternative solutions on a benefit-cost ratio. However, the public has a final responsibility to view, digest, and accept or reject the alternative choices elucidated by the experts.[30] Thus, informing the public, involving it in decision making, and insuring that the public interest is served are inherent goals of water quality management in the United States that must have a shared responsibility between the experts and an informed public.

Nonetheless, interest groups do stratify according to a number of factors that make policy decisions difficult to base on the needs expressed by the community or the expertise of the bureaucrats. Therefore, attitude measurement can be very important to predict the effect of changes on the majority of individuals who are not represented by any one interest group. Determining the effectiveness and responsiveness of a project to public needs may require a large public relations effort for which opinion polls must be conducted at various stages, first to determine what is needed and then to inform the people about the project. Another crucial issue, then, is when to involve the public. When the Corps of Engineers initially held hearings on the Chicago waste disposal project, few people were interested enough to attend. At a later stage, charges were raised both that hearings should have been held sooner and that it was too early to come to any conclusions since not enough information was yet available to make decisions on what could be done or where. Despite the delicate issues, critical timing, and the overall significance of a careful public information campaign, the need to educate and involve the public has not always been given proper consideration. Recognizing the sensitivity of an issue like wastewater reclamation, particularly in the initial stages where public acceptance is not assured, mandates greater budget allocations for public information and opinion polls. The role of the social scientist as well as the engineer and public health expert, must to be accorded its due place in the total scheme of an interprise that calls for the realignment of many social values. It is no longer possible for the engineers and city fathers to decide arbitrarily on what type of concrete construction should be built and where it should be located to rid an area of its wastes as unobstructively as possible. Now citizens are being called upon to review and ultimately accept a radical change in behavior from the idea of boundless quantities to air and water available for wasteful disregard to a new and more conservative stance toward the environment.

Wastewater recycling can also be a prototype of change for Americans who have not willingly accepted constraints that implied less than an endless bounty always at their disposal. With the recognition that many natural commodities: oil, wood, metals, and coal, for instance, are in limited supply, recycling is the wave of the future. Therefore, recycling of wastewater can help overcome inhibitions and alter the mentality of an industrial society to favor conservation and reuse rather than the previous throwaway mentality. To establish a receptive mind for change in the initial information seeking stages of planning, several specific goals can be implemented to involve and inform citizens who are not directly affected by threats of relocation, environmental or public health concerns or social isolation. Among these procedures, a referendum can bring an issue to the attention of voters and quasi-judicial hearings can illicit many facts about a project at the same time that the public is given a sense of participation.[29] Where these hearings are inadequate, court cases can also produce information and alleviate doubts by giving a legal sanction to the proceedings. This formal procedure may help overcome some negative public reactions. Such proceedings should be recognized as an ultimately healthy process. Otherwise, emotional issues like public health can generate heated debate that results in a stalemate. Since illiciting information frequently raises more doubts than it quells, some method of resolution is needed to prevent the retention of the status quo and override objections by the inevitable, if loyal, minority opposition. Thus, full revelation of information through hearings or the courts and voter participation in the final decision necessitate public relations campaigns to inform the citizens on a massive scale the likes of which has seldom been undertaken in the United States. The challenge represents a return to the fundamental principle of public responsibility expressed by Thomas Jefferson and his peers.

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Preliminary results with a pilot-plant waste recycling-marine aquaculture system*

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* Contribution No. from the Woods Hole Oceanographic Institution. This study was supported by National Science Foundation (RANN) grant GI-43884 and NOAA Sea Grants 04-4-158-5 and 04-5-158-8.

Abstract

A combined waste recycling-marine aquaculture system capable of complete nitrogen removal from treated domestic wastewater has been developed and tested on a pilot-plant scale over a one-year period. Effluent from secondary sewage treatment, mixed with seawater, is used to grow unicellular marine algae in large, continuous-flow, outdoor mass cultures. Harvest from the algae cultures is fed to oysters and other filter-feeding bivalve molluscs and to secondary crops of flounder or lobsters. Dissolved wastes produced by the animals are assimilated by cultures of commercially-valuable seaweeds.

Successful cultures of unicellular algae, mostly diatoms, and seaweeds have been sustained over long periods of time (months) with only minor problems. Algal yields and nitrogen removal capacity vary seasonally by 3-4 fold and are controlled by solar radiation but not by temperature.

Bivalve mollusc culture was unsuccessful during the first year of operation. Inability to control the species of unicellular algae in the mass cultures and other possible reasons are suspected and are now being investigated. Good growth of flounders and lobsters was obtained, but the carrying capacity of the system and potential yields of these secondary crops has not yet been determined.

An alternative nitrogen-removal system consisting only of seaweeds fed a continuous flow of secondary sewage effluent mixed with seawater has also been evaluated.

Assuming that problems with shellfish culture can be resolved, the combined unicellular algae-shellfish-seaweed system is capable of complete nitrogen removal from wastewater effluent within an area of 48 acres and with an estimated annual production of 183 tons of oyster meat 38,000 bushels of whole ' sters), 3,350 tons (wet weight) of seaweeds and undetermined quantities of flounder and/or lobsters per MGD effluent treated (wastes per 10,000 capita). A seaweed system alone is capable of nitrogen removal in an area of 60 acres with annual production of 16,300 tons wet weight of seaweeds.

The above figures are for year-around operation as would be possible in tropical to semi-tropical climates. Operation of the system in temperate climates is possible within the same areas but only on a seasonal basis (approximately six months) with half the above yields.

Introduction

A biological tertiary sewage treatment-marine aquaculture system has been developed, tested, and evaluated over a one-year period on a "pilot-plant" scale at the Woods Hole Oceanographic Institution's Environmental Systems Laboratory (ESL). (Fig. 1) The effluent from secondary sewage treatment, mixed with seawater, is used as a source of nutrient to grow single-celled marine algae (phytoplankton) in mass (35,000 gallon), continuous flow-through cultures. Harvest from the algal cultures (experimentally varied from 25% to 75% of the culture volume/day), diluted with seawater, is fed into 40' x 4' x 5' (deep) cement raceways containing stacked trays of shellfish. The latter, stocked at densities ranging from 75,000 to 150,000 animals/raceway (1,500-3,000 per tray) have consisted of the American oyster (*Crassostrea virginia*) and the hard clam (*Mercenaria mercenaria*), with smaller numbers of other shellfish species. The phytoplankton remove the nutrients from the sewage effluent, which has varied experimentally from 10% to 50% in the effluent-seawater mixture. The shellfish remove the phytoplankton from the water. Effluent from the shellfish cultures (i.e., the pond harvest and diluting seawater) prior to its discharge is passed through a culture of seaweeds, grown in suspended culture in raceways adjacent to the shellfish cultures, which serve as final polishing step, removing nutrients not initially assimilated by the phytoplankton and those regenerated by excretion of the shellfish and decomposition of their solid wastes. After initial experimentation with several seaweed species, research was concentrated on two red algae of potential commercial value, Gracilaria foliifera and Agardhiella tenera (which contain the polysaccharides, agar and carrageenan respectively).

Solid wastes produced by the shelifish and uneaten phytoplankton support dense populations of small invertebrates (amphipods, polychaete worms, etc.) These serve as food for secondary commercial crops of marine animals, the American lobster (Homarus americanus) and the winter or blackback flounder (Pseudopleuronectes americanus) which were stocked in respective raceways with the shellfish.

The primary objective of the research is to develop a biological tertiary sewage treatment process capable of removal of all inorganic nitrogen from secondary sewage effluent prior to its discharge into the environment. Earlier studies (1, 2) had established the fact that nitrogen is the nutrient limiting and controlling algal growth in and eutrophication of the coastal marine environment. Thus nitrogen removal may be considered as synonomous with tertiary sewage treatment of effluents to be discharged to the sea.

The second objective of the process is to develop a marine aquaculture system consisting of a primary crop of shellfish and secondary crops of other commercially-valuable marine organisms (seaweeds, lobsters, finfish), the value of which will pay for or help defray the cost of the tertiary sewage treatment process.

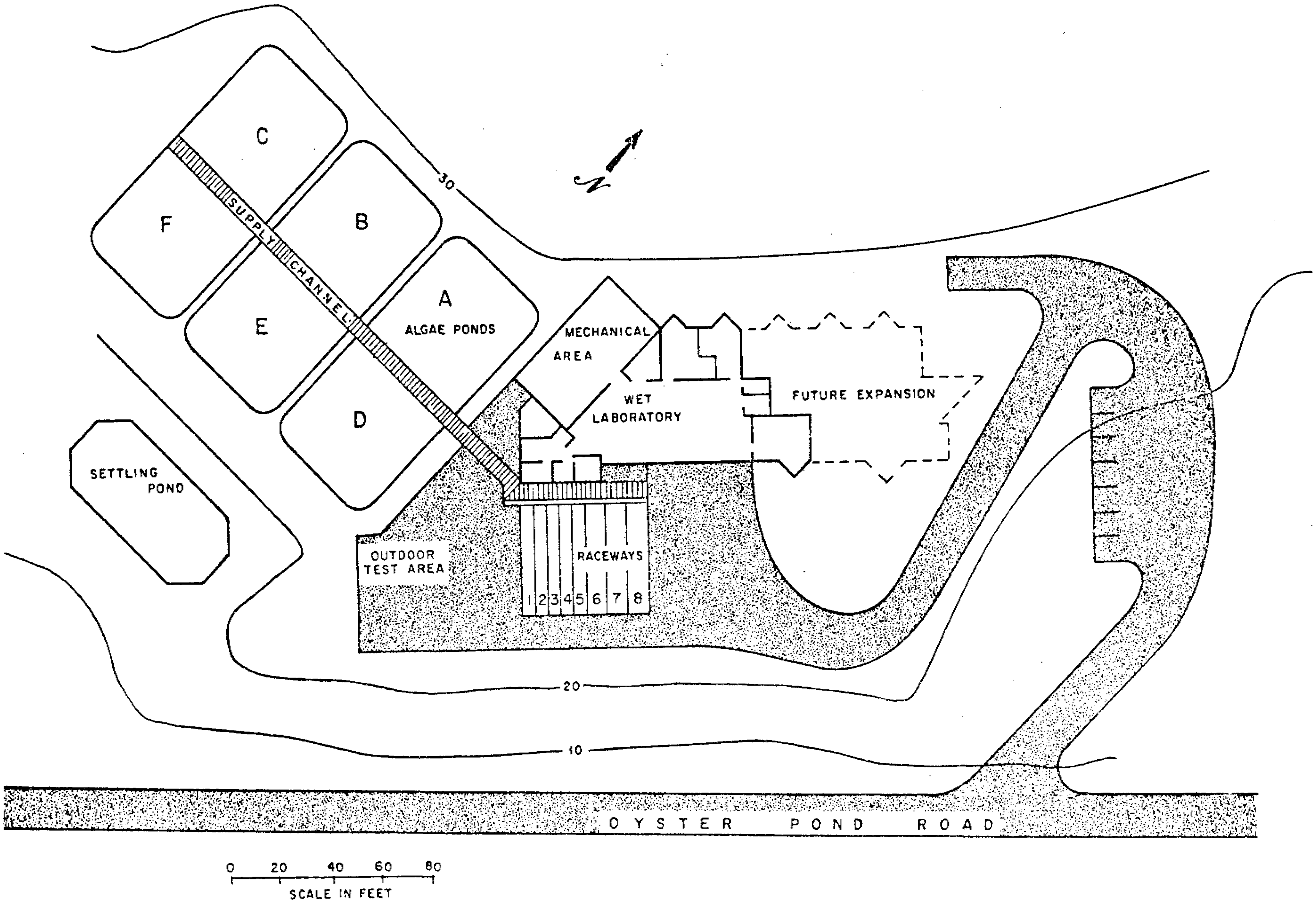

Figure 1 ENVIRONMENTAL SYSTEMS LABORATORY
WOODS HOLE OCEANOGRAPHIC INSTITUTION

Procedures and Results

A. Algae culture and nutrient removal.

Phytoplankton cultures have been maintained continuously in five of the six 35,000 gallon, 2,500 ft^2 algae ponds at ESL during the past year (the sixth pond was held out of production). Initially, the cultures were grown on inorganic chemical nutrients (commercial-grade ammonium chloride and monobasic sodium phosphate). Beginning in July, 1974, 8,000 gallons/day of effluent from the Town of Wareham secondary sewage treatment plant was trucked to ESL and discharged into one of the three buried 8,000 gallon fiberglass nutrient storage tanks. From there, the effluent was pumped to a head box in the ESL mechanical room and then distributed by gravity to the ponds.

For the remainder of the year, two to four of the pond cultures were grown on various mixtures of sewage effluent and seawater and the remainder on the inorganic nutrient medium, which was adjusted to the nitrogen and phosphorus levels of sewage effluent (typically 20-25 mg/l N and 10-15 mg/l P). The number of ponds operated on sewage effluent depended upon the sewage concentration and flow rate (% pond exchange/day) employed. For example, at 50% pond turnover with 25% sewage effluent and 75% seawater, over 4,000 gallons/day of effluent is required, over half the daily supply. Since it was desired to obtain maximum performance data of the algal cultures without any chance of their being nutrient limited, the usual procedure was to operate only two ponds with sewage effluent, at concentrations and turnover rates comparable to the above example, particularly during the high productivity period in summer, even although all nutrients were not removed.

Three times a week (M,W,F) the inorganic nitrogen and phosphorus input (sewage and seawater) and discharge (pond harvest) and the particulate (i.e., algal) carbon and nitrogen in the discharge were monitored. From these data, daily nutrient uptake and algal production could be calculated and expressed on a per volume and per area basis. This information is summarized in Table 1 on a seasonal basis, extrapolated to show areal requirements in acres per MGD of effluent (10,000 capita) for complete tertiary treatment (nitrogen removal). This ranges from 26 acres in summer to 77 acres in winter, with 19 acres for the best short-term performance in midsummer. Also shown in Table 1 are comparable data for production and nutrient removal by macroscopic algae (seaweeds), which will be discussed below.

In contrast to earlier experience with effluent from other treatment plants, in which the nitrogen is predominantly in the form of ammonia, the Wareham effluent is highly oxidized with 0-30% ammonia (depending upon time of year, performance of the plant, and perhaps other factors), the remaining nitrogen fraction being nitrate. This apparently does not affect algal production, though there is evidence that the ammonia is preferentially used first by the plants if a mixture of the two forms is present. To more nearly simulate sewage

Table 1. Mean algal production and nitrogen removal in effluent-enriched phytoplankton and seaweed cultures, on a seasonal basis. (Figures rounded) A = phytoplankton, B = seaweed*)

	Winter		Spring-Fall		Summer		Maximum	
	A	B	A	B	A	B	A	B
Mean algal production								
(g dry weight/m^2/day)†	3	3	6	5	9	13	12	16
Nitrogen removal								
g/m^3/day	0.3	0.1	0.6	0.2	0.9	.5	1.2	.6
lbs/acre/day‡	2.7	0.9	5.4	1.8	7.1	4.5	10.8	5.4
Equivalent volume effluent treated§								
MGD/acre	.013	.004	.026	.008	.039	.022	.052	.027
Area requirement								
Acres/MGD effluent ‖	77	223	37	112	26	45	19	37

*based on data for Gracilaria foliifera

† ash free

‡ assuming cultures one meter deep

§ assuming 24 mg N/l effluent or 200 lbs N/million gallons effluent

‖ or acres/10,000 capita

effluent in the cultures that were fed inorganic chemical nutrient medium, the ammonium chloride was replaced by an equivalent amount of sodium nitrate. However, the latter proved unsuccessful, possibly due to toxic contaminants in the industrial-grade chemicals used, so practice reverted to the use of ammonium chloride. Generally speaking, the performance of the cultures with respect to algal growth and nutrient removal were the same whether sewage effluent or the chemical nutrient medium, adjusted to the same nitrogen concentration, were used.

During a period of approximately two months in the late winter-early spring of 1975, due to malfunction or poor operation of the treatment plant, the effluent was of poor quality, containing large quantities of undigested suspended solids. The resulting turbidity inhibited algal production and the dissolved and particulate organic matter made monitoring of nutrient utilization and algal production impossible during that period. That experience points out the necessity for high quality, completely oxidized, and clear secondary effluent for the successful operation and monitoring of the algal growth system.

Despite considerable effort and experimentation, including filling the algae ponds with 1μ-filtered seawater and inoculation with large (several hundred liter) cultures of several different species of unicellular algae, no success was obtained in controlling the species of algae that developed and persisted in the ponds. Cultures were always virtually unispecific, the species varying with the season. In winter, at temperatures between 0° and 9°C, the diatom _Skeletonema costatum_ occurred. During most of the remaining part of the year, at water temperatures of 10°-25°C, the diatom Phaeodactylum tricornutum, was the persistent alga. During a brief period of about one month in midsummer, when pond temperatures exceeded 25°C, unidentified green flagellates replaced the Phaeodactylum cultures. It is unlikely that the species of algae present affects rate of algal production or nutrient utilization, so this is not an important factor with respect to the tertiary treatment role of the system. However, some species are well recognized and documented as better food organisms for bivalve molluscs (3). Although Skeletonema is generally regarded as one of the better shellfish foods, Phaeodactylum is variously reported as poor to indifferent. The implications of this problem will be discussed further below, but species control remains a problem that will continue to receive high priority in future research.

Two of the algae ponds may be heated by circulating their contents through heat exchangers in the laboratory. These were operated at 15°-20°C throughout the winter when temperatures in the unheated ponds ranged from 0° to 5°C. Surprisingly, there was no difference in algal production between the heated and unheated ponds. Seasonal variations in algal production of three-fourfold and even species succession and dominance are apparently due to changes in incident solar radiation, with temperature a second order factor, at least in winter. This is an important finding, as it eliminates the need to consider heating an extensive area of shallow algal ponds in winter in any commercial application of the process in temperature latitudes. Unfortunately, however, the algal culture must still be heated to 15°-20°C before it can be utilized by the shellfish.

The culture ponds, which are approximately 50' x 50' x 3' deep were constructed from shaped sand and fine gravel lined with 20 mm black PVC. The exposed edges of the PVC liners are further covered with a 10 mm PVC "sacrificial" sheet that may be replaced when and if sun damage occurs. When filled to a depth of three feet, the pond volume is 35,000 gallons.

The cultures are kept in gentle circulation with two one-third HP (40 gal/min) cast iron pumps on opposite corners of the ponds. These recirculate the culture, the return jets entering above the surface to provide both momentum and aeration. This action is normally sufficient to keep the algal cells in suspension.

The continuous-flow cultures may be maintained for months at a time with little or no maintenance. Gradually, the accumulation of organic matter on the bottom and the development of a fringe of epiphytic green algae (usually Enteromorpha) at the water's edge around the periphery of the pond causes a reduction in algal production. This is exacerbated if the sewage effluent contains significant amounts of suspended solids. When this occurs, normally at intervals of 3-6 months, the ponds are drained, cleaned, sprayed with dilute sodium hypochlorite, sun-dried, refilled, and reinoculated with an adiacent culture. This takes one to two man-days of effort per pond, and the new culture can be brought on line into production in about four days.

At pond temperatures exceeding 15°C when Phaeodactylum is the dominant alga in the cultures, an unidentified colorless protozoan flagellate, roughly the same size as the Phaeodactylum cells (i.e., 20-30μ in diameter) appears in the cultures and preys upon the diatoms. Unpredictably and very quickly, the flagellate at times proliferates throughout the culture and eliminates the algae. The cultures may be discarded and restarted, as described above, but if left alone, the flagellate population quickly subsides, presumably through lack of food, and the Phaeodactylum population reestablishes itself in about the same time (3-5 days) that it takes to start a new culture. This represents an undesirable interruption in algal production that should, if possible, be avoided. Studies have now been initiated and will be continued during the coming year on the identification, basic biology, and nutritional requirements of the flagellate predator so that, hopefully, conditions can be maintained that will inhibit its growth. Preliminary indications are that the organism favors high nutrient levels and perhaps the presence of dissolved organic compounds from the sewage effluent or excreted by the algae. It is hoped that the highest turnover rate and lowest nutrient level that are consistent with maximum algal production and nutrient removal (e.g., 10-20% effluent, 50% or more turnover per day) will also prevent growth of the flagellate population and thereby contribute to stability of algal production.

B. Bivalve mollusc culture

Harvest from the phytoplankton pond cultures (equivalent in volume to the daily turnover rate of the ponds) flows by gravity into respective cement raceways that are 40' x 4' x 5' deep. At its point of entry to the raceway, the algae culture is diluted with coarse-filtered seawater at ratios ranging from 1 to 5 parts seawater to 1 part culture, depending upon the season and other, related factors. Reasons for the dilution are: 1) to dilute the algal suspension to the degree necessary for the shellfish to filter and assimilate the food organisms most efficiently, a concentration believed to be of the order of 10^5 cells/ml; 2) to provide a more rapid flow of water through the raceway to enhance shellfish feeding; 3) to prevent the accumulation of metabolites of the animals, particularly ammonia, to toxic levels; and 4) through use of heated seawater when and as needed, to bring the combined flow of algae and seawater to a temperature at which the shellfish will feed and grow throughout the year. Phytoplankton will grow equally well on heated and unheated pond cultures in winter, as discussed above, but the unheated cultures must be heated to 15°-20°C or more (depending upon the shellfish species) before they are presented to the animals. One way of accomplishing this is to harvest the unheated cultures into the heated ponds where they can be raised to the desired temperature before being introduced to the shellfish. The other method, as mentioned above, is to dilute the culture with heated seawater to the extent necessary to bring the combined flow to the desired temperature. Both methods have been used successfully during the winter of 1974-75.

The relatively large ESL facility does not have the capacity to raise temperatures, by either of the above methods, of the combined algal culture-seawater mixture, at the desired flow rates, to levels above approximately 15°C in winter. Nor does it have the capability of providing a range of different temperatures to the raceway system while holding other factors (i.e., flow rates) constant. Finally, there is no capacity to cool water at our facility, and solar heating of the algal pond cultures together with the diluting seawater may result in peak summer raceway temperatures of 25°C. It has therefore not been possible to control temperatures in the animal culture system beyond a seasonal range of 15°-25°C This has led to some problems in attempting to assess shellfish growth over long periods of time as a function of other variables, such as food species, food concentration, flow rates, etc. Particularly, it has been difficult to assess the relative growth and potential value in our system of shellfish that have different optimal temperatures for feeding and growth. (e.g., The American oyster, Crassostrea virginia, grows more rapidly at 25°C or above while a strain of European oysters, Ostrea edulis, obtained from Maine grows best at about 15°C.)

The algae culture-seawater mixture enters one end of the 40-foot raceway and passes in a linear flow to the opposite end, where it enters the adjacent seaweed-stocked raceway, for final "polishing" of the effluent. Shellfish are stocked in wooden-frame, vexar-lined trays (mesh size depending upon size of the shellfish) at an initial density, for the 1/2-1" seed, of 1,500 to 3,000 animals per tray, which is later thinned appropriately as the bivalves grow. The trays are stacked vertically, 7-8 trays per stack, the raceways accommodating 8 such stacks of trays, holding a total of some 150,000 seed shellfish. An airline extends along the side of the raceway on the bottom to provide aeration and vertical mixing of the water throughout its length. This has been found essential for mixing thoroughly the algae culture and diluting seawater and preventing a stratified flow down the length of the raceway, particularly in cold weather when heated seawater is used. In addition, aeration is important in maintaining high levels of oxygen and low levels of metabolites, particularly ammonia, everywhere in the raceway and especially near the bottom.

In spite of the large amount of work that has been done with oysters and, to a lesser extent, clams, mussels and other bivalves, no one has yet successfully grown these organisms in a large-scale, artificial rearing system employing cultured food organisms. Our initial attempt, involving the stocking of three shellfish raceways with 300,000 seed oysters (Crassostrea virginica) from Flower Brothers Hatchery, Bayville, Long Island (N.Y.) and 150,000 seed hard clams (Mercenaria mercenaria) from Long Island Oyster Farms, Northport, Long Island (N.Y.) during the the winter of 1973-74, was largely unsuccessful. Neither species grew significantly during the following 18 months and most of the oysters died during the summer of 1974. Initially the cause of this failure was believed to be an improper or inadequate food source, a diet predominantly of the diatom Phaeodactylum tricornutum. Recent experience, however, has required reevaluation of this explanation. A new batch of 150,000 seed oysters from Long Island Oyster Farms stocked on April 28, 1975, and smaller numbers of C. virginica, of the European oyster (Ostrea edulis), and of the Manila clam (Tapes semidecussata) which were introduced at various times later in 1974 and 1975 have all grown well, in some cases on a diet exclusively of Phaeodactylum. Table 2 is a summary of the experience to date with shellfish growth and mortality in the raceway system.

It is suspected, therefore, that the initial group of oysters and clams may have been hatchery "culls", or individuals whose growth had been irreversibly checked for some reason. Perhaps, also, culture conditions during the early part of the operation (temperature, dissoloved oxygen, ammonia concentration, flow rates, food concentration, etc.) were unfavorable. Fortuitously, either or both of these problems may have been corrected, but if so, it is not yet clear exactly what problem was corrected or how its reoccurrence may be avoided.

It is planned during the coming year, therefore, to obtain smaller numbers of a large variety of seed shellfish from as many different sources (commercial hatcheries, research institutions, etc.) as possible and to evaluate their comparative growth under the same culture conditions. These will include experiments with different species of bivalves and with the same species (C. virginica) obtained at different times, of different sizes and ages, from different hatcheries, and from different geographical regions. It is hoped thereby to select the best species

Table 2. Growth and cumulative survival of shellfish in algae-fed raceways (based on samples of 200 individuals).

Species	*Crassostrea virginica*			*C. Virginica*			*C. virginica*			*C. virginica*			*Ostrea edulis*			*Mercenaria mercenaria*			*Tapes semidecussata*		
Raceway No.	1			5			1			1, 3			1			3			1		
No. stocked	150,000			150,000			5,000			150,000			3,000			150,000			10,000		
Date stocked	12/73			12/73			7/74			5/75			3/75			1/74			11/74		
Date	Length(cm)		% survival	Length(cm)		% surv.	Length(cm)		% surv	Length(cm)		% surv.	Length(cm)		% surv.	Length(cm)		% surv.	Length(cm)		% surv.
	$\bar{m}$	σ		$\bar{m}$	σ		$\bar{m}$	σ		$\bar{m}$	σ		$\bar{m}$	σ		$\bar{m}$	σ		$\bar{m}$	σ	
12/73	2.7	.8	---	2.7	.8	---															
1/74																1.5	.1	---			
2/74																1.7	.2	100			
3/74	2.8	.7	96	2.8	.8	100															
4/74	3.1	1.7	71	3.1	.8	100										1.7	.2	100			
5/74	3.2	.8	60	3.3	.9	96										1.6	.4	99			
6/74	2.7	.9	34	3.5	.9	78										2.0	.3	99			
7/74				3.4	.5	78	2.3	.3	---												
8/74																2.0	.2	97			
9/74							2.7	.2	100												
10/74	3.9	.6	24				3.5	.3	100							2.1	.2	97			
11/74				3.9	.7	2													.5	.1	---
12/74	3.3	.8	24				3.6	.5	100							2.1	.3	97	.7	.1	100
1/75	3.8	.6	24				4.2	.4	100												
2/75																			.9	.2	100
3/75	3.1	1.3	24				3.8	.8	93				4.1	.9	---	2.1	.3	90	1.1	.2	100
4/75				5.3	.8	2	4.0	.7	77				5.8	1.1	100	2.0	.2	87			
5/75				5.2	.8	2				1.2	.3	---	7.5	.6	100				1.5	.2	100
6/75										1.9	.3	100	7.4	.8	100				1.8	.2	100

and define the best size and condition and the best stocking time for optimal growth under normal operating conditions of the system. Particularly, organisms capable of rapid growth on a unialgal diet consisting, for sustained periods of time, of Phaeodactylum tricornutum will be looked for.

It is also planned to conduct experiments with the same batch of seed oysters (i.e., obtained at the same time from the same hatchery) in which such factors will be varied as food concentration, flow rate, aeration, temperature, stocking density, size and configuration of trays, and any other conditions that are amenable to experimental manipulation in the raceway system. Many of these factors have been varied during the past year, but lack of significant growth of the shellfish under any conditions and infrequent monitoring of their size, condition, mortality, etc. made it impossible to reach any meaningful conclusions. During the coming year, the shellfish will be monitored much more frequently (at least once a week) and more intensively (from several different trays in each raceway) so that changes in growth and mortality may be attributed to both experimental and natural variations in the culture environment. It is also planned to carry out small scale experiments, using a few hundred to a few thousand animals in individual trays, water tables, or other containers where the effects of a wide range of experimental conditions (food concentration, flow rate, etc.) may be examined simultaneously. The object of these experiments, both large and small scale, will be to define, within the limits that can be achieved and controlled in the culture system, the optimal mode of operation for the best possible growth and survival of the shellfish.

C. Seaweed culture.

Seaweeds are used in the polyculture system as a "polishing step" to remove nutrients not initially assimilated by the phytoplankton and those put back into the culture system by excretion of the shellfish and other animals and the decomposition of their solid wastes. The objective is to achieve a nutrient-free final effluent that will meet standards of tertiary sewage treatment at the same time producing a crop of commercially valuable plants.

During the past year, seaweed research was restricted to red algae of several species that are of existing or potential commercial value for their content of agar or carrageenan. These have included Chondrus crispus, Gracilaria foliifera, Agardhiella tenera, and Hypnea musciformis. Of these, Gracilaria and Agardhiella have proved most successful. The following discussion concerns primarily the results obtained with Gracilaria.

As explained in the preceding section, water leaving the shellfish raceways passes through the adjacent raceway in the opposite direction where it is exposed to suspended cultures of seaweed before being discharged back to the ocean. The latter have the same dimensions as the shellfish raceway (40' x 4' x 5' deep) but have been modified with a sloping plywood bottom with a depth ranging from two feet, on the high side to the bottom (five feet) on the low side.

An air line on the bottom at the five-foot depth provides the vigorous circulation needed to keep the seaweed in suspension and to bring it continuously to the surface and to exposure to sunlight. The sloping bottom eliminates a dead area in the circulation cell in the corner opposite the air line, in which the seaweed would otherwise settle and collect.

Once a week, the seaweed population is harvested from the raceways with dip nets, drained, and weighed. Net production over the previous week is removed, returning a constant starting biomass of 50 kg/raceway. This routine has varied experimentially during the year, but the preceding figure was found empirically to be optimum for maximum daily production, which ranged from a mean of 3 grams dry weight (organic matter) m^2/day in winter to 10 grams/m^2/day in summer (dry weight is 10% of wet weight and contains an average of 40% ash in Gracilaria foliifera). The harvested seaweed is dried and packed in bales which have been sent to commercial seaweed firms for evaluation and assay for polysaccaride content. Information has not yet been received back from these organizations. Performance of the seaweed raceway as a polishing step in nutrient removal will be considered in the following section, in which nutrient mass balance for the whole system is discussed.

Occasionally fouling organisms, in particular the green alga Enteromorpha, invade seaweed cultures and grow epiphytically upon the cultured species. Under extreme conditions, the cultures must be discarded. Epiphytic growth is probably the single greatest problem in and constraint to commercial seaweed culture, particularly in the tropics and subtropics where conditions are otherwise ideal for such practices. For reasons not fully understood, this problem has not developed in the Woods Hole experiments. Occasionally, in smaller-scale experiments, the seaweeds have become overgrown with Enteromorpha, but this has never happened to any significant extent in the raceway cultures. Earlier, when Chondrus crispus was being grown, it became seriously epiphitized by filamentous red algae (Ceramium rubrum, Spermothamnion sp.). For that and other reasons, principally the relatively slow growth of Chondrus in our system, it was replaced by Gracilaria and Agardhiella. These two seaweeds have remained remarkably clean and free of epiphytes and epizoa and, in fact, when contaminated specimens from other, small-scale experiments or collected from nature are introduced to the raceways, they normally lose their epiphytes. Presumably, some fortuitious accident in the design or operation of the seaweed growth system results in the supression of epiphyte growth. It is hoped, in the near future, that the responsible factor or factors can be identified and defined.

During 1974, new experiments were initiated in which seaweeds have been grown alone, in a single-step waste recycling system, using mixtures of seawater and secondary sewage effluent in a continuous flow-through mode of operation. A series of plywood tanks 8' x 6' x 3' painted with white epoxy have been used in these experiments. As in the seaweed raceways, the bottom of the tank slopes from a depth of one foot to the three-foot bottom on the long (8') dimension. Because the tanks are wider and shallower than the raceways, the slope of the bottom is more gentle. Again, aeration is provided through an air line extending the length of the tank along the bottom, three-foot deep edge. Because of the more gentle slope, the seaweed is not carried over into the shallow side of the tanks, most of it sinking one to two feet before reaching the edge. As a result, some 15 ft^2, or almost one-third of the area of the tank, is not in production and yield per unit area is correspondingly lower than in the raceways. A bottom slope approaching 45° would appear to be an optimal design for such units.

Despite this design deficiency, high yields of as much as 16 grams ash-free dry weight/m^2/day have been achieved for short periods of time in summer, while average yields of 3 g/m^2/day in winter and 12 g/m^2/ in summer were sustained over long periods of time. Table 1 shows, in addition to the yields and nitrogen removal of the unicellular algae in the phytoplankton culture ponds, the corresponding data for the seaweeds grown on sewage effluent and seawater mixtures in the experimental tanks described above. Again the data have been extrapolated to show the potential and areal requirement of such a system in nutrient removal per MGD effluent. It may be seen that seaweed production is comparable to and, in summer, slightly better than unicellular algae production. However, because the seaweeds contain on the average less nitrogen per unit of ash-free dry weight (4% for seaweeds and about 10% for unicellular algae), the equal or higher rate of growth of seaweed is more than offset by its lower capacity for nitrogen removal per unit growth.

In one experiment, three of the above seaweed tanks were operated in series, with an input of 25% sewage effluent - 75% seawater mixture introduced into the first tank and then passing through the second and third tanks at flow rates equivalent to 50% of the individual tank volume turnover per day. The three tanks were initially stocked with 5,000, 3,000, and 1,000 grams respectively of Gracilaria, and the growth increment allowed to accumulate during the one-month period of the experiment. Inorganic nitrogen and phosphorus were monitored in the water entering and leaving each of the tanks. The data from this experiment is summarized in Table 3, where it may be seen that the three tank cultures progressively removed 99% of the incoming nitrogen. Nitrogen deficiency of the Gracilaria in the third tank was evident both in its pale yellow coloration, in contrast to the deep reddish-brown color of the plants in the first tank, and in its carbon:nitrogen ratio, which was 28 in contrast to 10 in the first tank. This has some practical significance, as the commercial product of the seaweeds (agar in Gracilaria) is reportedly elaborated more

Table 3. Nitrogen removal in experimental seaweed (*Gracilaria foliifera*) tanks operated in series, April 15 - May 15, 1975, under steady-state, continuous-flow conditions*

Tank No.	Effluent N concentration† (ppm)	%N removal†	Seaweed production ($g/m^2/day$)⁑	C:N in seaweed
1	0.96	60	3.4	10
2	0.07	77	2.5	12
3	0.02	99	1.2	23

*See text for description of experimental conditions

†Initial N concentration (input to Tank 1) = 2.41 ppm.

⁑ Ash free dry weight

rapidly and to a greater degree in nitrogen-deficient plants. In a commercial seaweed culture application, using a raceway or channel-type culture configuration with a linear flow of water and nutrients, the seaweed should presumably be moved downstream in the system, away from the source of nutrients, and harvested from the far end following a period of exposure to nitrogen-free conditions.

The same experiment as described above is now being repeated, at the time of this writing. Conditions are identical except that the input of both sewage effluent and seawater has been doubled (one tank volume turnover per day and twice the daily flux of nutrients). Complete nitrogen removal is still being observed in this experiment, the results of which are not yet ready for publication. Ultimate capacity of the system will eventually be evaluated by this means.

D. Nutrient removal efficiency of the system as a whole.

As pointed out earlier, algal pond cultures were operated during the first year deliberately at nutrient (sewage effluent) concentrations higher than could be completely utilized by the phytoplankton. This was done to develop information on the maximum potential growth and nutrient assimilative capacity of the algae under non-nutrient limited conditions. The amount of nitrogen taken up by the algae from solution or the amount contained in the algal harvest, by direct measurement, could then be used to calculate the daily assimilative capacity of the system and this, in turn, to calculate the daily input of sewage effluent per unit area of algal pond for complete nitrogen removal. That information, based on a year's observation, is presented in Table 1, also including the comparable data for a seaweed-based tertiary treatment system.

The above data, interpreted in terms of the ESL pond culture system, means that complete nitrogen removal could be expected in winter operating at a 25% pond volume turnover per day with an input of 10% sewage effluent and 90% seawater. In spring and fall, the effluent strength can be increased to 20% or the turnover rate doubled (50%), resulting in either case in doubling the nutrient input rate. In summer, the system should be able to assimilate completely the nitrogen from 30% effluent - 70% seawater mix at 25% turnover, or a 10% effluent - 90% seawater at 75% turnover rate per day.

Since it is costly to pump seawater, the higher effluent concentration at the lower exchange rate is the more economical mode of operation. There is some evidence, however, that stability of the cultures may be enhanced by low nutrient levels at high turnover rates, so the costs of labor (for cleaning and restarting cultures) and of building and operating stand-by cultures to provide for down-time may exceed the cost of pumping additional seawater.

Beginning in April, 1975, a series of pond experiments was initiated to test and evaluate the above conclusions. Two of the ponds have been operated continuously on a 10% sewage - 90% seawater mixture at 50% pond volume turnover per day and 20% sewage effluent - 80% seawater at 25% turnover per day respectively (i.e., the projected "spring" operating conditions discussed above), with the objective of achieving complete nitrogen removal.

At the same time, a new supply of 5-10 mm seed oysters (Crassostrea virginica) was obtained from Long Island Oyster Farms. These oysters had set in the hatchery in February, 1975, and were new, vigorously-growing animals. Growth has continued in the ESL raceways up to the present time with no detectable mortality. In addition, by the spring of 1975 the seaweed raceway culture system had been improved through experience to the extent that a dense, clean, vigorously-growing culture of Gracilaria was well established with stable, uninterrupted production of the seaweed.

Conditions were appropriate, therefore, for evaluation of the nitrogen balance and mass flow through the entire system. In so doing, half the algal harvest from one pond was fed into one shellfish raceway and its discharge into one seaweed raceway, the three units serving as one module of a prototype tertiary sewage treatment-aquaculture system.

Table 4, shows the daily mass flow of nitrogen through the three-step system under the operating conditions defined above, considering only the half of the pond culture that was fed to the shellfish. Of the nitrogen (nitrate, nitrite, and ammonia) daily entering the pond as sewage effluent (84 grams) and seawater (1 g), over 98% (83.5 g) was removed by the phytoplankton. The remaining 1.5 g, together with the algae, was fed to the shellfish raceway, where it was mixed with twice its volume of seawater. Since the latter contained the same concentration of inorganic nitrogen as the pond effluent (0.04 ppm), the seawater contributed twice as much nitrogen as the effluent (total 4.5 g). To this, the shellfish raceway added 22.5 g of dissolved inorganic nitrogen through excretion, decomposition, or other sources, roughly 25% of the amount that entered the raceway as phytoplankton. Of the total output of 27 g nitrogen from the shellfish raceway, 18 g were removed by the seaweeds, leaving a final residual of 9 grams, 10% of the initial input of the sewage effluent and seawater, for a total removal efficiency of the system as a whole of 90%.

The complete system, as described, was not "balanced" with respect to the size and biomass of the seaweed culture relative to the other components, since the experiments were designed to determine, among other things, what that balance should be. (i.e., It was not known at the outset what fraction of the unicellular algal nitrogen would be regenerated by the shellfish.) Since the seaweed removed two-thirds of the regenerated nitrogen, it can be assumed that expansion of the seaweed culture by one-third (from 160 ft^2 to 240 ft^2 in the pilot facility) would result in complete nitrogen removal of the final effluent.

The above results are typical of those that were obtained in over one month of continuous operation, with extremely little variability with the exception of one five-day period, when predation by colorless flagellates (discussed in an earlier section) temporarily

Table 4. Mass flow of inorganic nitrogen (ammonia, nitrite, and nitrate) through the phytoplankton-oyster-seaweed system; May, 1975.

	grams N/day	
1. Phytoplankton pond input		
sewage effluent	84	
seawater	1	85
2. Phytoplankton pond output		1.5
3. Shellfish raceway input		
phytoplankton pond harvest	1.5	
seawater	3.0	4.5
4. Shellfish raceway output		27
(= seaweed raceway input)		
5. Seaweed raceway output		
(final effluent from system)		9.4
Total N removal efficiency (including seawater)		89.3%
Effluent N removal efficiency		93.6%

reduced phytoplankton production. It should be pointed out, however, that this monitoring period, in April-June, 1975, coincided with the and took place under conditions of the best possible performance of the system, and is not representative of average performance throughout the year.

In both modes of operation of the pond cultures (i e., 10% effluent-50% turnover and 20% sewage-25% turnover), 95-99% nitrogen removal by the unicellular algae has consistently been achieved except for brief periods mentioned above when algal production is depressed by the protozoan predator. The latter has been more frequent and pronounced in the more heavily enriched (20% effluent) culture. More recently, at the time of this writing, the nutrient concentration in the sewage effluent has unaccountably increased by about 25%, giving a total daily input of nitrogen to the ponds of about 210 grams, in contrast to the 170 g reported in Table 4. This is equivalent to roughly 25% effluent of the strength used up to this time. Nitrogen removal is still virtually complete, ranging from about 90% on cloudy days to as much as 99.9% on clear sunny days, at which times the effluents from the ponds contain less nitrogen than the incoming seawater.

E. Culture of secondary animal crops.

Solid wastes (feces and pseudofeces) produced by the shellfish and/or uneaten phytoplankton cells which settle out from suspension in the shellfish raceways provide sources of food for large quantities of several species of small, invertebrate detritovores that presumably enter the system as larvae in the coarse-filtered seawater used to dilute the phytoplankton pond harvest. Prominent among such invertebrates are amphipods (Corophium, Jassa, and Gammarus), polychaetes (Capitella capitata), bryozoans, tunicates, and mussels. This small invertebrate fauna serves the dual purpose of preventing the accumulation of solid organic wastes in the raceways and providing a source of food for secondary crops of carnivores or omnivores of potential commercial value. The latter has included the American lobster (Homarus americanus) and the winter or blackback flounder (Pseudopleuronectes americanus) (Table 5).

In July, 1974, 474 juvenile (0 and 1 year class) flounder were collected locally and stocked in one of the oyster raceways. Their size distribution was, of course, bimodal for the two-year classes, but averaged 7.0 cm. In October, 1974, the raceway was drained and 124 fish recovered, averaging 11.0 cm in length. In April, 1975, 69 fish were recovered, averaging 16.75 cm in length. The surviving fish thus more than doubled in size in 9 months. If the observed linear growth rate were to continue, the fish would reach a marketable size of 25 cm (1/2-1 lb) in another 9 months, or 18 months from the time of stocking as juveniles.

The fish were obtained by beach seine some distance from the laboratory, measured immediately, and stocked. The smaller (0-year

Table 5. Growth and survival of winter flounder and American lobsters in oyster raceways.

Winter flounder (*Pseudopleuronectes americanus*)			
Date	Number	% survival	Size (mm)
7/74	474	----	70
10/74	99	21	110
4/75	69	14.5	167

American lobster (*Homarus americanus*)			
Date	Number	% survival	Size (mm)*
9/74	390	----	9.0
11/74	256	66	13.4
4/75	124	32	25.0

*carapace length

class) fish are very delicate and subject to injury during collection and transport, and they very likely suffered a large initial mortality. Some fish may also have escaped, since flounder were found in adjacent raceways during the summer of 1974. Thus, the small survival (15%) of flounder is not necessarily representative of the carrying capacity of the raceway. The fish recovered in April, 1975, were extremely fat, healthy in appearance, with good natural coloration, and with no sign of disease. There was still an abundance of the small, invertebrate food organisms in the raceway, so it is unlikely that food was limiting.

The surviving 69 fish represented a density in the raceway of 0.4 fish/ft^2, which is a dense population if the bottom of the raceway alone were considered. However, many flounder were observed in the shellfish trays resting on the oysters and presumably feeding on the invertebrates associated with the shellfish. The area of the trays (483 ft^2 for 64 trays) increases the total habitat of the raceway by fourfold and should thereby increase the habitat of the system for bottom-dwelling fishes such as flounder proportionately, if sufficient food is available. A more careful and heavy stocking of the larger size juvenile fish (1-year class) that have been held long enough to eliminate mortality due to initial injury should provide the needed information on carrying capacity and potential rate of production of flounder in the system.

Egg-bearing lobsters were obtained from commercial fishermen, by special permit, and were held in the laboratory until the eggs hatched (i.e., in spring, when water temperatures reach 15°-20°C). The larvae were transfered to specially-constructed larval rearing tanks where they were fed live or frozen brine shrimp (*Artemia salina*). After metamorphosis to juvenile lobsters (10-14 days), they were segregated into small containers, to prevent cannibalism, and fed the same food until they had molted an additional 3-4 times and attained a mean size of 9 mm carapace length and 0.18 grams. A total of 390 of these lobsters were then stocked in September, 1974 in segregated (screened-off) portions of two oyster raceways, each group together with two stacks (16 trays) of oysters. In April, 1975, a total of 124 lobsters were recovered which had a mean size of 25 mm carapace length and a mean weight of 18 grams. These ranged widely, however, in their size distribution, from 10 to 52 mm carapace length. The larger individuals, some 150 mm total length, attained a size in eight months that is not reached by wild lobsters in New England in less than three years, and is comparable to the best growth obtained with segregated lobsters held in captivity at elevated temperatures and fed artifically.

A survival rate of 32% in an animal as cannibalistic as the lobster is remarkedly good. Post-larval lobsters can be produced in vast numbers quite inexpensively, so even quite a small percentage survival to adult animals could be economically attractive. The important factor, however, is not survival or mortality of the original stock, but the carrying capacity of the system. Lobsters were found living in the oyster trays and on the bottom of the segregated portions of the raceways, a total area of 365 ft^2 or about 0.3 lobsters/ft^2. On that basis, a complete 40' x 4' raceway with 64 trays of oysters can support some 200 lobsters for their first eight months. How long it will take for them to reach legal marketable size (81 mm carapace length in Massachusetts), and how many will survive to that size must await further observations.

Other lobster experiments were conducted in which post-larval animals were stocked at various densities in individual trays or screened-off portions of trays of oysters. Growth, again on the natural food that developed in the oyster trays, was comparable to that reported above, but after reaching a size of 13-17 mm carapace length (10th-12th molt), cannibalism reduced the experimental populations essentially to one lobster per tray or per compartment.

Although there is much current interest in commercial lobster culture, the logistics and economic problems of maintaining lobsters individually in separate containers (because of cannibalism) and feedint each lobster individually a prepared, artificial food has discouraged any serious undertaking. If, however, post-larval lobsters could be segreated in compartmentalized trays of oysters and allowed to feed on natural food, so that they would not have to be handled or inspected except at relatively long intervals (e.g., 3-6 months) when the oysters would need to be thinned and culled in any case, the economic prospects would be considerably more attractive.

New experiments are now being initiated in oyster culture using plastic, commercial grow-out trays (Nestier Division, Vanguard Industries, Cincinnati, Ohio) that measure 23" x 23" x 2 7/8". Six hundred and forty such trays will fit into one shellfish raceway. Even at a stocking density of one lobster per tray, this would be equivalent to 175,000 lobsters per acre of shellfish raceways five feet deep. Conceivably, two or even four lobsters per tray could be stocked by compartmentalizing the trays. The value of the secondary crop of lobsters could thus rival if not exceed that of the primary crop of shellfish. Such speculation, however, is premature until more is known about the space requirements of the lobsters and most important, the amount and rate of production of food in such a system, for it is the latter that will ultimately determine the potential yield of lobsters. These subjects will be investigated in the coming year.

Summary and Conclusions

1. Tertiary treatment requirements.

Nitrogen removal is equivalent to tertiary sewage treatment of domestic wastewaters discharged into coastal marine environments, if the objective is to prevent algal growth in and eutrophication of the receiving waters. Repeated bioassays have demonstrated that effluent-seawater mixtures from which nitrogen has been removed are incapable of supporting the growth of algae, despite residues of phosphate and perhaps other nutrients.

2. Nitrogen removal by unicellular marine algae.

Complete nitrogen removal may be accomplished by growing unicellular marine algae in continuous, flow-through cultures in effluent-seawater mixtures. Shallow (1 meter), 2,500 ft^2, PVC-lined ponds, gently circulated to maintain the algae in suspension, have been used for this purpose. Optimal and maximum possible size of ponds to achieve the same or an improved performance have not been determined.

3. Factors controlling algal growth and nutrient removal.

Algal growth and nitrogen removal are independent of temperature (0°-25°C) but controlled by incident solar radiation, ranging in magnitude seasonally by three-fourfold in temperature latitudes. For the same reason, nitrogen removal is a function of surface area of the algae ponds and is largely independent of depth.

4. Algal production and nitrogen removal.

Sustained algal production in pond cultures has ranged from 3 grams ash-free dry weight per square meter of pond surface per day in winter to 9 g/m^2/day in summer with intermediate levels in spring and fall. Maximum production for short periods of time in summer has reached 12 g/m^2/day. A production of 10 g/m^2/day is equivalent to nitrogen removal (assimilation) of 1 g/m^2/day or 9 lbs/acre/day.

5. Area requirement for nitrogen removal.

At the latitude of Woods Hole, Massachusetts, the pond area required for complete nitrogen removal from one million gallons per day (1 MGD) of secondary sewage effluent (10,000 capita) ranges from 26 acres in summer to 77 acres in winter. At lower latitudes, as in Southern United States, a seasonal range of 26-37 acres per MGD effluent would be required.

6. Seawater pumping requirement.

A total of 7-9 MGD of seawater must be pumped per 1 MGD sewage effluent treated to dilute the effluent and provide a suitable medium for growth of the marine algae.

7. Algal species.

Pond cultures consist of uni-specific populations of single-celled algae, usually diatoms. At Woods Hole, Mass. cultures have consisted of the diatoms Skeletonema costatum in winter and Phaeodactylum tricornutum the rest of the year except for a brief period (ca. 1 month) in midsummer, at pond temperatures > 25°C, when green flagellates appeared. Efforts to control the species of algae in the ponds have been unsuccessful.

8. Culture stability.

Cultures are stable with respect to cell density (biomass), growth rate, and nutrient removal for long periods of time (months). Gradual accumulation of sediment requires periodic draining and cleaning of ponds every 3-6 months, requiring 1-2 man-days effort per 2,500 ft^2 pond with down-time of 3-5 days.

Occasionally and unpredictably, predation of the algae by an unidentified protozoan depletes the algal populations and temporarily reduces or stops cell production. This situation normally corrects itself within 3-5 days. Operating conditions that will inhibit development of the protozoan predator without depressing algal growth (i.e., low effluent concentrations at high turnover rates) are currently being investigated.

9. Algal removal and shellfish culture.

To complete the tertiary sewage treatment process, the algal cells that have assimilated nutrients from the sewage effluent must themselves be removed from the water. Filter-feeding bivalve molluscs are used for this purpose. The continuous-flow algal pond harvest is fed by gravity to cement channels or raceways containing stacked trays of oysters, clams, or other bivalves. Algal cultures must be diluted with coarse-filtered seawater to reduce cell densities to the degree that they can be used efficiently by the shellfish and to prevent accumulation of toxic metabolites of the shellfish (e.g., ammonia). Shellfish must also be supplied with vigorous aeration to mix cultures with diluting seawater, to prevent stratified flow through raceways, and to insure an adequate oxygen supply.

10. Seawater pumping requirement for shellfish culture and for entire system.

Algal cultures are diluted with an equal volume of seawater in winter, as much as five times the culture volume in summer. Total seawater requirements for the algal and shellfish cultures, per MGD effluent treated, range from 17 MGD in winter to 50 MGD in summer.

11. Shellfish production.

Shellfish culture during the first year of operation of the pilot plant facility was largely unsuccessful, with poor growth and high mortality of seed clams and oysters. This was due to unresolved problems believed to be: 1) unfavorable culture conditions in the raceway system; 2) unfavorable algal food (a diet for most of the year of the diatom, Phaeodactylum tricornutum, variously reported in the literature as a poor to indifferent food for bivalves); or 3) an inferior stock of shellfish that were stunted or whose growth was irreversibly checked prior to acquisition.

12. Projected shellfish prduction.

Based on data from small-scale experiments and from the literature (4), the algae produced from 1 MGD sewage effluent is sufficient to produce eleven million market-sized (3-4") oysters per year, which is equivalent to 183 tons of oyster meat or 38,000 bushels of whole oysters, assuming continuous feeding and growth throughout the year. Held in raceways or channels five-feet deep in stacked trays, the annual crop would require an area of approximately six acres assuming maintenance of a mixed population of juvenile and adult animals with periodic stocking and harvesting. These data are speculative until a successful method of shellfish production is demonstrated and evaluated in the pilot plant project.

13. Temperature requirements for shellfish production.

Indigenous bivalve molluscs require a minimum temperature of about 15°C and an optimum temperature of about 20°C or more (depending upon species) for feeding and growth. Algal cultures may be grown at ambient seawater temperatures throughout the year, with no advantage from heating (i.e., growth is controlled by solar radiation), but unheated algal cultures cannot be fed upon by the shellfish if temperatures of the culture and diluting seawater are together less than about 15°C. Diluting seawater could be heated more practically than could the shallow, extensive algal cultures, but neither could be done economically. Use of cooling water effluent from power generating stations (Δt normally 10°-15°C above ambient) is not sufficient to raise temperatures of the algal food to the degree necessary in winter in regions of temperate climate, but at best could only extend the shellfish growing season.

14. Implications of thermal constraints to shellfish culture in operation of the system.

Because of the temperature requirements for shellfish growth (see 13), year-around operation of the system is restricted to tropical to semi-tropical regions. In temperate latitudes, operation is restricted to 4-6 months per year. This may be an acceptable alternative in coastal resort areas, where both the need for tertiary treatment and population pressure are greatest in summer. Over two-thirds of annual algal production occurs during the six warmer months (i.e., in Woods Hole, Mass.), so losses in nutrient-removal capacity and shellfish growth during the remaining six months would be minimized. Winter storage of effluent in lagoons, as is now practiced in some terrestrial waste recycling systems, is a possible alternative to discharge of such wastes when the aquaculture system is inoperative.

15. Regeneration of nutrients by shellfish and final nutrient removal by seaweeds.

In a balanced system in which there are enough shellfish to consume all of the unicellular algae provided as food (10^8-10^9 cells/animal/day depending upon size) approximately 25% of the nitrogen contained in the algae cells is regenerated as inorganic nitrogen, principally ammonia, by excretion of the shellfish and other animals in the shellfish culture system and decomposition of their solid wastes. These may be removed by a final "nutrient polishing" stage consisting of cultures of one or more species of seaweeds. Red algae of existing or potential commercial value for their hydrocolloid (carrageenan or agar) content are used for this purpose.

Seaweeds are grown in cement channels or raceways similar to those used for shellfish culture but with a sloping (ca. 45°) bottom and with vigorous aeration to maintain the plants in suspension and exposure to sunlight.

16. Seaweed production and nutrient removal capacity.

On the basis of inorganic nitrogen mass flow data in the complete pilot system, an area 8.5% that of the unicellular algal pond requirement is needed for total nitrogen removal of regenerated wastes by the animals. This is equivalent to 2.2 acres per MGD effluent treated. However, the mass flow data are based on short term measurements in May, when the system was operating near maximum efficiency and therefore represent "best performance" conditions.

Continuous, long-term seaweed production during late spring, summer, and early fall (i.e., when shellfish production is possible) averages 10 grams (ash-free dry weight)/m^2/day or 89 lbs/acre/day. This is equivalent to a nitrogen removal capacity (assuming N = 4% ash-free dry weight) of 3.5 lbs/acre/day.

The residual nitrogen produced by the animal system may therefore be removed by seaweed culture in an area of 14 acres per MGD effluent treated during a six-months growing season at temperatue latitudes, presumably throughout the year in tropical to semi-tropical environments.

Seaweed production from the "polishing stage" is 3,350 tons wet weight per year per MGD effluent treated, half of that figure for a six-months growing season.

17. Total areal requirement for complete system.

Considering only a six-months operating cycle in Northern climates and a year-around operation in the South, the areal requirements per MGD effluent treated for the complete nitrogen removal-aquaculture system consists of approximately 28 acres of algae ponds, 6 acres of shellfish cultures, and 14 acres of seaweed culture, for a total of 48 acres/MGD effluent. Mean depth of algae and seaweed cultures is three feet, that of the shellfish cultures, five feet.

18. A nitrogen-removal system based on seaweed culture only.

A tertiary sewage treatment (N-removal) system consisting only of seaweed culture may be used as an alternative to the combined unicellular algae-shellfish-seaweed culture. Production of seaweeds, as ash-free organic matter per unit of culture area per day, is comparable or perhaps slightly higher than that of unicellular algae. However, seaweeds contain less nitrogen per unit weight and therefore assimilate less nitrogen per unit growth than unicellular algae. Consequently a larger culture area is required for seaweeds than for unicellular algae to accomplish the same nitrogen removal.

19. Seasonal aspects of seaweed culture.

Experience with the pilot plant facility to date has been restricted to the culture of semi-tropical seaweed species (forms that are also summer annuals in New England), such as Gracilaria foliifera, Agardhiella tenera, and Hypnea musciformis. In winter, this was done in heated (ca. 15°C) water. Sustained production of Gracilaria has ranged approximately fourfold, from 3 grams (ash-free dry weight)/m^2/day in winter to 13 g/m^2/day in summer, presumably due to changes in solar radiation. Neither the above species nor the colder-water species Chondrus crispus, grows in nature or in unheated culture in winter. It is therefore assumed that seaweed culture is restricted to seasonal operation in the North but could be carried out throughout the year in tropical to semi-tropical climates.

20. Areal requirements and yields of seaweed culture.

Areal requirement for a seaweed culture system for nitrogen removal is approximately 60 acres per MGD effluent with an annual production of 16,300 tons (wet weight) of seaweed, half of that for a six-month seasonal operation.

21. Seawater pumping requirement for a seaweed-based waste recycling system.

Although not yet conclusively determined, tentative evidence is that best growth of and nitrogen removal by seaweeds occurs at nitrogen concentration of 2.5-3.0 mg/l, equivalent to approximately 10% wastewater effluent, at a flow rate of one culture volume exchange per day Seawater required per MGD effluent treated is therefore 9 MGD.

22. Production of other animals grown in the shellfish cultures.

Shellfish cultures produce large quantities of solid, organic wastes (feces and pseudofeces). Small invertebrates (worms, crustacea, etc.) entering the shellfish culture as larvae in the diluting, coarse-filtered seawater, establish themselves and multiply in the shellfish trays and on the bottoms and sides of the raceway system. This small invertebrate fauna keeps organic sediment from accumulating in the shellfish culture and may also serve as a source of food for larger animals. Winter (blackback) flounder (Pseudopleuronectes americanus) and American lobsters (Homarus americanus), stocked as juveniles with the shellfish, have shown good growth and reasonable survival with no supplemental feeding. Carrying capacity of the system and potential yields of such animals with respect to food supply and its rate of production and habitat have not yet been determined.

23. Trace contaminate and pathogen uptake in organisms cultured in sewage effluent and related public health problems.

Problems associated with the uptake of trace contaminates (heavy metals, organic compounds) and pathogens (principally viruses) from the treated sewage effluent by the shellfish and other cultured organisms, and depuration of these substances by the organisms are subjects of an independent study now in progress. Results, when available, will be presented separately.

24. Preliminary results of research in Florida.

Smaller scale studies are currently being undertaken in Fort Pierce, Florida under support of the Harbor Branch Foundation, Inc. Growth yields, and nutrient removal by both unicellular algae and seaweeds are being studied on a seasonal basis. While it is unrealistic to compare the performance of the pilot-scale Woods Hole facility with that of the smaller Florida project, and results from the latter are not yet available for a complete year, preliminary indications are that growth and nutrient removal of both unicellular and macroscopic algae are, on the average considerably greater throughout the year in Florida than for the Spring-Fall period in Woods Hole (5, 6). On that basis, extrapolation of the seasonal performance of the Woods Hole system to a year-around operation in a climate such as Florida is not unreasonable and may be conservative.

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THE CULTIVATION OF FISH IN MUNICIPAL WASTEWATER LAGOONS AS AN AVAILABLE PROTEIN SOURCE FOR HUMAN BEINGS WITH EMPHASIS ON SALMONIDS

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INTRODUCTION

Fertilizing fish ponds with animal manures is known to antiquity (Borgese 1975). There is a relatively small but steadily expanding scientific literature documenting the levels of increased fish production using domestic wastewaters (sewage) (Allen 1969, 1972; Mortimer and Hickling 1954; Bardach et al 1972; Ryther et al 1972; Thorslund 1971). Today most utilization of domestic wastewaters in aquaculture occurs in areas where human populations have animal protein deficiencies in their diet, where energy and fertilizer resources are not available for food production due to low income levels, or where water is in short supply. Usually the area also has a cultural heritage of fish consumotion or fish husbandry. Thus in highly industrialized areas today there is little wastewater aquaculture, although part of the restriction is due to the poor quality of the wastewaters due to industrial pollutants. In the United States, an abundance of energy and fertilizer has not made waste-water aquaculture economically attractive, although these conditions are changing (Steinhart and Steinhart 1974; Pimentel et al 1973). In addition, strong cultural attitudes have limited wastewater aquaculture in the United States. Kildow and Huguenin (1974) indicate that the average

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American has been acculturated to have repugnance for wastes, especially human, and shows this distaste as a concern both for modesty and cleanliness.[3] A sophisticated medical science reinforces the attitude, which is reflected in a relatively conservative approach among regulatory agencies dealing with water and food supplies, waste management, and especially public health (Bryan 1974). Most wastewater reutilization in food production in the United States has been confined to agriculture (Sullivan 1974). No wastewater-grown aquaculture products go directly into human consumption, as most states have laws tending to restrict such practices.

Opportunities for utilization of wastewaters in aquaculture are now appearing in the United States. A major impetus comes from a general conservation movement, especially that part emphasizing recycling of inorganic materials such as glass and metals where markets for the material remain strong. Popular reports suggesting the use of algae in waste treatment as part of the recycling potential of organic materials such as sewage (Weinberg 1975) help build a broad public understanding on the subject. The recent documenting of the variety of research, pilot, demonstration, and production projects on wastewater agriculture-aquaculture (Boivo 1972; Carpenter 1974) helps create the data bank necessary to form political and economic support required for completion of large-scale wastewater aquaculture efforts. Potential markets for wastewater-grown fish are now appearing in the United States due to the rising costs of fish protein which has been the basic ingredient in diets fed to the major species of fish cultured in the United States (salmon, trout, catfish)

[3] The senior author frequently refers to this attitude as the "American soap syndrome" when discussing wastewater aquaculture in public.

(Brown and Hussen 1974; Greenwalt 1975; Regier 1975).

To assist in these general objectives, we present in this paper some positive results from two pilot projects employing wastewater to produce fish. One project (Quail Creek, near Oklahoma City, Oklahoma) is testing the use of a fish polyculture system in six municipal lagoons operated in series as a method of achieving advanced levels of wastewater treatment. The second project (Humboldt Bay, Arcata, north-coastal California) is testing empirically the feasibility of rearing juvenile salmonids (trout and salmon) on natural food chains in saline ponds fertilized with secondary treated domestic wastewaters.

The two projects have fundamental objectives in common: first, to reduce energy and material inputs to current high-cost, high-technology enterprises; and second, to convert into something useful to society valuable resources currently being wasted. The two projects face widely varying sets of conditions (Table 1), and thus illustrate the extensive range of possibilities open to fish production in municipal wastewater treatment and other systems (Carpenter op. cit.).

In addition to biological information, we will present some of the non-technical considerations surrounding wastewater aquaculture in one of these regions (California) since these factors influence funding for both research and production projects in municipal wastewater aquaculture.

ARCATA WASTEWATER TREATMENT SYSTEM

The wastewater treatment system operated by the City of Arcata services about 11,000 inhabitants, plus Humboldt State University with about 7,000 students. Most of the wastewater is domestic, with minor industrial inputs from a few lumber and plywood plants. The Arcata sewage collection system is separated from a storm drain system; however, the collection system is old and a sizeable degree of infiltration occurs during rainstorms. Thus the low lows during summer (dry season) months varies around one million gallons per day, while peak flows during the winter (wet season) is around two million gallons per day. Incoming wastewater enters a grit removal chamber, then passes into clarifiers, thence into an aeration pond, through a partially completed facultative pond, and then finally into a 55-acre oxidation pond. Effluent from the oxidation pond enters Humboldt Bay after chlorination-dechlorination. The system is required to meet water pollution control standards to protect general biota in the area, but especially commercial oyster beds located about two miles from the point of discharge (23 MPN/100 ml total coliforms; 0.1 mg./1 residual chlorine). Wastewater is drawn into our fish ponds from the oxidation pond either by a pipe or by pumping. With aeration, oxidation pond water is not toxic to Pacific salmon fingerlings in the winter months (Allen and O'Brien 1967), and is non-toxic with only slight dilution with seawater in summer months (Allen 1974).

TARGET SPECIES IN ARCATA SYSTEM

The initial target species has been the chinook salmon (Oncorhynchus tshawytscha). In northern California, the major runs of this species return from the ocean to freshwater to spawn in the fall months (fall chinook). The fry emerge from stream gravel in late winter, generally remaining in the rivers as feeding juveniles (parr) only a few months before migrating downstream to saltwater in the spring. The physiological change leading to migration to the sea is termed smolting, with the downstream-migrating juveniles called smolts. Smolting occurs in fall chinook stocks in the Mad River (immediately north of Arcata) at about 70 mm fork length (Taniguchi 1970). In chinook rearing studies, fry are placed into the ponds in a winter (January-February) period, and smolts captured for release in a spring (late April-early June) period.

Our second priority target species has been the coho salmon (O. kisutch). This species enters freshwater during late fall-winter periods but somewhat later than chinook salmon. Under natural conditions the parr of the coho remain in freshwater for a year, migrating to the sea as smolts in the spring at sizes rarely less than 100 mm fork length (Shapovalov and Taft 1954). Six experiments with coho have involved short-term rearing of fingerling, with four experiments using a late fall to winter rearing period, and two experiments involving summer rearing.

During the summer and fall of 1974 rearing of steelhead rainbow trout (Salmo gairdneri) and coastal cutthroat trout (Salmo clarki) in conjunction with coho salmon was initiated. From our experience to date, these species may eventually prove the more successful in the system.

DESCRIPTION OF EXPERIMENT VII

During July 1971, two 0.15-hectare ponds (Figure 1) located within the perimeter of the Arcata oxidation pond were placed into operation (Allen et al 1972). The rearing of salmonids in these ponds has been grouped into ten periods (Experiments) based upon the species studies and season of rearing (Table 2). There has been a steady improvement in the survival rates of salmon reared in the ponds (Table 3). Experiments with local stocks of fall chinook salmon showed the greatest improvement, except for Experiment X completed in May 1975 (Table 3A). The 27 percent survival (fry to smolt) with fall chinook in South Pond (Experiment VII, spring 1974) approaches a level sufficient to propose a wastewater aquaculture system for a production operation in either a private or public salmon program. As this level of survival would have to be maintained, and hopefully improved, it is valuable to document the conditions found in this system, and to compare them with conditions in other years. Information on chinook rearing in 1972 and 1973 has been published (Allen and Dennis 1974). Data reports on chinook rearing in 1972 and 1974 are also available (Allen 1973, 1975).

During the summer of 1974, extensive modifications were made to North Pond to test ways of reducing predation from shore-wading birds (egrets, blue herons, night-crowned herons), and to study pond aeration by forced air. A two-foot vertical wall was constructed along North Pond banks, except those portions layered with oyster shell. Aeration pipes were laid about a foot away from the base of the pond bank. This created a bubble field immediately adjacent to the bank to provide both circulation, aeration, and to hinder feeding activities of shore birds. An air line was also laid to a manifold located in the lowest point of the pond (fish collecting basin immediately in front of pond headgates) which insured total circulation of pond waters (Figure 1-A). South Pond was left unmodified and was aerated by AIR-O-LATOR spray units mounted over 55-gallon drums to bring bottom water to the surface thus preventing stratification (Figure 1-B).

Saltwater from Humboldt Bay and wastewater from the City of Arcata oxidation pond were introduced into the ponds during late December 1973. The ponds were aerated until late January 1974. Due to additions of freshwater by rainfall, the relative amount of sewage effluent in the ponds was considerably less than the 2:1 ratio indicated by the salinities in the ponds when fish were introduced (ca 10 parts per thousand, °/oo). On January 25, 1974 both ponds were planted with 7,500 chinook salmon fry. The fry were from eggs of fall chinook taken by the California Department of Fish and Game Hatchery at Iron Gate on the Klamath River. Eggs were incubated and hatched at the Humboldt State University Hatchery. An additional 7,500 fry of the same stock were added to each pond on February 15, 1974. The ponds were drained on May 7, 1974 and surviving salmon enumerated and planted directly into Humboldt Bay in the outlet channels from the fish ponds.

Each pond is equipped with four 2-meter square nylon pens located over four available substrates in the ponds (river-run gravel; oyster shell; muds; and Hookton soil, a yellow sandy loam used in pond bank construction) (Figure 1). These pens were originally intended to exclude fish from these substrates (Sharp 1974), but since have been used to rear known numbers of fish over each substrate. For Experiment VII one pen in each pond (gravel bottom) was modified to increase the ratio between substrate and water volume. This was accomplished by suspending nine stringers of oyster shell in North Pond pen and nine stringers of folded nylon strips in South Pond pen (Bates 1975). On February 15, 1974 all pens were planted with chinook fry. Densities ranged from 10 to 90 fry per pen, with 50 fry per pen about the same density as fry planted into the ponds (Table 4).

During the experiment, samples of salmon were trapped to obtain fish for studies of Na^+- and K^+-ATPase enzyme activity in the gills as part of studies on smolting (Collins 1975). The traps were single funnel, and of several designs. Traps were fished in various parts of the ponds. One trap in each pond, however, was consistently fished in the area immediately adjacent to the pond headgates. Information on salmon activity in the ponds was available from the catch in these traps.

PHYSICAL-CHEMICAL CONDITIONS

Salinity values during spring 1974 chinook rearing was about 10 ppt during the early months, then rising steadily during the latter portion of the experiment through evaporation and introduction of higher salinity water from Humboldt Bay (Figure 2). Temperature varied between 8 and 10°C during the first part of the experiment, then suddenly rose to around 14°C where it remained until early May (Figure 3). The transparency of each pond was similar at the beginning of the experiment; however, North Pond phytoplankton became much denser than South Pond as the experiment progressed (Figure 4). This was reflected in a slightly higher level of dissolved oxygen in surface waters (measured during the morning) in North Pond than South Pond (Figure 5), but with no values below 7 mg/l. The higher phytoplankton levels in North Pond resulted in higher oxygen production, and caused a much higher pH level (around 8.5) in North Pond than South Pond (Figure 6).

SURVIVAL, GROWTH, AND PRODUCTION OF SALMON

After three months of rearing, 16% of the fry planted in North Pond were recovered and 27% in South Pond with total smolt production of 6,400 salmon (Table 4). The salmon grew from an average weight of about 0.5 gm to about 4.0 gm. In all pens, the survival of fry was equal to or higher than the corresponding survival in the pond (Table 5). The difference in survival between North Pond (16%) and South Pond (27%) resulted in a net loss in fish flesh in North Pond as compared to a net gain in fish flesh in South Pond (Table 6). The higher survival of the pen-reared fish also resulted in correspondingly higher rates of production. The highest production rate occurred in the Mud pen in South Pond (5 kg/ha/day). The highest survival rate in pens in an individual pond occurred in pens modified by hanging stringers of oyster shell or nylon (64 and 94%). All fish reared in pens in South Pond showed higher survival rates than the corresponding pen in North Pond, which followed the survival rates obtained for unpenned fish.

Catch of salmon in fixed traps showed a steep rise in April, then a sharp drop in early May just prior to pond draining (Figure 7). This drop in catch initially was considered the result of a major mortality in the population. However, Na^+- and K^+-ATPase specific activity in gill microsomes of the salmon doubled in value during late April (Collins, op. cit.) These results were consistent with increased enzyme activities during smolting reported for other salmonids (Zaugg and McLain 1972; Zaugg and Wagner 1973).

The increase in catch in fixed traps in April we feel is active searching by the salmon for higher salinities (i.e. active migration to the ocean). We hope this behavior will aid in trapping salmon as they become smolts to allow for releases in a natural sequence.

CAUSES OF FISH MORTALITY

The sewage-saltwater ecosystem is very complex and it has been difficult to document precisely the causes of mortality. We have correlated periods of low dissolved oxygen with mortalities of coho salmon in floating pens (Allen 1973, Allen and Dennis op. cit.). In chinook rearing experiments, no periods of low oxygen occurred in 1972 and 1973, although a few short periods were recorded when values reached 4-5 mg/l. No oxygen levels below 7 mg/l were recorded in either 1974 or 1975 under forced air aeration system.

Salmonid fishes are extremely sensitive to unionized ammonia under high pH regimes (Spotte 1970). There are no simple routine methods for ammonia analysis in sewage-seawater mixtures, and the unsophisticated methods we have employed have not given reliable data. High pH values, however, may be used in highly eutrophic systems as indicators of times when pH-ammonia mortalities might have occurred. In 1972 and 1973, pH values near 9.0 occurred near the end of each experiment, while in 1975 these levels occurred at the beginning of the experiment. In 1974 (Experiment VII), consistently lower values were recorded, especially in South Pond (about 7.5). A heavy mortality in late February in 1975 (Figure 8) in groups of salmon held in floating pens generally correlated with pH values approaching 9.5-10.0. However, the oxygen levels were high, and the appearance and behavior of the salmon suggested mortalities may have been from gas bubbles in the blood (Renfro 1963).

It would appear that physical-chemical conditions in our ponds are marginal for chinook salmon when pH values approach 9.0. This can be inferred from the consistently higher survival of chinook salmon in many of the pen experiments as compared to the same fish reared in the ponds (Table 7). It is difficult to conceive that all salmon in the ponds were not subject to the same physical-chemical conditions, especially under forced air aeration. If, however, conditions were marginal, then any slight advantage through either better nutrition or reduction of stress through habitat improvement, might allow salmon to survive periods of poor chemical conditions. This possibility was most forcefully demonstrated with completion of Experiment X, in which virtually no smolts were produced, but saw 67 percent survival in an experimental pen fitted with underwater reefs (Table 7). Size and location of these movable pens is shown in Figure 1B. This was not a local phenomenon because the pen on gravel substrate near the pond center showed a 16 percent survival, while a 20 percent survival occurred in a pen over oyster shell located near the shore on the opposite side of the pond. In addition, floating pens located at the headgate showed survivals of 12 and 36 percent. Thus use of underwater reefs, plus new concepts in the design of pond bottoms, may be crucial to obtaining consistent survivals with chinook salmon.

Loss of fish to birds has been a difficult problem to assess although we have established losses ranging from virtually nothing to 25% (Blankinship 1974). Possible losses from birds that feed at night have been particularly hard to establish.

Crawford (1975) undertook studies on nitrite levels on chinook salmon because of the know deleterious effects of low concentrations of this ion to salmonids in freshwater. Seawater ions, plus calcium shell, used in the ponds provide the salmon protection from nitrite toxicity.

DISEASE

The direct discharge of unchlorinated sewage waters into Humboldt Bay is prohibited by the North Coast Regional Water Quality Control Board. Fish pond waters, however, may be discharged after a month's retention of any introduced oxidation pond water, although earlier discharge is permitted if monitoring of total coliform contents of pond waters to be discharged shows levels sufficient to meet discharge requirements for a receiving water where shellfish for human harvest occurs. Single grab samples of water and mud from the oxidation pond and the fish ponds were studied in Experiment VII for total bacteria and total coliform levels (Table 8). Coliform levels were very low in fish pond water. Both a low and a high value were associated with fish pond muds. Although no water from Humboldt Bay was sampled in this experiment, Humboldt Bay waters generally have had coliform values higher than the effluent from the fish ponds, especially during winter periods when heavy runoffs from beef and dairy pasture lands occur.

Fish diseases were not considered a major source of mortality in this experiment. Although regular monitoring of salmon for bacterial and viral infections is currently underway, under the direction of Dr. Robert Busch in charge of fish pathology in the Humboldt State University fisheries department, personnel and facilities were not available during the spring of 1974 for such work. However, it is difficult to conceive of salmon living freely throughout the ponds would suffer losses from disease, whereas salmon reared in the pens would not, unless the pen enviroments produce healthier fish able to resist infections. The mercury content of chinook salmon smolts (0.2 ppm) has been found no higher than in marine species in Humboldt Bay, with such values below current FDA standards for fish products (Nelson 1975).

FUTURE RESEARCH ON ARCATA SYSTEM

Although a Humboldt Bay Wastewater Authority has been recently formed from five local political entities with wastewater responsibilities to collect and treat all Humboldt Bay area wastewaters at a central plant for discharge into the Pacific Ocean, all proposed plans have recognized the huge storage capacity in the existing 55-acre Arcata oxidation pond. Thus wastewater should be available for aquaculture in the future. The current lack of a continuous flow from the system into Humboldt Bay will complicate our ability to capture returning adults as there will be no water to which the salmon have become imprinted for homing (Donaldson and Allen 1957). To overcome this difficulty, salmon reared in the Arcata system (beginning with Experiment VII) have been planted into the lower reaches of nearby streams. Special attention will be paid to Jolly Giant Creek which discharges into Humboldt Bay immediately adjacent to the fish ponds. This small flowage begins in the hills east of the Humboldt State University campus, flows through University property, and through a series of underground pipes and channels directly through the City of Arcata before entering Humboldt Bay. Establishing this stream as a point-of-return for pond-reared smolts has three important fish management considerations:

1. The stream has no existing anadromous fish runs, therefore any returning adults would not displace or become confused with natural runs, thereby avoiding possible conflict with sport and commercial salmon fishermen.

2. Release of smolts into the stream would not produce any displacement of native fish ("residualization", Royal 1972), a situation which is causing increasing concern in Pacific coast states (Bakke 1975).

3. Decoy systems using morpholine (Cooper and Hassler 1974) could be employed easily to maximize homing success in planted smolts.

The quality of smolts produced from the system will determine the level of adult returns to a selected point-of-return. Part of this quality is proper imprinting, while another is release of disease-free fish (Fryer 1973). Vibrio-diseases have been typically severe in saltwater reared salmonids. Immunization techniques are currently under study as part of the Humboldt State Sea Grant salmonid-aquaculture program. A 4,000-gallon, four tank, recirculating fish holding system is partly completed for use in future programs of imprinting and immunization of pond-reared smolts.

The original concept of the Arcata system of a continuous mixing of Humboldt Bay saltwater and oxidation pond effluent is yet to be tested. Recently, however, the City of Arcata has authorized the addition of two 1-acre ponds to the system. By pumping effluent from pond to pond, and then the final effluent back into the oxidation pond, we hope eventually to produce a semi-continuous system, with the terminal unit with salinities approaching those in Humboldt Bay. This should allow incorporation of final effluent polishing with techniques employed by Huguenin and Ryther (1974).

Steelhead trout will be given much more attention in future work since it is a species which is a minor portion of the catch in saltwater by either sport or commercial fishermen. Thus conflict over ownership will be minimized and maximum return to a "fish farm" seems possible.

SITING RESTRAINTS ON HUMBOLDT BAY AQUACULTURE

Kildow and Huguenin (op. cit.) have thoroughly outlined the non-technical problems facing wastewater aquaculture in the United States. The establishment of an aquaculture venture in the Humboldt Bay region now involves consultation with at least eleven governmental agencies having jurisdictional responsibilities under law (Table 9). An enlightened political climate, a supporting bureaucracy, and an informed public will be needed to overcome opposition (rational, irrational, or from vested interests) to a technically feasible wastewater operation.

The political status of wastewater mariculture in California can be inferred from current thinking in coastal zone management. On November 7, 1972, the voters of California approved the Coastal Initiative (Proposition 20) to plan for the use and protection of the California coast. With passage of the proposition, the California Coastal Zone Conservation Commission, with six regional commissions, came into existence. These commissions were charged with developing recommendations for presentation to the Governor and Legislature by December, 1975. On March 6, 1975, A Preliminary Coastal Plan, Hearing Dreaft, was issued (Lane 1975). The concept of waste utilization in aquaculture in coastal waters is stated as follows:

> Some species can even thrive in heated waters in the vicinity of energy generating plants. Other species may be able to utilize nutrients from properly treated sewage discharges. p. 20

This is encouraging to wastewater aquaculture since the concept has now appeared in a public document prepared under stimulus of the vote of the entire population of the state. On the other hand, the recommended policy concerning evaluation of new aquaculture operations states:

> New or expanded aquaculture operations in coastal waters shall be permitted only if they would preserve, restore, or enhance areas for public use and would not displace productive natural habits. p. 23

It would appear that the number of potential sites meeting this criteria would be limited.

Further limitations on aquaculture ventures are readily inferred from the section on Coastal Water Quality. This section outlines the magnitude of the problem in maintaining coastal water quality in face of the tremendous percentage of the California population which lives adjacent to coastal areas and therefore discharges a major percentage of its wastewaters into coastal areas. The public momentum on the issue has been sufficiently strong to allow the California State Water Quality Control Board to adopt a policy of no waste discharges into California bays, estuaries, and lagoons, with some major exceptions such as San Francisco bay. The policy was applied in toto to Humboldt Bay by the North Coastal Regional Water Quality Control Board. The North Coastal Board recently adopted language, however, which indicated aquaculture discharges might be permissible if there was some "enhancement" to bay waters or local values. Presumably aquaculture water released along with fish into Humboldt Bay from an aquaculture enterprise might qualify, but the possibility will have to be tested as the regulations are so new they were not distributed publicly at the time of writing this paper. Such a strict imposition of a no-discharge policy may be reconsidered as indicated by language in the section on policies designed to phase out discharges to enclosed bays and estuaries:

> North Coast: additional study should be conducted before a total prohibition is imposed against further sewage discharge in Humboldt and perhaps other bays in the North Coast Region. p. 26

The State of California has had a long history of permissive legislative statements on wastewater reclamation, and similar general language appears in the coastal zone draft as follows:

> 7. Stress Reclamation of Waste Water. Reclamation and reuse of adequately treated waste water (for agricultural, industrial, recreational, fish and wildlife, or domestic use) should be fully considered as a preferred alternative to discharge into coastal waters for, or as a desirable component of, all water and waste water management programs. Projects incorporating reclamation should be given funding priority over those that do not. p. 27

Although reuse is mentioned frequently, the legislative intent remains permissive. It requires strong citizen action to develop and promote innovative programs, as for example the agricultural use of wastewater as part of open space programs to counter urban sprawl (Marin and Sonoma Counties, California) (People for Open Space, 1975).

One of the major public issues leading to the passage of the Coastal Initiative was concern over the degree of destruction of estuarine wetlands by uncontrolled growth (75 percent in southern California, 50 percent in San Francisco bay area; in Humboldt Bay 90 percent of intertidal marshes have been converted to other uses). Thus the draft's policy recommendations are for strong protection of existing wetlands, and for restoration of degraded wetlands (p. 40). It is precisely these degraded areas that have the greatest potential for mariculture, with or without wastewater fertilization (Hepher and Schroeder 1974). Thus aquaculture in California coastal areas will have to be evaluated against other potential uses of the same site.

It seems realistic to conclude that due to the equivocal language of the draft, should it be accepted by the legislature, establishing aquaculture ventures in California will involve a great deal of astute politics.

QUAIL CREEK RESULTS

The description and operation of the Quail Creek lagoon system operated under polyculture with fish is described by Coleman et al (1974). The most important wastewater parameter under study in the system is that of Suspended Solids, since the effluent from lagoons generally have values for this parameter in excess of federal standards due primarily to algal cells. The use of herbivorous and other fishes is one method of reducing algal concentrations. The Quail Creek lagoon system operated with fishes drastically reduced Suspended Solids, as well as eleven other parameters studied (see Table 1 in Coleman et al, op cit).

To further document the effects of fish on the system, a study is currently underway monitoring the final cell of the lagoon system in order to compare water quality of the system obtained without fishes to that when the system contained fishes. Five parameters have been monitored in the final cell of the system operated without fish (Table 10). These values may be compared with values found for incoming raw sewage established in 1973, and with current federally defined secondary treatment levels set for nationwide implementation in 1977 (Table 10).

The Quail Creek data demonstrates that extended lagooning in conjunction with aquaculture is more than capable of meeting the existing federal secondary treatment definition. Without aquaculture the Quail Creek system did not consistently meet secondary treatment standards. In addition to the reduction in nutrient levels obtained in extended lagooning, the addition of aquaculture to the system is capable of reducing nutrient content to levels normally achievable only through physical-chemical means.

DISCUSSION AND SUMMARY

Mortimer and Hickling (op cit) wrote of:

> the paradox in the present practice of allowing large quantities of nurients to flow to waste in sewage effluents, while at the same time agriculturists must buy fertilizers to maintain and increase production, ...

Municipal lagoons are efficient when not overloaded in reducing human pathogens (Carpenter et al 1974), and have the capacity of meeting federal water quality standards for this parameter. A major benefit of such an effluent would be elimination of chlorinated hydrocarbons from chlorination processed or not having to install costly dechlorination units.

The most difficult effluent standards that the lagoon method of treatment faces is that of suspended solids. This parameter does not now, however, distinguish between the type of particulate matter in the incoming raw sewage and that found in lagoon effluents. The latter includes mainly algal cells, which may actually be beneficial to aquatic life in a receiving water. The culture of fish in reducing algal cells thus can make municipal wastewater lagoons one of the most efficient and least costly methods for meeting increasingly stringent federal standards in processing domestic wastewaters.

Although federal law and California state law have language that makes methods of wastewater reclamation in agriculture or aquaculture eligible for financial support, the degree of planning, authorizing and supporting of such systems by wastewater authorities is rather slim. Part of this reluctance stems from long familiarity with such advanced treatment facilities as physical-chemical methods, and from with only limited data in the sanitary engineering literature on advanced biological treatment.

Environmental concern for clean public waters is forcing a continuous improvement in the level of treatment of all types of domestic and industrial wastes. Consequently, the availability of such water suitable for many types of reuse is increasing. Aquaculture is seriously lagging in demonstrating the utility of wastewater for fish and fish-food production, as well as the utility of such systems for water purification. Aquaculture must disseminate more effectively the public and private benefit available in the "water plus fertilizer" resource represented by domestic sewage or lose the resource to other more established and progressive wastewater reclamation industries.

The design, operation, and study of wastewater aquaculture systems for fish production will involve interdisciplinary teams to obtain economically viable operations. Such disciplines as fish culture (Prowse 1966, Ryther et al 1972) will be combined with sanitary engineering pond technology (Dornbusch et al 1971), to undertake proposals such as that suggested for Hungary (Donaszy 1974), and in operation in Munich, Germany for years (Allen op cit). The normal variability to be expected from fish-pond production (Buck et al 1970) will have to be established for low-technology systems, such as the Arcata system, as the reliability of the system will determine the degree of financial support for any proposed production units.

With rising water quality standards, the standards of operation in wastewater aquaculture schemes, either for the primary goal of wastewater treatment or fish for human consumption, will be higher than current economically feasible treatment systems. Thus a long-term commitment to support pilot and demonstration projects will be required to develop comparative operational data for comparison with standard acceptable techniques which sanitary engineers, public health and water quality control authorities, and elected public officials support as a matter of prudence.

Highlights of the current successes and limitations in the two municipal wastewater lagoon systems producing fish as discussed in this paper are shown in Table 11. In a real sense, these research efforts are attempting to apply in the public realm what innovative new organizations such as the New Alchemy Institute (Wade 1975) is promoting for private organizations.

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TABLE 1

CHARACTERISTICS OF TWO EXPERIMENTAL MUNICIPAL WASTEWATER LAGOON SYSTEMS REARING FISH AS DESCRIBED IN TEXT.

	Name and Location	
Factor	Quail Creek, Oklahoma City, Oklahoma	Arcata, Humboldt Bay north coastal California
Local Climate	Cool winters, hot and dry summers.	Cool marine climate all seasons, wet winters.
Local Community	Suburban population; no industry.	Small town with university; small number lumber and plywood plants main industry.
Population served by sewage system	10,000	11,000, plus university with 7,000 enrollment.
Number and arrangement of Lagoons	Six Ponds in series.	Two ponds inside 55-acre ponds acting as terminal unit in advanced secondary treatment system.
Average Daily Flow	1 MG/Day.	1-2 MG/Day.
Total Acreage in Fish Production	25	0.7
Primary Objective of System	Wastewater Treatment.	Production of trout and salmon smolts.
Species Cultured	Channel catfish, golden shiners, fathead minnows, Tilapia, black bullhead green sunfish, mosquito fish.	Chinook and coho salmon, steelhead and cutthroat trout, plus variety of marine species introduced as eggs or larvae.
Use of Product	Provide advanced biological wastewater treatment.	Smolts released to sea for catch as feeding adults in ocean by sport and commercial fishermen; possible future sale of adults captured on return to point of release.
Major Technology	Hinde "Air-Aqua" system of forced air aeration in two fish ponds.	Several systems of pond mixing; forced-air aeration system of local design and fabrication preferred.
Sponsoring Agencies	Oklahoma State Dept. of Health.	Fisheries Dept., Humboldt State University; City of Arcata; NOAA Sea Grant Program; Calif. Dept. Fish and Game.

TABLE 2

SUMMARY OF REARING EXPERIMENTS IN ARCATA FISH PONDS WITH JUVENILE PACIFIC SALMON AND TROUT USING HUMBOLDT BAY SEAWATER MIXED WITH WASTEWATER FROM OXIDATION POND

Experiment No.1/	Rearing Period	Species	Size of Fish	Days of Rearing	Aeration Mixing System	Salinity (parts per thousand)
VII	25 Jan - 7 May 74	Chinook	Fry	101	Air 2/ Spray 3/	10
VIII	3 Jul - 20 Sep 74	Chinook Coho Steelhead Cutthroat	Fingerling Yearling Fry Yearling	79	Spray 3/	15-23
IX	23 Oct 74 - 11 Jan 75	Coho Steelhead	Fingerling Fingerling	79	Air 4/	11-17
X	4 Feb - 10 May 75	Chinook Steelhead	Fry Yearlings	85 "	Air 4/ "	6-10 "

1/ Experiments I - VI summarized in Allen and Dennis (1974), Table 2, p. 178.

2/ North Pond

3/ South Pond

4/ North and South Ponds

TABLE 3

SUMMARY OF SURVIVAL OF SALMONIDS REARED IN SEA WATER FERTILIZED WITH SECONDARILY-TREATED DOMESTIC WASTE-WATERS, JULY 1971 - JANUARY 1975

A. Chinook Salmon, Fry to Smolt

Exp. No.	Period of Rearing	Days of Rearing	Percent Survival of Salmon Planted	Remarks
II	Spring 1972	45-111	3	North Pond
V	Spring 1973	30-119	1-6 0.1-2	North Pond South Pond
VII	Spring 1974	90 90	16 27	North Pond South Pond
VIII	Summer 1974	79	58	South Pond (Fingerlings)
X	Spring 1975	85	Trace	North & South Ponds

B. Coho Salmon, Fingerlings

Exp. No.	Period of Rearing	Days of Rearing	Percent Survival of Salmon Planted	Remarks
IA	Summer 1971	-	None	North Pond. Unstable water conditions.
IB	Winter 1971-72	45	55	North Pond. 25% of loss due to birds.
III	Summer 1972	45	4	North Pond. Out-competed by marine species.
IVA	October 1972	-	None	North Pond. One period unstable water conditions.
IVB	November 1972	14	84	North Pond. Experiment primarily to assist bird predation study.

TABLE 3 (CONT'D)

B. Coho Salmon, Fingerlings (Cont'd)

Exp. No.	Period of Rearing	Days of Rearing	Percent Survival of Salmon Planted	Remarks
VI	Fall 1973	43	96	North Pond
		43	78	South Pond
VIII	Summer 1974	79	64	South Pond Yearlings
IX	Fall 1974 - Winter 1975	82	56	North Pond
		82	85	South Pond

C. Trout - South Pond

Exp. No.	Species	Period of Rearing	Days of Rearing	Percent Surv. of Trout Reared	Remarks
VII	Cutthroat	Spring 1974	92	100	Yearlings, pen-reared.
VIII	Cutthroat	Summer 1974	79	17	Yearlings, pond-reared.
	"	"	83	50	Yearlings, pen-reared.
VIII	Steelhead	Summer 1974	79	26	Fry, pen-reared.
IX	Steelhead	Fall 1974-Winter 1975	82	55	Fingerlings, North Pond.
			82	63	Fingerlings, South Pond.
X	Steelhead	Spring 1975	85	66	South Pond, yearlings, pen-reared.

TABLE 4

SURVIVAL OF CHINOOK FRY AND AVERAGE GROWTH IN WEIGHT, EXPERIMENT VII, JANUARY-MAY, 1974

	North Pond			South Pond		
	Start	End	Percent Surv.	Start	End	Percent Surv.
Number	15,000	2,400	16	15,000	4,000	27
Weight in grams:						
25 Jan. plant	0.5	3.6		0.5	4.3	
15 Feb. plant	0.7			0.7		

TABLE 5

SURVIVAL OF CHINOOK FRY REARED IN 2-METER SQUARE PENS OVER FOUR SUBSTRATE TYPES, EXPERIMENT VII, FEBRUARY-MAY, 1974

	North Pond			South Pond		
Pen Site	Start	End	Percent Surv.	Start	End	Percent Surv.
Hookton Soil	10	- 1/	-	30	8	26
Mud	70	20	28	90	69	77
Oyster Shell	50	20	40	50	28	56
Gravel-Sand	50	32	64	50	47	94

1/ Hole in webbing; fish escaped.

TABLE 6

COMPARISON OF SURVIVAL, GROWTH, AND PRODUCTION OF SALMON FLESH IN PENNED AND UNPENNED FISH, EXPERIMENT VII, JANUARY-MAY, 1974

		Average Size Attains		Production (kilo/ha/day)	
Rearing Area	Percent Survival	Weight (grams)	Length (fk. ln. mm)	Based on Survivors	Change in Biomass
Mud Pen, North Pond	13	2.4	57	0.5	Loss
Shell Pen, N. Pond	12	4.5	70	0.7	Loss
North Pond	16	3.4	66	0.5	Loss
South Pond	27	4.1	68	1.1	0.6 gain
Seven Pens Combined	47	3.7	63	2.5	1.8 gain
Mud Pen, South Pond	77	3.4	62	5.0	5.0 gain

TABLE 7

COMPARISON OF SURVIVALS IN CHINOOK SALMON FRY REARED IN PONDS WITH FRY REARED IN 4-SQUARE METER PENS FIXED ONTO POND SUBSTRATES, 1973-1975

Experiment Number	Year	North Pond		South Pond	
		Pond	Pens 1/	Pond	Pens 1/
V	1973	1-6	44 (2)	0.1-2	18 (1)
VII	1974	16	27 (3)	27	64 (4)
X	1975	0	1 (4)	.01	13 (3)
		0	26 (6) 2/	.01	34 (3) 3/

1/ Number of pens shown in parentheses.

2/ Six, 6'-square fixed pens fitted into northwest corner of North Pond; two pens each fitted with oyster shell, gravel, and bay mud bottoms.

3/ Three, 4'-square movable pens. Two pens without reefs: 18% survival; one pen with six underwater reefs: 67% survival.

TABLE 8

TOTAL BACTERIAL AND TOTAL COLIFORM COUNTS PER 100 ML IN WATER AND MUD[1] FROM ARCATA OXIDATION POND, AND NORTH AND SOUTH FISH PONDS, MAY 7, 1974

Location and Factor	Total Bacteria[2]	Total Coliform[3] MPN	95% levels confidence Upper	Lower
North Pond				
Water	1.39×10^1	33	11	93
Mud	7.00×10^1	1609	640	5800
South Pond				
Water	4.25×10^0	5	0.5	13
Mud	1.79×10^1	46	16	120
Oxidation Pond				
Water	5.00×10^3	1609	640	5800
Mud	6.00×10^3	1609	640	5800

1/ Small core of mud 3" in depth mixed with 1-½ gallons water and sampled.

2/ Pour plate method on TSA agar with serial dilutions of samples in sterile 0.5% tryptone water.

3/ Procedure in Standard Methods utilizing the most accurate and sensitive 5/10 - 5/1 - 5/0.1 dilution series (Table 37), with cultures incubated at 37°C.

TABLE 9

PARTIAL LIST OF GOVERNMENTAL AGENCIES WITH JURISDICTION OVER THE SITING OF A POTENTIAL WASTEWATER AQUACULTURE OPERATION IN THE HUMBOLDT BAY REGION, NORTHERN CALIFORNIA

Level of Governmental Agency		Name of Agency	General Area of Jurisdiction
U. S. Government	1.	Environmental Protection Agency	Water quality coastal and navigable waters
	2.	Food & Drug Administration	Public health
	3.	Army Corps of Engineers	Coastal zone land and water administration.
State of California	4.	Regional Water Quality Control Board	Water quality
	5.	Dept. of Fish & Game	Regulation of commercial fishing and aquaculture
	6.	Regional Coastal Zone Conservation Commission	Coastal zone planning and management
	7.	State Land Commission	Ownership tidal lands
Humboldt County	8.	Humboldt Bay Harbor, Conservation and Recreation District	Humboldt Bay planning, development and conservation
Appropriate local government depending on specific site	9.	Planning Commission	General land use
	10.	Environmental Review Board	Conservation
	11.	County Board of Supervisors, or City Council	Elected local authority with final authority

TABLE 10

MEAN VALUE OF SELECTED WATER QUALITY PARAMETERS IN TERMINAL UNIT OF SIX-LAGOON WASTEWATER TREATMENT FACILITY, QUAIL CREEK, OKLAHOMA CONTAINING FISHES COMPARED TO SYSTEM OPERATING WITHOUT FISH

Parameters 1/	Raw Sewage	Terminal Unit: With Fish 2/	Terminal Unit: Without Fish	Current EPA Standards 3/
Biochemical Oxygen Demand (5 day)	164	6	21	30
Suspended Solids	197	12	37	30
Total Nitrogen (as N)	19	3	10	
Total Phosphorus (as P)	9	2	8	
pH (standard units)	7.3	8.3	7.9	
Fecal Coliforms/100 ml	3×10^6	20	25	200
Sampling Schedule	Weekly	Weekly	Biweekly	
Sampling Period	June - October 73	June - October 73	July 74 - April 75	
Number of Samples	17	17	13	

1/ Values mg/l except where noted.

2/ Data from Coleman et al 1974, Table 1.

3/ Secondary effluent standards as objectives to be attained by United States wastewater treatment facilities by July 1977. Effluents not to contain in excess of stated values.

TABLE 11

COMPARISON OF MAJOR LIMITATIONS AND SUCCESSES IN MUNICIPAL WASTEWATER SYSTEMS EMPLOYING FISH CULTURE, QUAIL CREEK, OKLAHOMA AND ARCATA, CALIFORNIA

Factor	Quail Creek, Oklahoma City, Oklahoma	Arcata, California
Limitations to Date	System requires cold-adapted species for winter operation. Direct use of fish for human consumption not certified as yet. Correct stocking density to obtain consistent water quality treatment, a major design parameter, not yet defined. Scientific literature has little supporting information on human pathogens in fish.	Consistent operation of system for acceptable levels of survival of initial target species (fall chinook salmon) not yet achieved. Lack of flow-through operation to Humboldt Bay prohibited, thus point-of-return for adults yet to be established.
Successes to Date	System with fish polyculture has met defined EPA standards for secondary treatment. No major evidence of human pathogens in fish.	Four species of salmonids have been successfully cultured. Evidence on fish mortalities due to pathogens slight. High calcium-saltwater solution gives fish protection. Successful rearing of salmonids in system at elevated temperatures previously believed unsuitable.

Figure 1-A. North fish pond, located inside oxidation pond, City of Arcata, Humboldt County, northern California, showing modified pond banks with adjacent aeration pipes, underwater shell and brush reefs, and pens over four pond substrates. Future facultative pond in Arcata wastewater system in background.

Figure 1-B. South fish pond, located inside oxidation pond, City of Arcata, Humbold County, northern California, showing spray-type aeration (AIR-O-LATOR), fixed pens over substrates, with pens for crab rearing in center foreground and movable pens in left foreground. Authorized site for additional fish ponds in oxidation pond in background.

Figure 2. Salinities, North and South ponds, fall chinook rearing (Experiment VII), February-May, 1974.

Figure 3. Morning surface water temperatures, North pond, fall chinook rearing (Experiment VII), February-May, 1974. (South pond temperatures essentially same as North pond not shown.)

Figure 4. Water clarity, North and South ponds, fall chinook rearing (Experiment VII), February-May, 1974.

Figure 5. Morning surface water oxygen content, North and South ponds, fall chinook rearing (Experiment VII) February-May, 1974.

Figure 6. Hydrogen ion concentration (pH) surface waters, North and South ponds, fall chinook rearing (Experiment VII), February-May, 1974.

Figure 7. Catch of salmon in 1-meter long, cylindrical, single-funnel stationary trap fished adjacent to headgate, North and South ponds, fall chinook rearing (Experiment VII), February-May, 1974.

Figure 8. Cumulative mortalities from 25 chinook salmon fry held in 1-meter square floating pens located near headgates, North and South ponds, February-May, 1975 (Experiment X).

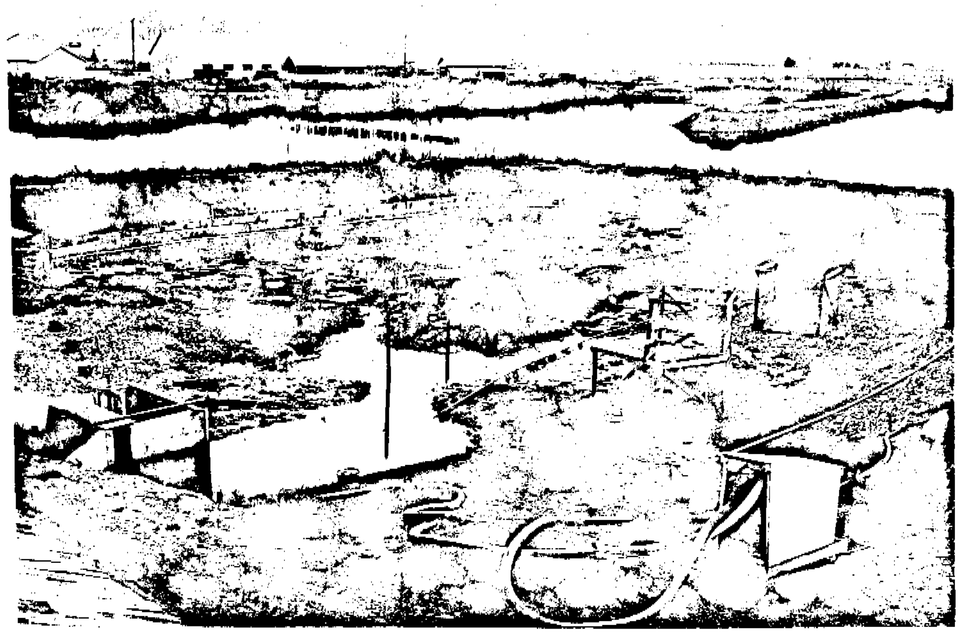


Figure 1-A North Pond

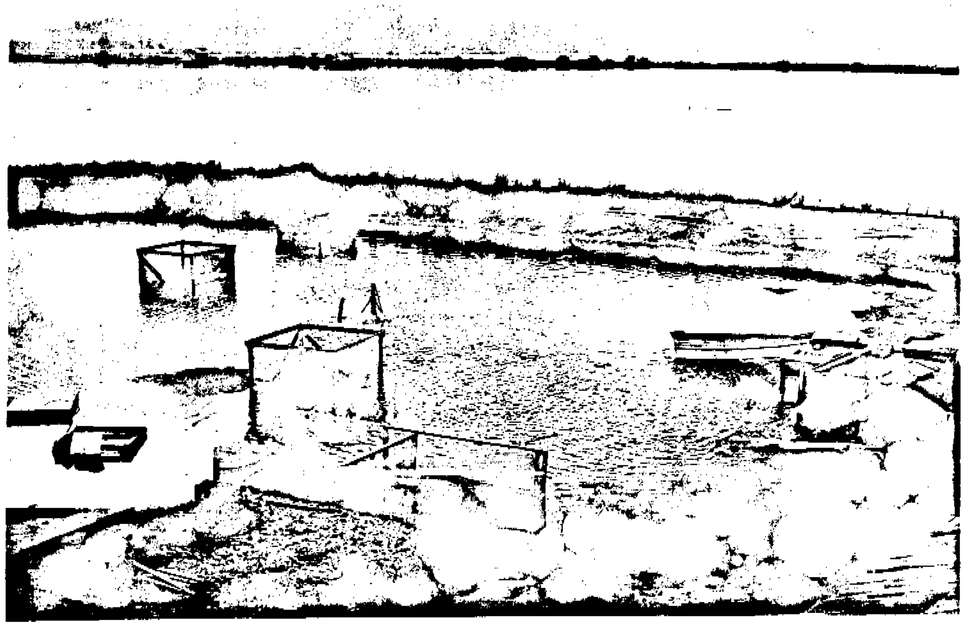
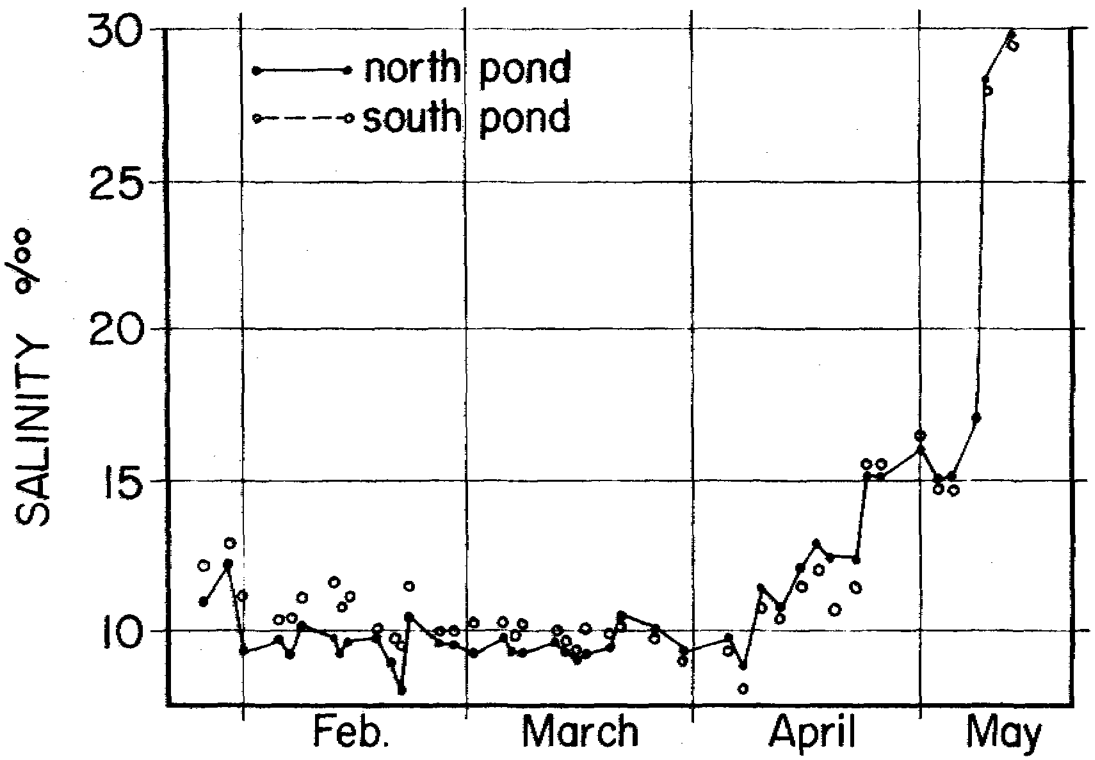


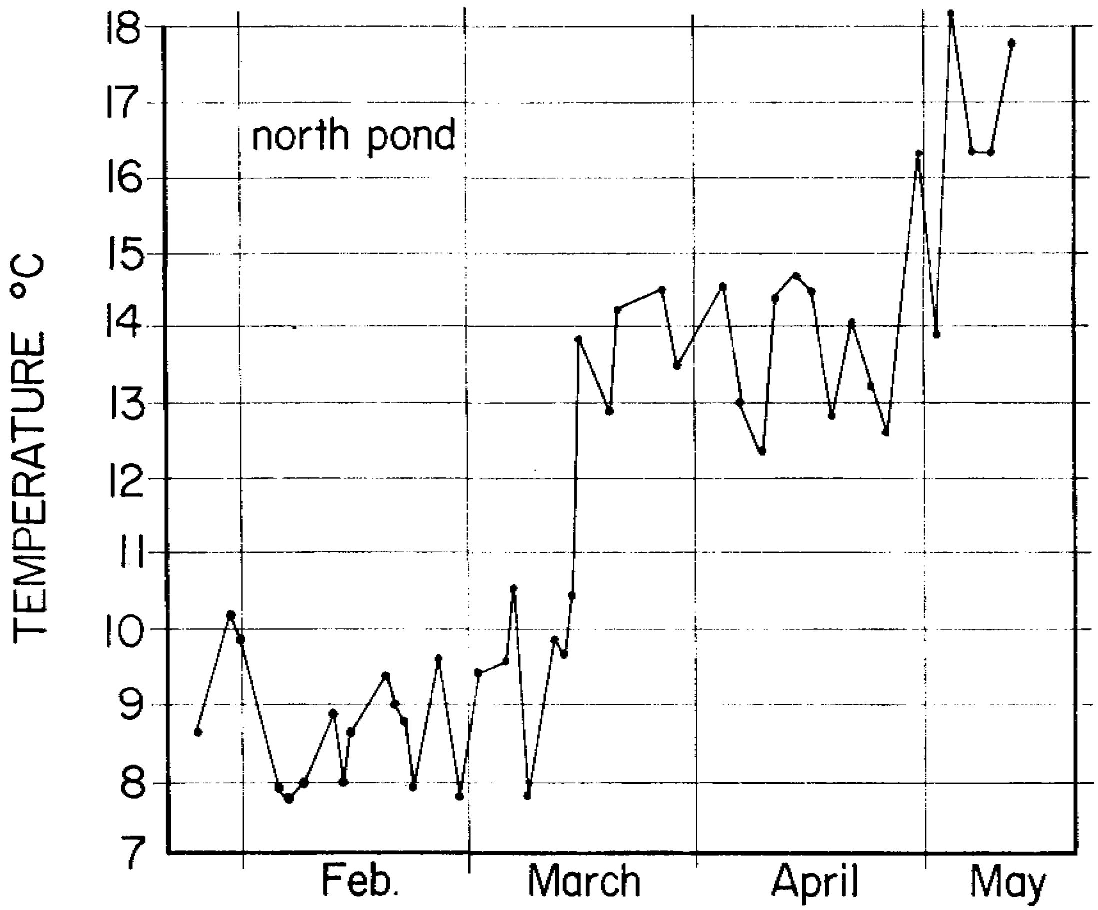
Figure 1-B South Pond

Figure 2



north pond
south pond
30
25
20
15
10
SALINITY ‰
Feb.
March
April
May

Figure 3



TEMPERATURE °C
18
17
16
15
14
13
12
11
10
9
8
7
north pond
Feb.
March
April
May

Figure 4

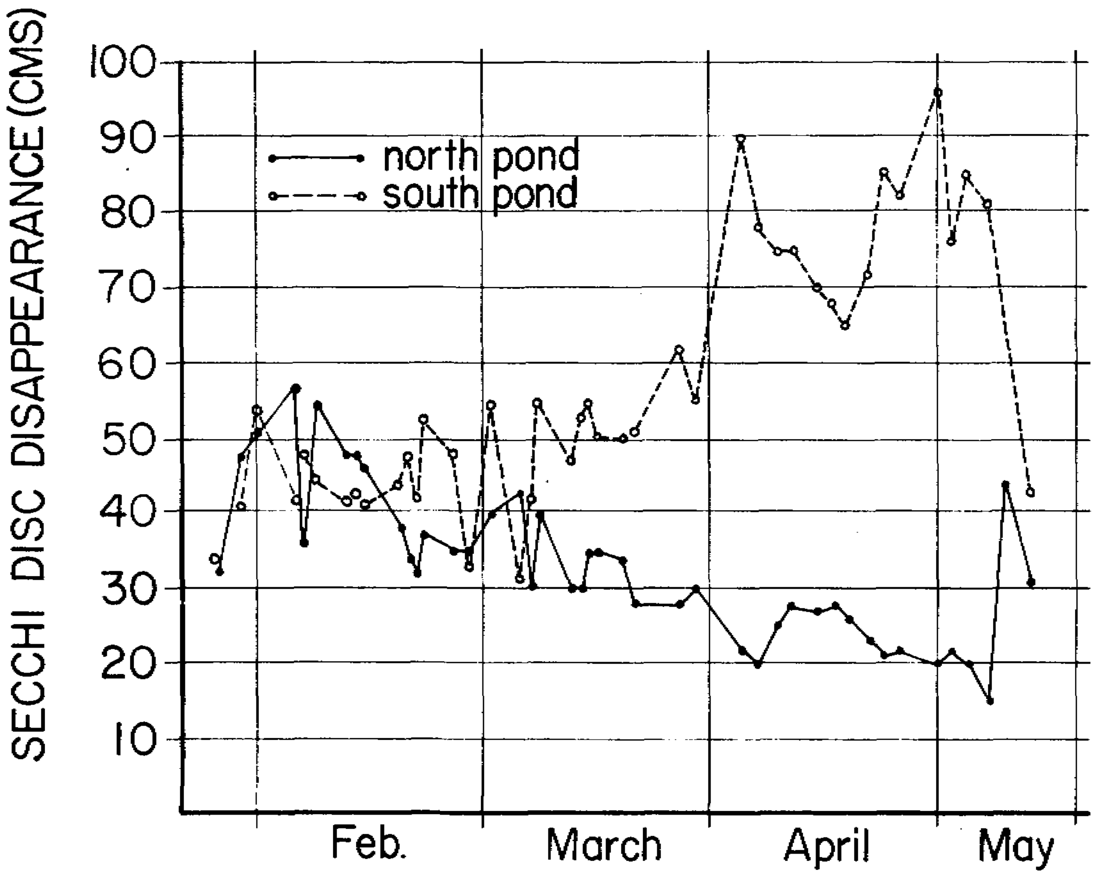
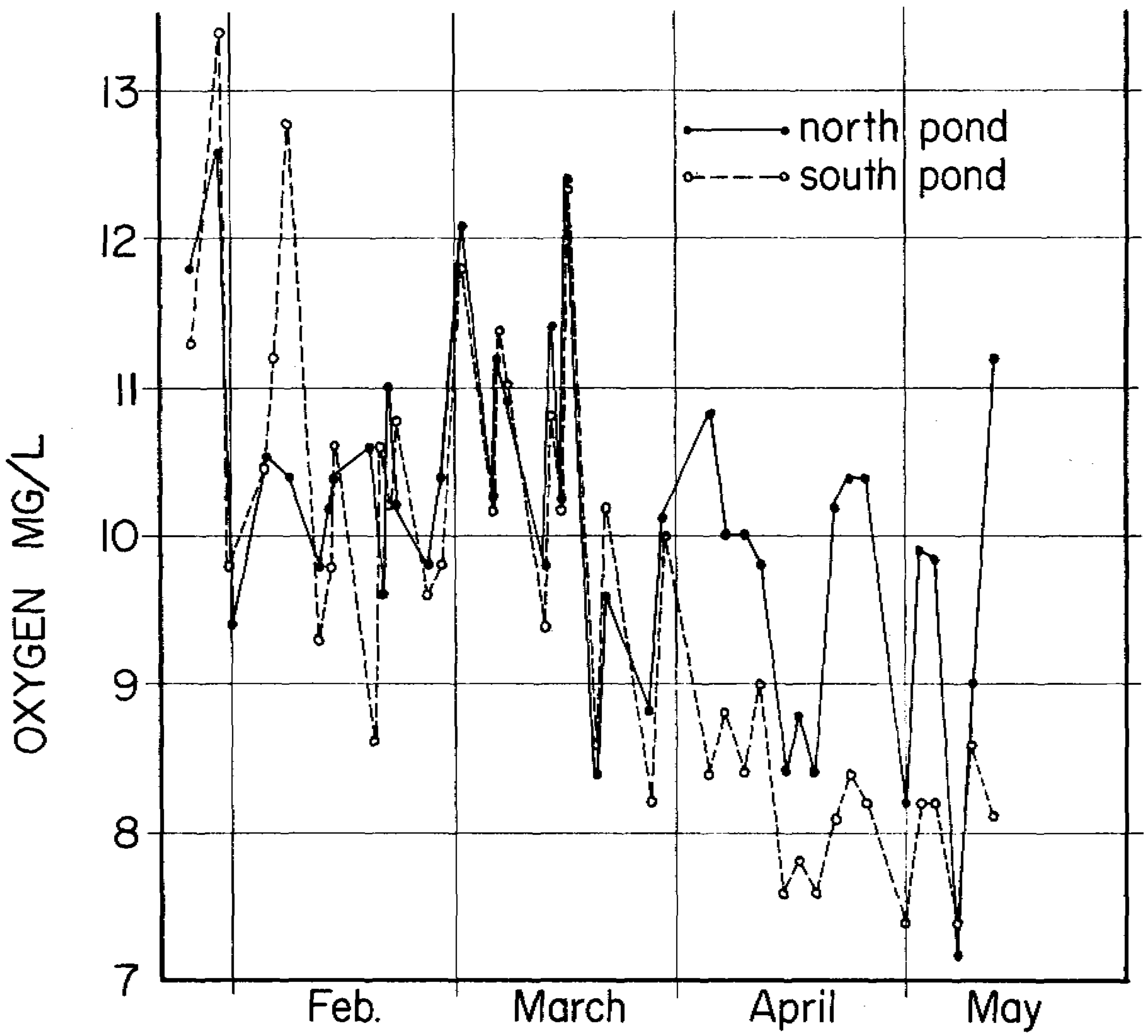
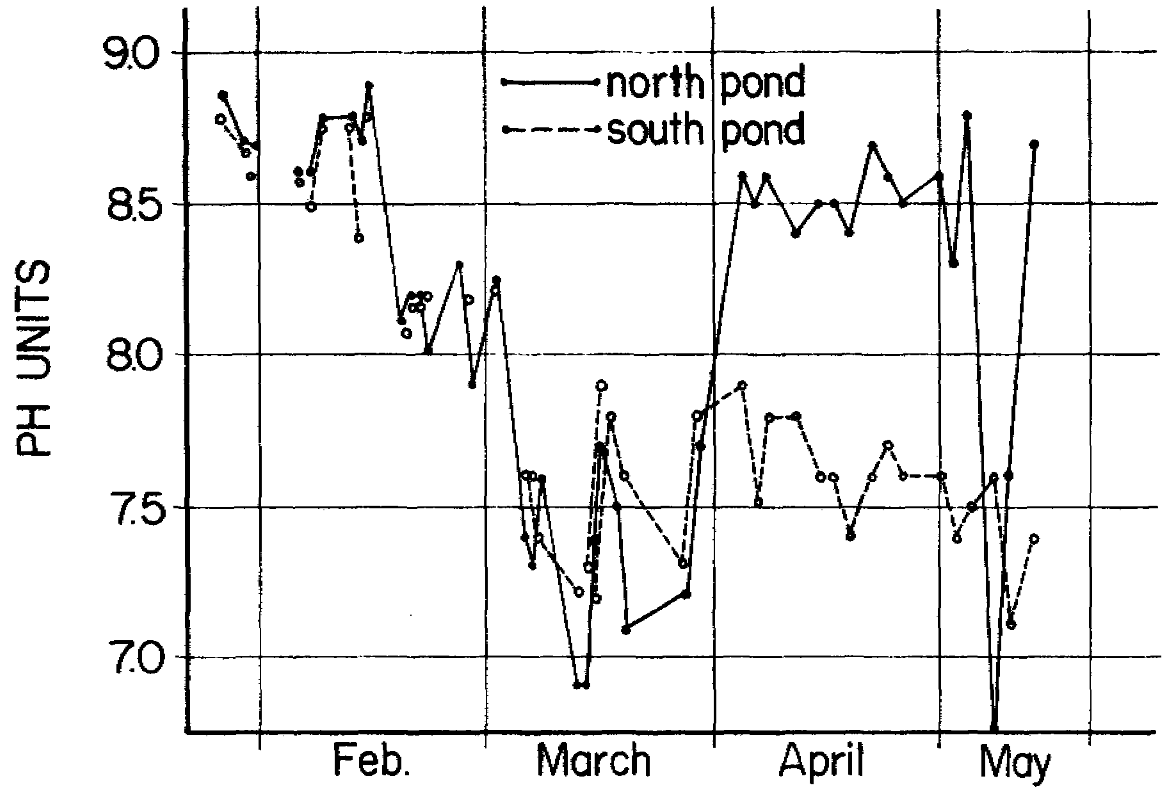

SECCHI DISC DISAPPEARANCE (CMS)
100
90
80
70
60
50
40
30
20
10
north pond
south pond
Feb.
March
April
May

Figure 5



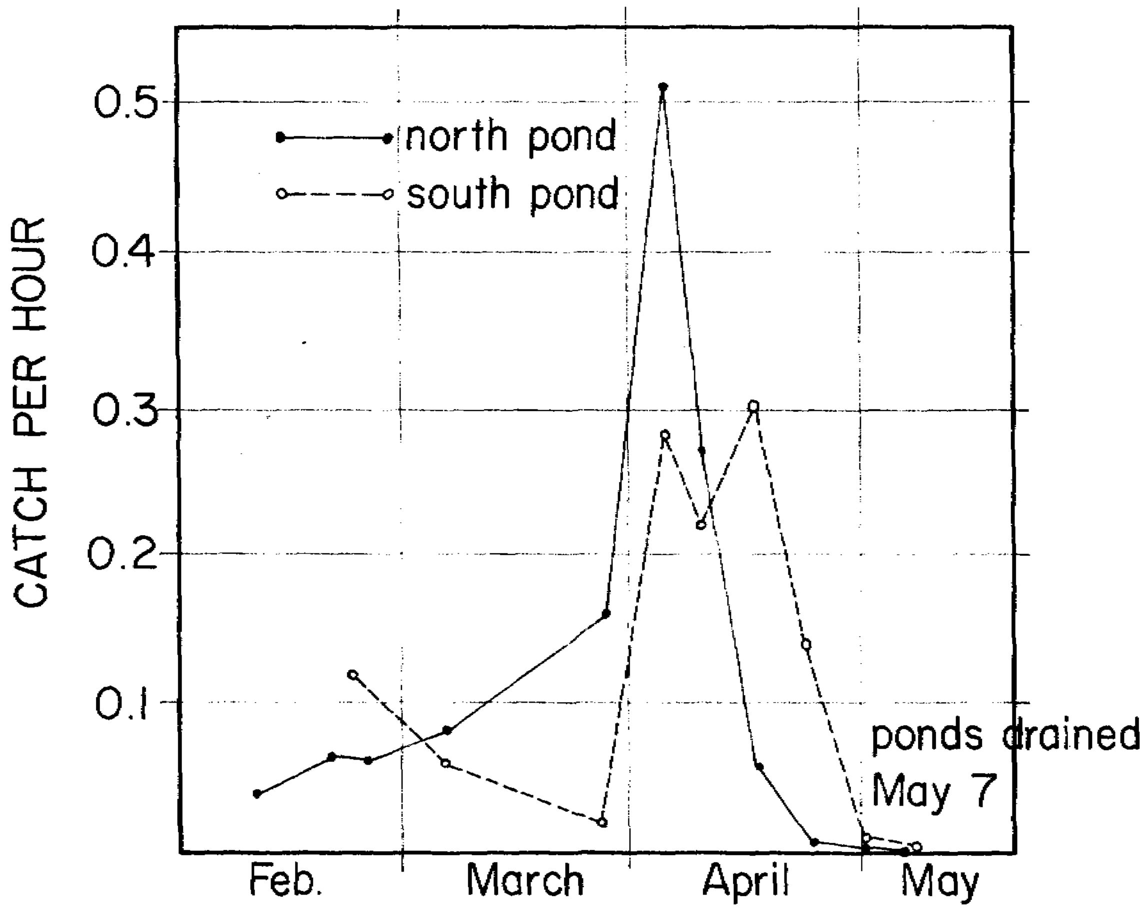
north pond
south pond
OXYGEN MG/L
13
12
11
10
9
8
7
Feb.
March
April
May

Figure 6



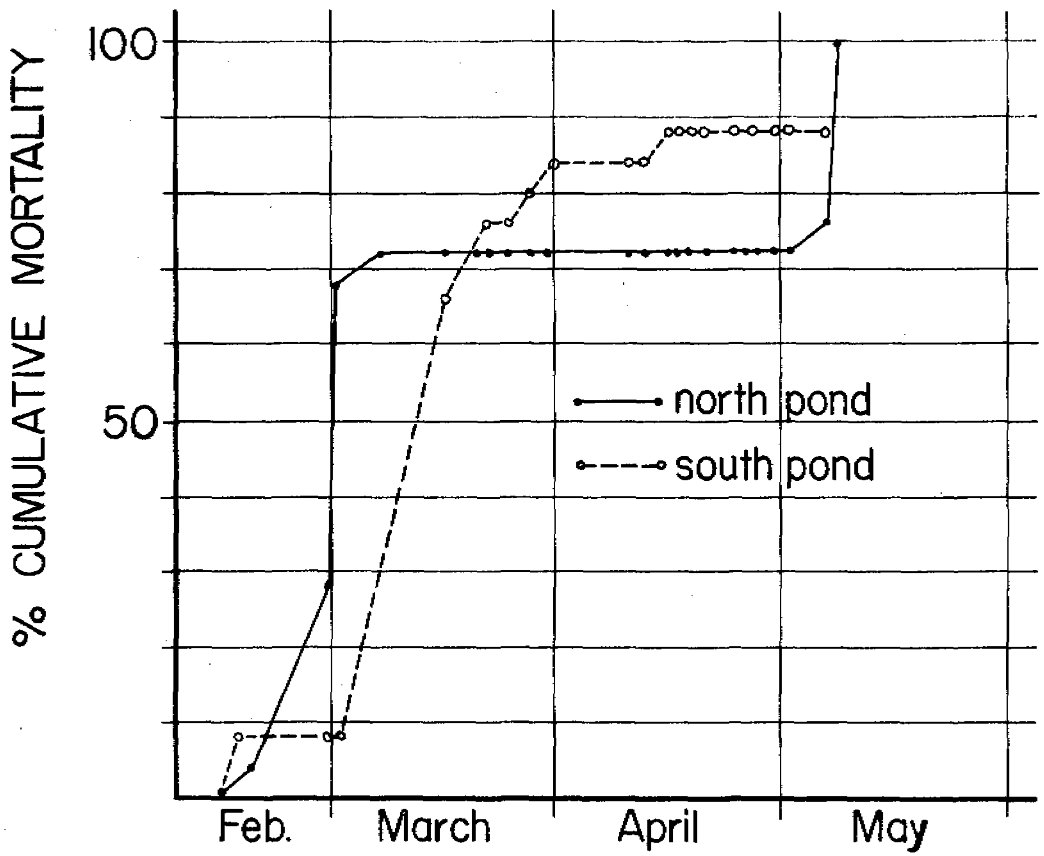
9.0
8.5
8.0
7.5
7.0
PH UNITS
north pond
south pond
Feb.
March
April
May

Figure 7



north pond
south pond
CATCH PER HOUR
0.5
0.4
0.3
0.2
0.1
ponds drained
May 7
Feb.
March
April
May

Figure 8


100
50
% CUMULATIVE MORTALITY
north pond
south pond
Feb.
March
April
May

RECYCLING TREATED SEWAGE THROUGH CYPRESS WETLANDS IN FLORIDA

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*Studies supported by Rockefeller Foundation and RANN Division of the National Science Foundation through a joint project - Cypress Wetlands for Water Management, Recycling, and Conservation.

A natural water management system exists in Florida: its cypress wetlands catch and hold excessive rains, letting them percolate slowly to the ground water, thereby exerting water quality control. The needles drop in the dry season and the dense trunk biomass shades the waters, reducing water loss during this critical time. A test of the feasibility of recycling treated sewage through cypress wetlands in its second year in Gainesville, Florida (see Fig. 1). Wastewater from a secondary sewage treatment plant at a trailer park is being routed into two of the cypress domes which are found in large numbers in many of the counties of Florida (40,000 in some counties). A dome is a roughly circular cypress swamp 1-25 acres in size occupying a saucer-shaped depression which receives water from the surrounding higher ground. The trees are tallest in the center and shorter at the sides like an inverted bowl, or dome.

One of the domes receiving waste was severely burned prior to flow and one is typical of the wet ponds that have only slight effect of fire. Normal acid water ponds are being used as controls. Another burned cypress dome is receiving hard ground water, a condition found in many disturbed areas in South Florida.

The burned, unburned, soft water, hardwater, and wastewater variations represent major classes of conditions in Florida. Phases of research are measuring water chemical uptake of nutrients, heavy metals, organic matter and microbes; tree growth, ecological communities, microclimate, mosquitoes, and economic potentials in stimulating land productivity and use in lieu of tertiary treatment.

Figure 2 is an aerial photograph of the experimental area six years prior to the study. The dark isolated areas are cypress domes; the light areas are cutover pine plantations. This degree of interspersion of the two ecosystem types is very typical of the north Florida landscape. The current layout of the experimental areas is outlined in Figure 3, which shows the location of the three domes affected by fire. The fourth is about ten miles from this site.

The geologic strata were determined in thesis work by Cutright (1974), and are shown in Figure 4. His studies showed the movement of superficial ground water through the sands beneath the dome. During the winter dry season, the ponds of the cypress domes are perched several feet above the ground water; in the summer rainy season, the ground water contacts the lower surface of the dome. The dome has some of the properties of a giant pressure filter. During the summer, it receives about an inch per week in rainfall and two inches in runoff. The experimental domes receive an additional inch per week of wastewater or groundwater. One inch per week is estimated to pass from the pool in the dome to the groundwater.

The diagram in Figure 5 shows the nutrient concentrations in the wastewaters flowing into the dome in the standing water, and in the groundwaters emerging from under the dome. Preliminary indications are that most of the nutrients are being taken out of the waters, although we don't know yet whether the nutrients are being routed from sediments to increased tree growth. Sediments below domes are calcareous and may be depositing phosphorus from percolating waters by differential precipitation as shown by Gilliland (1973).

Similar changes in coliform bacteria counts are shown in Figure 6. Concentrations for the various flows and for the standing water are given in Table 1. Some leakage around wells drilled into the first dome to receive sewage resulted in high bacterial concentrations in the ground water; no wells or other deep installations were put into the second dome, preventing this artificial leakage.

Cooperative studies by F.M. Wellings (1974) also showed leakage of viruses into the groundwater. So far none have appeared in the groundwater wells surrounding the unburned dome that has not been perforated.

The main features of the cypress dome ecosystem are shown in Figure 7. The energy circuit symbols used (Odum, 1971) imply specific mathematical relationships. The major features shown include the autotrophic compartments of cypress, pine, and understory. Nutrients, water, and organic peat are important in the sediments. Interactions with the outside include fire, logging, drainage, and nutrient loading.

The ecosystems with normal acid water are fairly diversified with some aquatic plant growth particularly when cypress leaves are still off in early spring. Dissolved is about one fourth of saturation. After fire the waters developed a floating algae and duckweed growth stimulated by the nutrients, but this decreased as fire-resistant cypress trees regained their leaves. The burned dome receiving treated sewage, however, developed a solid duckweed cover which reduced aeration causing near zero oxygen levels.

Nitrogen was depleted there presumably due to denitrification and duckweed growth.

Normal cypress domes are characterized by submerged aquatic vegetation, bladderwort (*Utricularia* sp.) in this case. Emergent vegetation such as lizard's tail (*Saururus cernuus*) and maidencane (*Panicum hemitomum*) are common in shallower areas. Most of the vegetation is characteristically clumped around the bases of the trees and knees; fetterbush (*Lyonia lucida*), Virginia willow (*Itea virginica*), and Virginia chain fern (*Woodwardia virginica*) are common species throughout the dome. The thick cover of duckweed included *Lemna purpusilla*, *Spirodella oligorhiza*, *Azolla caroliniana*. This growth soon disappeared in the dome receiving groundwater, but has persisted in the sewage dome and proliferated in the second dome receiving sewage almost as soon as flow began. The *Azolla* returned to the groundwater dome when a rookery of immature white ibis took up temporary residency. The duckweed have elevated nitrogen concentrations 5-6% - much higher than the other species of vegetation.

Baldcypress seedlings planted in all the domes grew more slowly at first in the sewage domes, but all seedlings showed a high survival rate, and in the second year the seedlings in the sewage dome are growing faster than those in the groundwater dome. Cypress seeds require drawdown to regenerate, so natural regeneration may have stopped in the three experimental areas except around the edges. New tupelo seedlings have been found growing in the sewage dome.

The intensity of the fire, increased by considerable draining in the area during the last few years, was so great that all the hardwoods and most of the pines in the two badly burned domes were killed. Over

95% of the cypress trees survived, however. Foliage put out in the spring following the fire was very abnormal, springing adventitiously from the trunk and hanging close to the trunks, opening up the canopy considerably. New limbs were red barked. The second year's growth has been normal, however, and many of the branches show the spread needles characteristic of baldcypress rather than pondcypress.

The organic cycle within the dome is shown in Figure 8. Measurements of litterfall and tree metabolism indicate that net primary productivity is greater than zero and that the trees are still growing. The high input from autochthonous organic material is due to the proliferation and deposition of duckweed. Cypress has a small leaf biomass per unit area although the photosynthesis and transpiration per area of leaf is normal. See rates in Figure 9 by S. Bayley and Fig. 10 by S. Cowles.

Mosquitofish, as well as several other species are commonly found in cypress domes. All the ponds are being seeded with a combination of common freshwater species. Self-design properties of the ecosystem may result in an interface ecosystem with organisms using all the resources; seeding will eliminate the delay caused by species having to gain access by natural means.

The duckweed cover is changing the insect population considerably, at present fostering moth and shore flies which are common in sewage areas. Many migratory songbirds were observed in the waste domes attracted by the flies. Birds are carriers of viral encephalitis. The mosquito species with disease potentials are listed in Table 2.

Surrounding the domes are slash pine seedlings planted by Owens-Illinois Inc., from whom the land is leased. One of the beneficial effects of maintaining domes wet during the dry season, as natural domes tend to be, may be in keeping surrounding forests damp, reducing the damage by fire and increasing growth of pine trees. Preliminary data gathered by P. Okorie on the effects of the increased water levels on soil moisture in the surrounding area are shown in Figure 11.

The possible interplay of sun, nutrients, water, fire, and harvesting over the long range was studies by W.J. Mitsch (1975) in computer simulation models which incorporated much of the information already discussed. The model simulated is in Figure 12 and samples of the graphs are given in Figures 13 - 15. Combination of cutting and draining so as to increase the effect of fire has a major effect in reducing productivity, whereas fire alone or cutting along (without draining) is not a major effect and may accelerate the dominance of the taller trees.

Field workers on the project have commented on the pleasant micro-climate within the dome; measurements are being made to determine the differences between radiation balance, temperature, saturation deficit and wind velocity, in the domes and surrounding pinelands (see Figure 16). Cypress lands are only half as expensive as uplands in Florida, and they are aesthetic attractions in many places. Savings in housing costs may be realized by building in or near cypress swamps. Architects associated with the project seized the need for an equipment shed as an opportunity to test the concept of building houses within domes. Houses have been built in Naples, Florida, with cypress swamps as yards. They are elevated on pilings or earth pads, and driveways are elevated as well. Drainage ditches are common, lowering the groundwater in the vicinity to some extent.

Another part of the project is monitoring cypress growth and nutrient relations in riverine and strand swamps where waters flow in from larger drainage areas and out again, more as in a river floodplain. Here the nutrients available per tree are believed to be more, water may be more regular in dry season, and faster growth per tree can be shown. Trees are of the bald cypress variety. See Fig. 17.

At the virgin Corkscrew Swamp site, Collier Co. Fla., Mike Duever as part of this project has documented the hydroperiod and ground water levels that go with cypress as contrasted with those that generate marshes, wet prairies and ponds without cypress. See Fig. 15 (Duever, Carlson, and Riopelle, 1974). The cypress is in the sites with the lower position and longer period of submergence.

At Wildwood, Fla. wastes from the town have passed into a floodplain swamp for 19 years. Recent analysis of these by Brown, Bayley, and Zoltek (1974) showed coliform reduction from 1.6 million to 300 MPN per 100 ml, phosphorus reduction from 7 ppm to 0.1 ppm and increased growth of the trees by a third.

A new energy investment ratio principle can also be used to estimate economic worth. The average purchased energy (in fossil fuel equivalents) that an area can attract depends on a free resident resource that supplements bought energy in generating a yield. It is the basis for attracting economic investment in the first place and the resident energy helps keep prices competitive. An activity that supplements bought energy with less than 1.0 kcal fossil fuel equivalent (2000 kcal solar energy) for every 2.5 kilocalorie bought (directly or indirectly from 2.5 kcal of fossil fuel work) will not be competitive.

Based on the nation's economy as a whole, 1 kcal fossil fuel equivalent (FFE) of sunlight on the average has been attracting 2.5 kcal FFE of fossil fuels to the resident economy. The ratio will change as the nation's energy intensiveness changes. Figure 19a shows a general diagram for the disposal of 2.8 million gallons per day of nutrient wastewater. The high energy quality of the nutrients gives that flow a value of 8.0×10^8 kcal FFE/year if it is matched by enough sunlight (intensity x area) to get a net increase in 8.0×10^8kcal FFE/yr from the natural system. This increase in resident natural energy flow can therefore theoretically attract 20.0×10^8 kcal FFE/yr from external sources. The model shows that the disposal of the 2.8 mgd of secondary sewage into a natural system has the capability of increasing the money flow in the local economy by $80,000/yr or approximately $0.08/1000 gallons of secondary sewage. This assumes no limitations of sunlight to the natural area.

In a cypress system disposal scheme (Figure 19b), a loading rate of one inch per week allows the water to percolate slowly through the soil to the aquifer, leaving nutrients behind. The nutrients increase the metabolism of the cypress trees over a large area, and the economic flow is amplified with the production of high quality cypress wood. The purchased energies attracted to this system include the work of harvesting, the work of finishing the wood, and possibly even the work of building a cypress wood house of some other manufactured goods. Here the free work of the sun, water, wind, and land have supplemented the purchased economy. Preliminary calculations show the investment ratio in this case to be 2.1.

A cost comparison of nutrient removal processes for domestic waste by Eliassen and Tchobanoglous (1969) found that for both nitrogen and phosphorus removal efficiencies of 80% or more the costs ranged from \$0.17 to \$1.00 per 1000 gallons treated. Table 3 gives the unit cost for cypress wetland disposal at \$.25 per 1000 gallons, but it should be noted that using a higher loading rate with the existing system would lead to a much smaller unit cost.

TABLE 1

Coliform Concentrations in Various Flows and in Cypress Dome Receiving Sewage

Sample	Total Coliforms, #/100 ml	Fecal Coliforms, #/100 ml
Rainfall	8.6×10^2	6.8×10^2
Stemflow	1.0×10^2	6.7×10^{-1}
Runoff to Dome	1.3×10^4	5.7×10^3
Sewage to Dome	6.1×10^5	2.9×10^5
Weir Flow from Dome	3.4×10^4	2.2×10^3
Groundwater Seepage	3.2×10^2	3.4
Standing Water	3.0×10^5	4.6×10^4

TABLE 2

MOSQUITOES CAUGHT IN RAMP TRAPS IN SEWAGE DOME (OVER 1% OF CATCH)

Species	% of Catch		Disease Vector
	April	July	
Aedes atlanticus	-	4.9	
Anopheles crucians	16.6	7.5	pest
Anopheles quadrimaculatus	-	-	malaria
Culex nigropalpus	-	4.3	encephalitis virus
Culex spp. (other)	43.2	13.5	virus
Culiseta melanura	16.6	53.0	encephalitis in birds
Mansonia perterbans	3.3	1.8	
Psorophora ferox	6.6	1.1	
Psorophora confinnis	-	1.8	
Uranotaenia sapphirina	3.3	16.4	
Uranotaenia lowii	-	1.1	

TABLE 3

COST ANALYSIS FOR CYPRESS WETLANDS DISPOSAL @ 25,000 GPD

Cost Items	Cost Data
Capital and Installation Cost	
Wetwell (6 ft. dia., 10 ft. deep)	$ 1,200
Pumps (2 ea., 1-1/2 hp.)	2,000
6" P.V.C. Pipe and Fittings (1500 ft.)	4,500
4" P.V.C. Pipe and Fittings (3100 ft.)	7,750
	$15,450
Annual Operating Cost	
Power @ 5¢/kwh	75
Maintenance	
Parts	300
Labor (1-1/2 hrs./week @ $10.75/hr.)	840
	$ 1,215
Total Unit Costs	
Annual Capital Cost	
Wetwell and Pump (20 yrs. @ 6%)	279
P.V.C. Pipe (40 yrs. @ 6%)	815
Annual Operating Cost	1,215
	$ 2,309
TOTAL UNIT COSTS $/1000 gal.	$ 0.25

Legends for the Figures

Fig. 1 Generalized profile of a cypress dome ecosystem showing surrounding pinelands and wastewater disposal scheme.

Fig. 2 Aerial view of cypress domes among planted pine before forest fire or sewage disposal.

Fig. 3 General site plan of cypress domes receiving treated sewage and groundwater.

Fig. 4 Idealized east-west cross-section through the cypress dome receiving sewage showing geological strata, test wells, and general groundwater flow.

Fig. 5 Nutrient concentrations of treated sewage, standing water, and groundwater of cypress dome receiving sewage. Little or no surface outflow is assumed at an application rate of one inch per week. Groundwater percolation values are from a well 3m directly below the dome while surface groundwater is from a shallow well 50 m downstream of the dome.

Fig. 6 Coliform budget (total and fecal) for cypress dome receiving treated sewage (Price, pers. comm).

Fig. 7 Energy flow diagram of major compartments of a cypress dome ecosystem including interactions of fire, tree logging, and drainage.

Fig. 8 Major organic storages and flows in the cypress dome receiving sewage. Flows are in g-organic matter/m^2-yr while storages are in g-organic matter/m^2.

Fig. 9 Productivity and transpiration of cypress leaves as measured by CO_2 gas metabolism unit. Data are from October 1973 (Bayley, Burns, and Burr, 1974).

Fig. 10 Productivity and transpiration of cypress leaves as measured by CO_2 gas metabolism unit. Data are from October 1974 (Cowles, 1974).

Fig. 11 Soil moisture as a function of distance from edge of cypress dome receiving sewage. Curves are for 3 depths in the soil (Okorie, pers. comm).

Fig. 12 Cypress dome model used for computer simulation.

Fig. 13 Simulation results for model in Fig. 11 for undisturbed conditions.

Fig. 14 Simulation results for model in Fig. 11 in which cypress harvesting occurs when cypress biomass reaches 15 kg/m^2.

Fig. 15 Simulation results for model in Fig. 11 where cypress harvesting and 10 year fire occur, the latter due to low water conditions.

Fig. 16 Microclimate differences between interior of an undisturbed cypress dome and the interior of the surrounding pineland in March, 1975. (Heimburg, pers. comm.).

Fig. 17 Relationship between cypress net productivity and tree density for three different cypress associations in the Withlacoochee State Forest in west-central Florida. Cypress-mixed hardwood associes generally indicate riverine or strand swamps.

Fig. 18 Relationships between seasonal water levels and ecosystem type found in the Big Cypress Swamp in Southwest Florida (Deuver, Carlson, and Riopelle, 1974).

Fig. 19 a) Theoretical investment ratio model showing energy flows to be expected (in kcal fossil fuel equivalents x 10^8/yr) for the disposal of 2.8 million gallons per day treated sewage into a natural system. b) Calculated natural and purchased energy flows applied to the disposal of 2.8 mgd treated sewage into cypress domes. For every natural calorie fossil fuel equivalent of increased productivity, 2.1 calories fossil fuel equivalent are purchased in this case.

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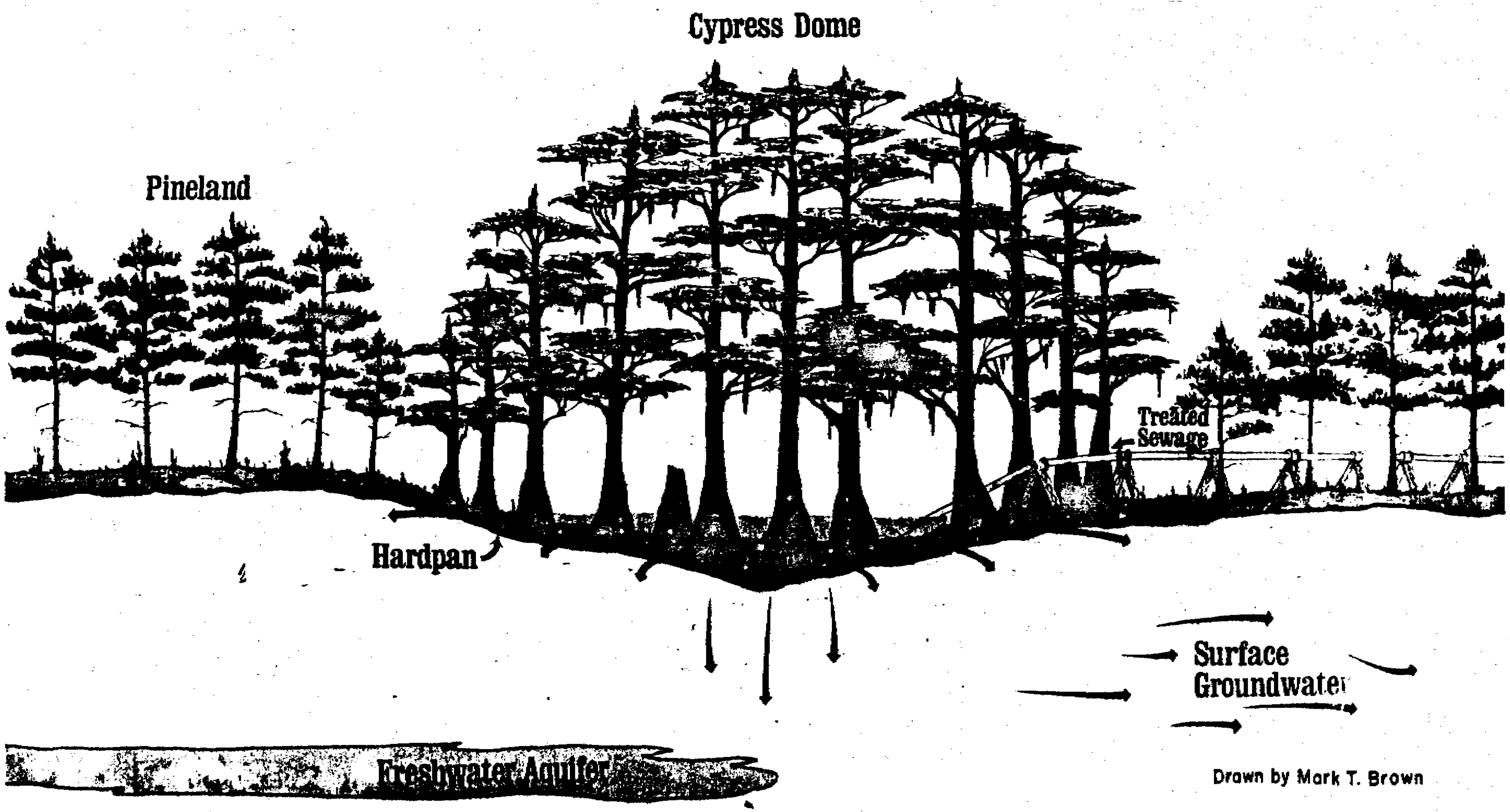
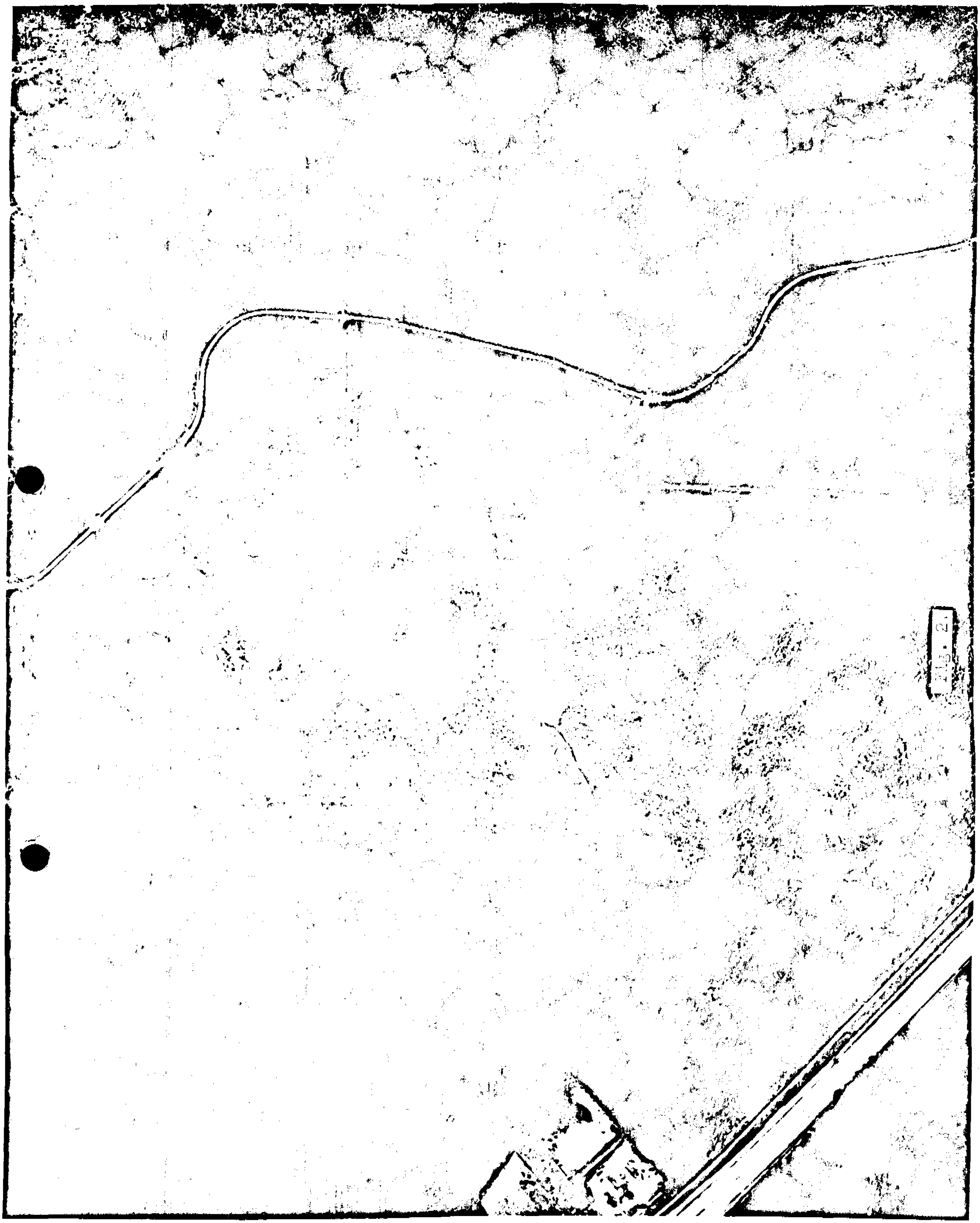

Cypress Dome
Pineland
Treated Sewage
Hardpan
Surface Groundwater
Freshwater Aquifer
Drawn by Mark T. Brown

Fig. 1.



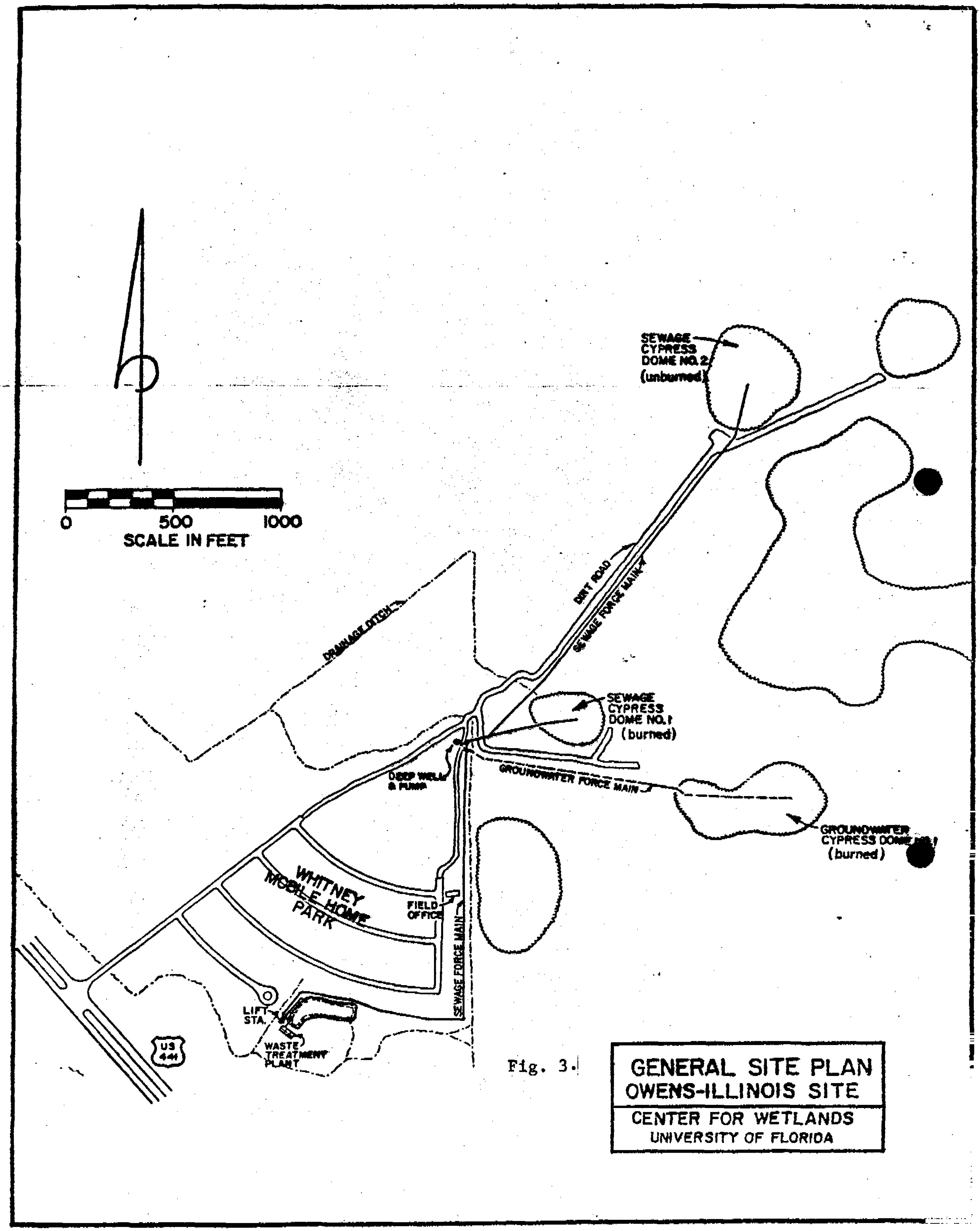
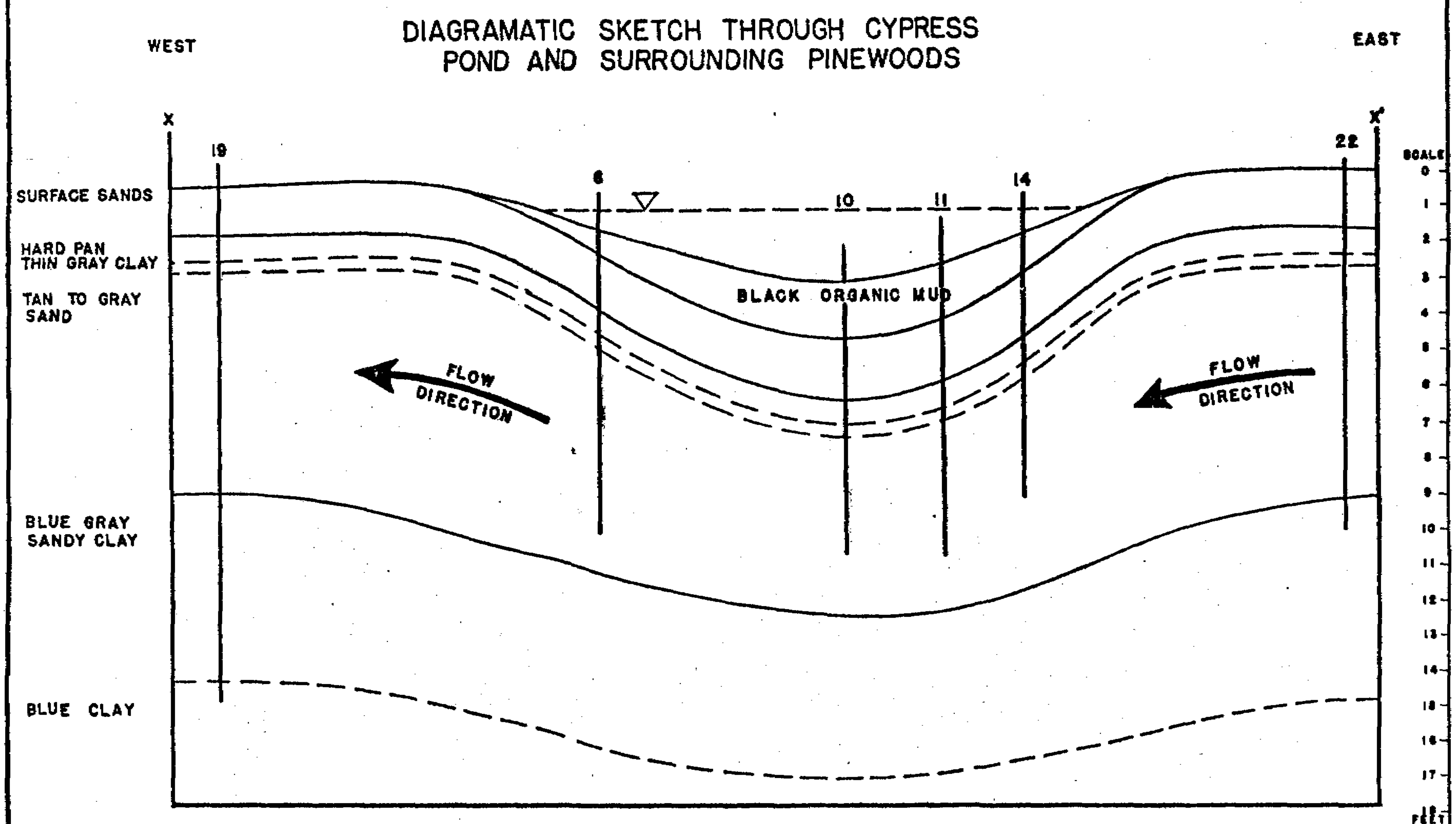
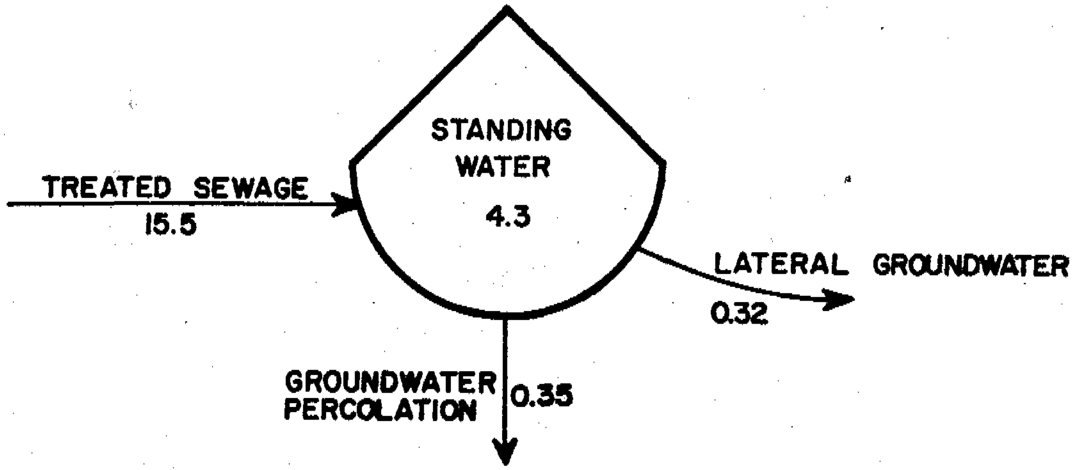

0
500
1000
SCALE IN FEET
SEWAGE CYPRESS DOME NO. 2 (unburned)
DIRT ROAD
SEWAGE FORCE MAIN
DRAINAGE DITCH
SEWAGE CYPRESS DOME NO. 1 (burned)
DEEP WELL & PUMP
GROUNDWATER FORCE MAIN
GROUNDWATER CYPRESS DOME NO. 1 (burned)
WHITNEY MOBILE HOME PARK
FIELD OFFICE
SEWAGE FORCE MAIN
LIFT STA.
WASTE TREATMENT PLANT
US 441
GENERAL SITE PLAN
OWENS-ILLINOIS SITE
CENTER FOR WETLANDS
UNIVERSITY OF FLORIDA

Fig. 3.



DIAGRAMATIC SKETCH THROUGH CYPRESS POND AND SURROUNDING PINEWOODS
WEST
EAST
X
X'
19
6
10
11
14
22
SURFACE SANDS
HARD PAN
THIN GRAY CLAY
TAN TO GRAY SAND
BLACK ORGANIC MUD
FLOW DIRECTION
FLOW DIRECTION
BLUE GRAY SANDY CLAY
BLUE CLAY
SCALE
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
FEET

Fig. 4.



TOTAL NITROGEN CONCENTRATION, mg-N/ℓ

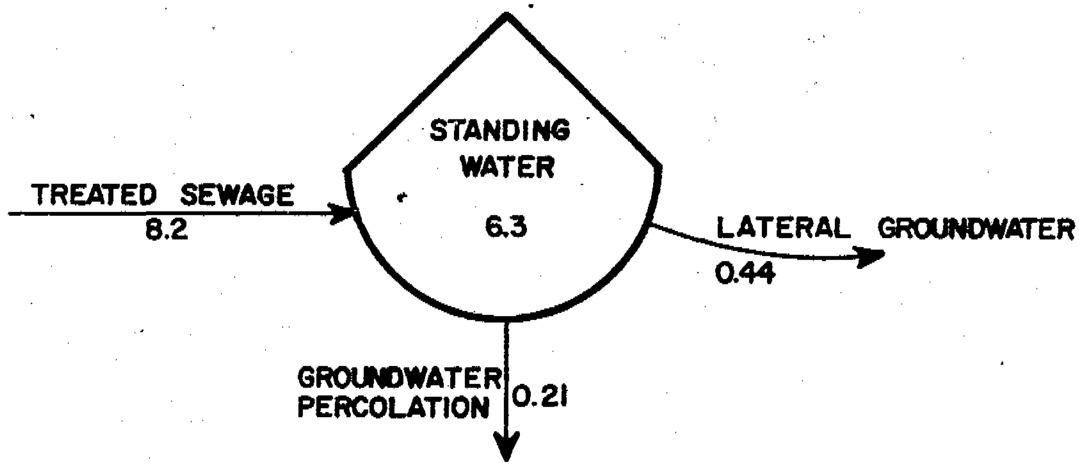


Fig. 5. TOTAL PHOSPHORUS CONCENTRATION, mg-P/ℓ

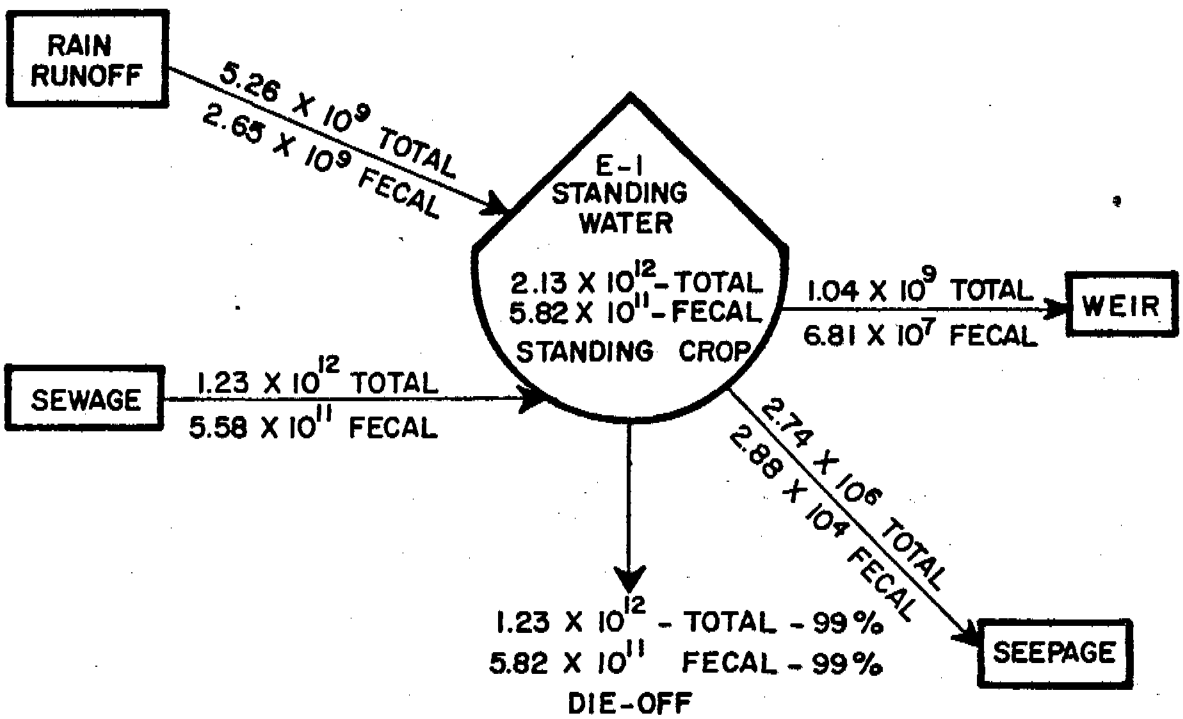
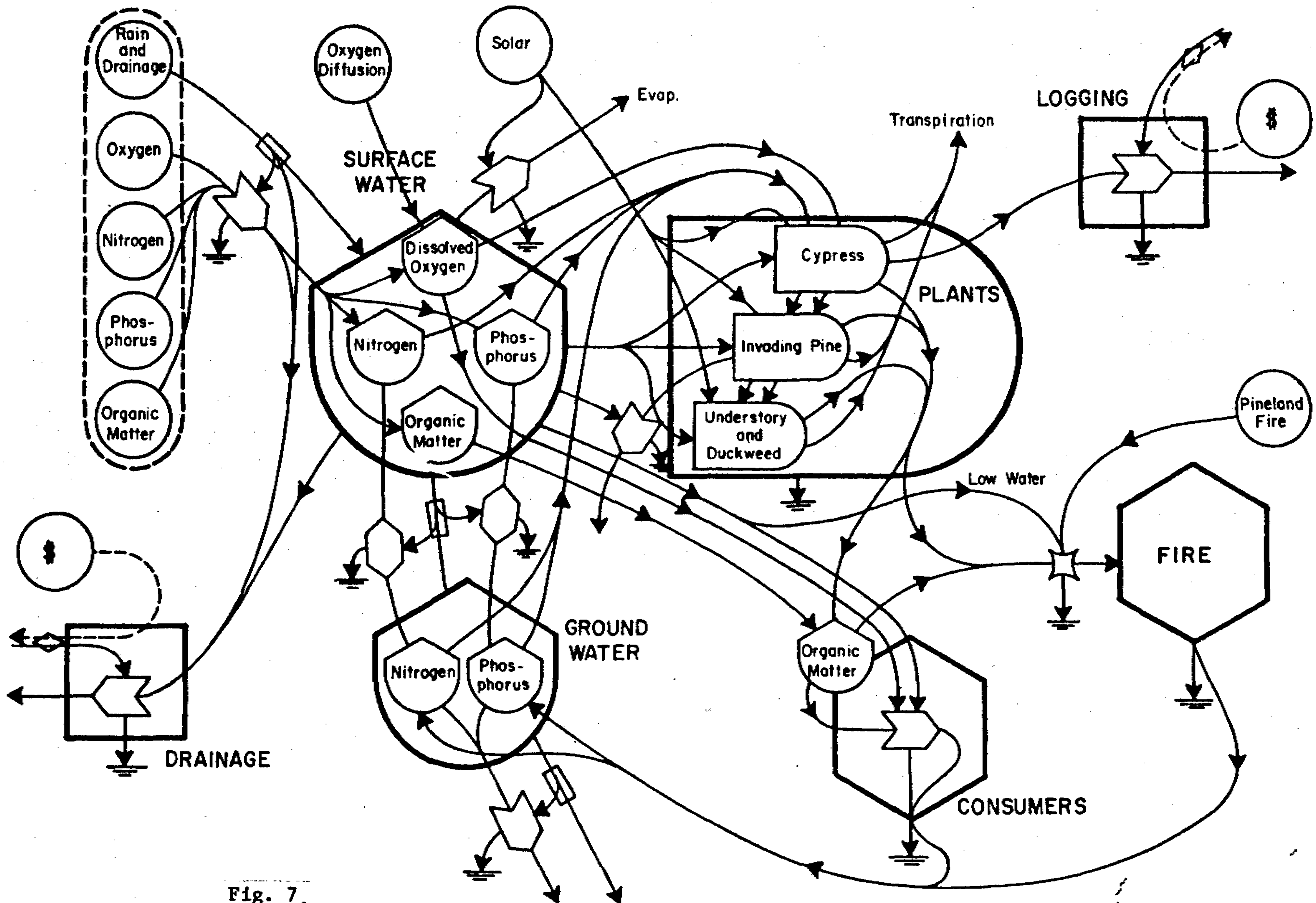
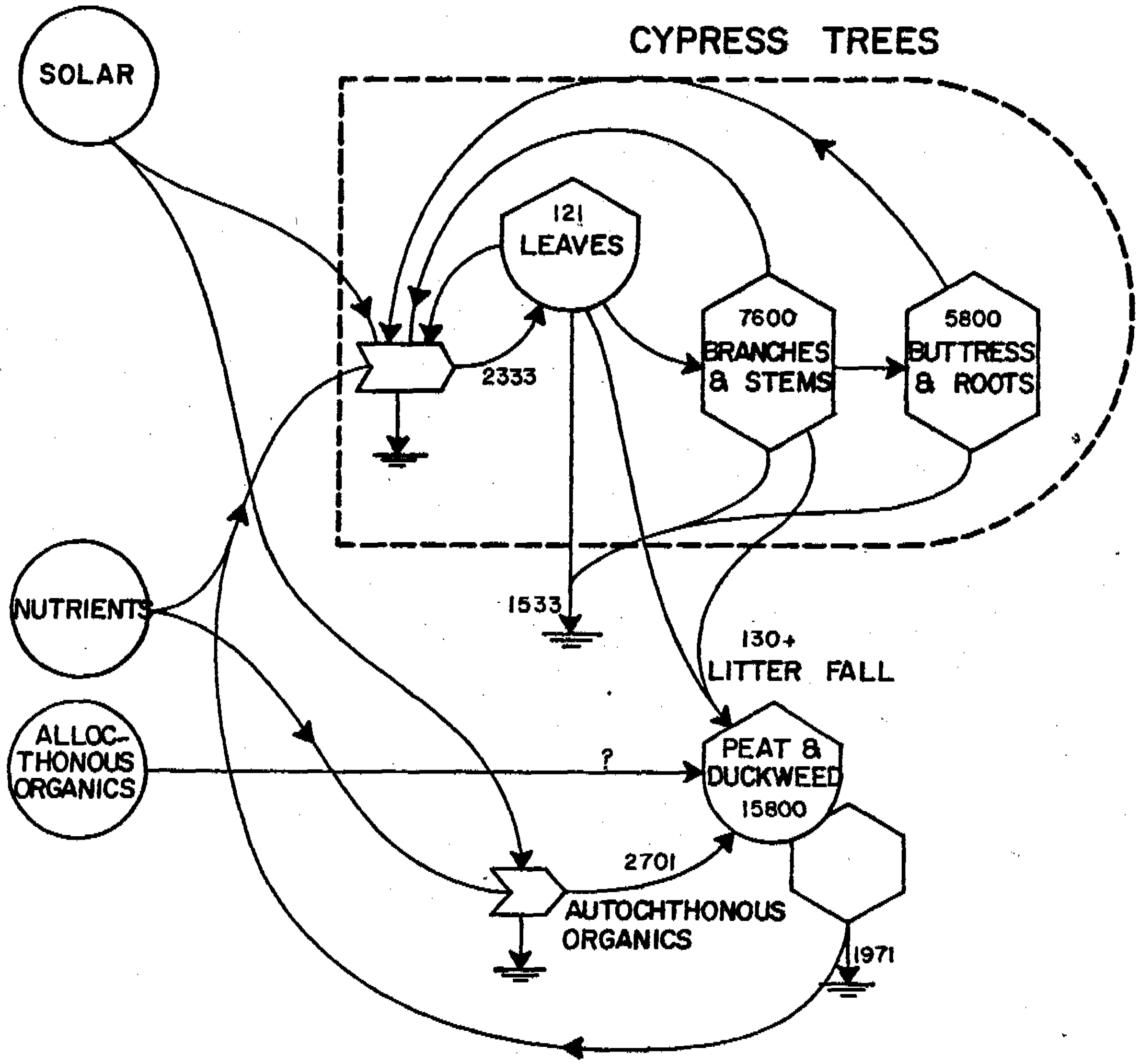


Fig. 6.1 COLIFORM BUDGET, # COLIFORMS/MONTH



Rain and Drainage
Oxygen
Nitrogen
Phos-phorus
Organic Matter
Oxygen Diffusion
Solar
Evap.
SURFACE WATER
Dissolved Oxygen
Nitrogen
Phos-phorus
Organic Matter
Transpiration
LOGGING
$
Cypress
PLANTS
Invading Pine
Understory and Duckweed
Pineland Fire
Low Water
FIRE
$
DRAINAGE
GROUND WATER
Nitrogen
Phos-phorus
Organic Matter
CONSUMERS

Fig. 7.



CYPRESS TREES
SOLAR
121
LEAVES
2333
7600
BRANCHES & STEMS
5800
BUTTRESS & ROOTS
NUTRIENTS
1533
130+
LITTER FALL
ALLOC-THONOUS ORGANICS
?
PEAT & DUCKWEED
15800
2701
AUTOCHTHONOUS ORGANICS
1971

Fig. 8.

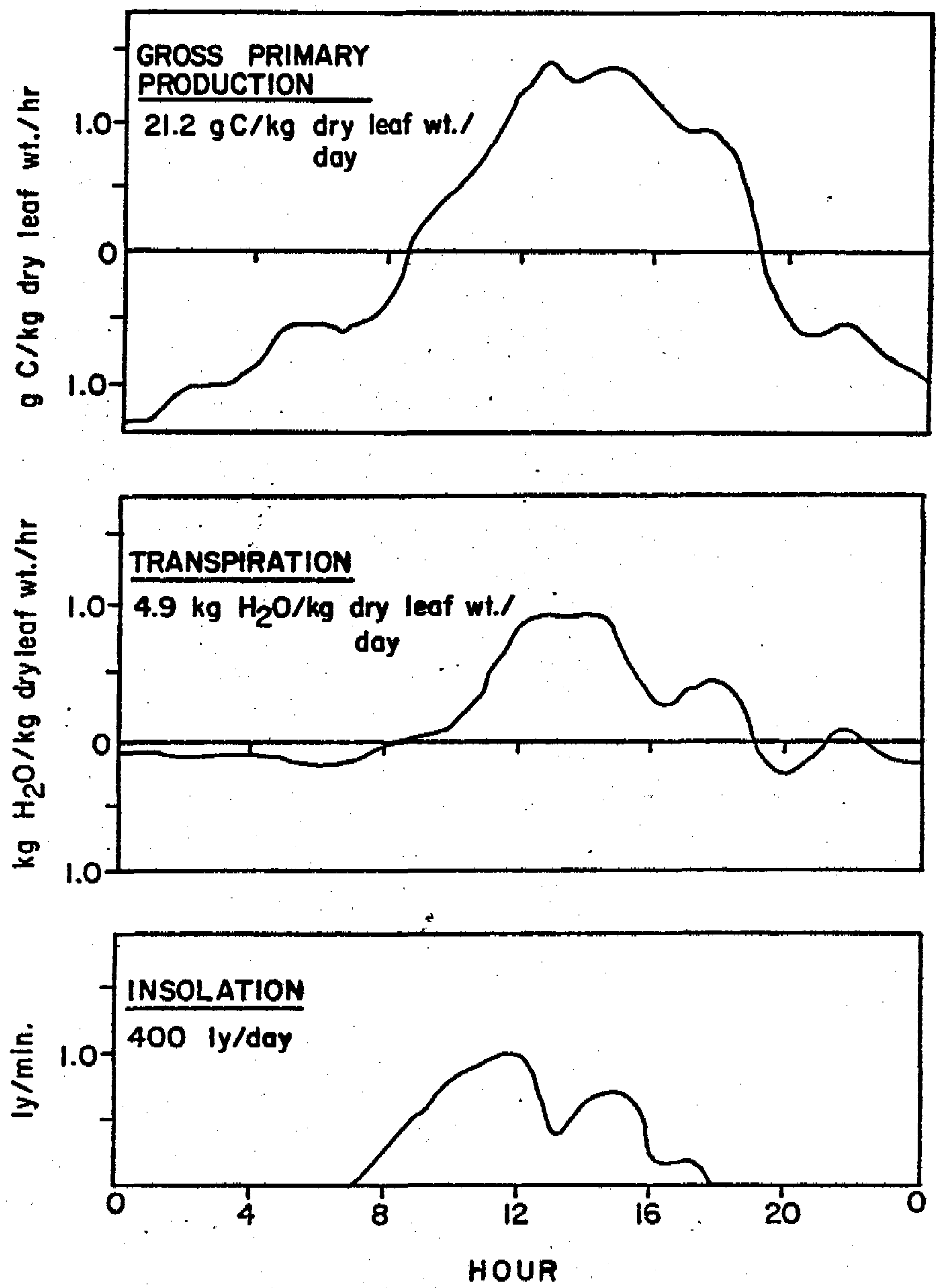

GROSS PRIMARY PRODUCTION
21.2 g C/kg dry leaf wt./day
g C/kg dry leaf wt./hr
1.0
0
1.0
TRANSPIRATION
4.9 kg H2O/kg dry leaf wt./day
kg H2O/kg dry leaf wt./hr
1.0
0
1.0
INSOLATION
400 ly/day
ly/min.
1.0
0
4
8
12
16
20
0
HOUR

Fig. 9.

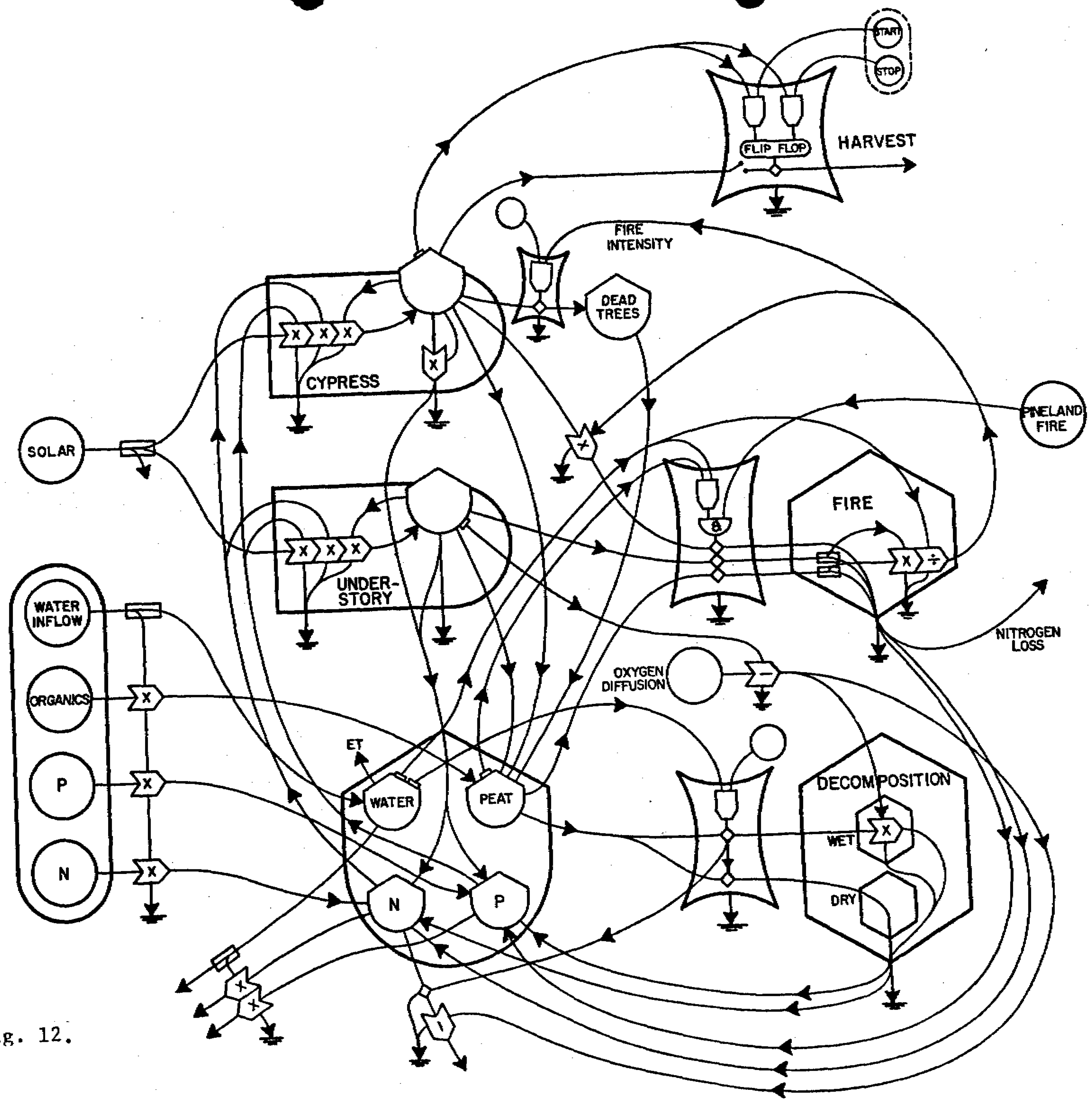
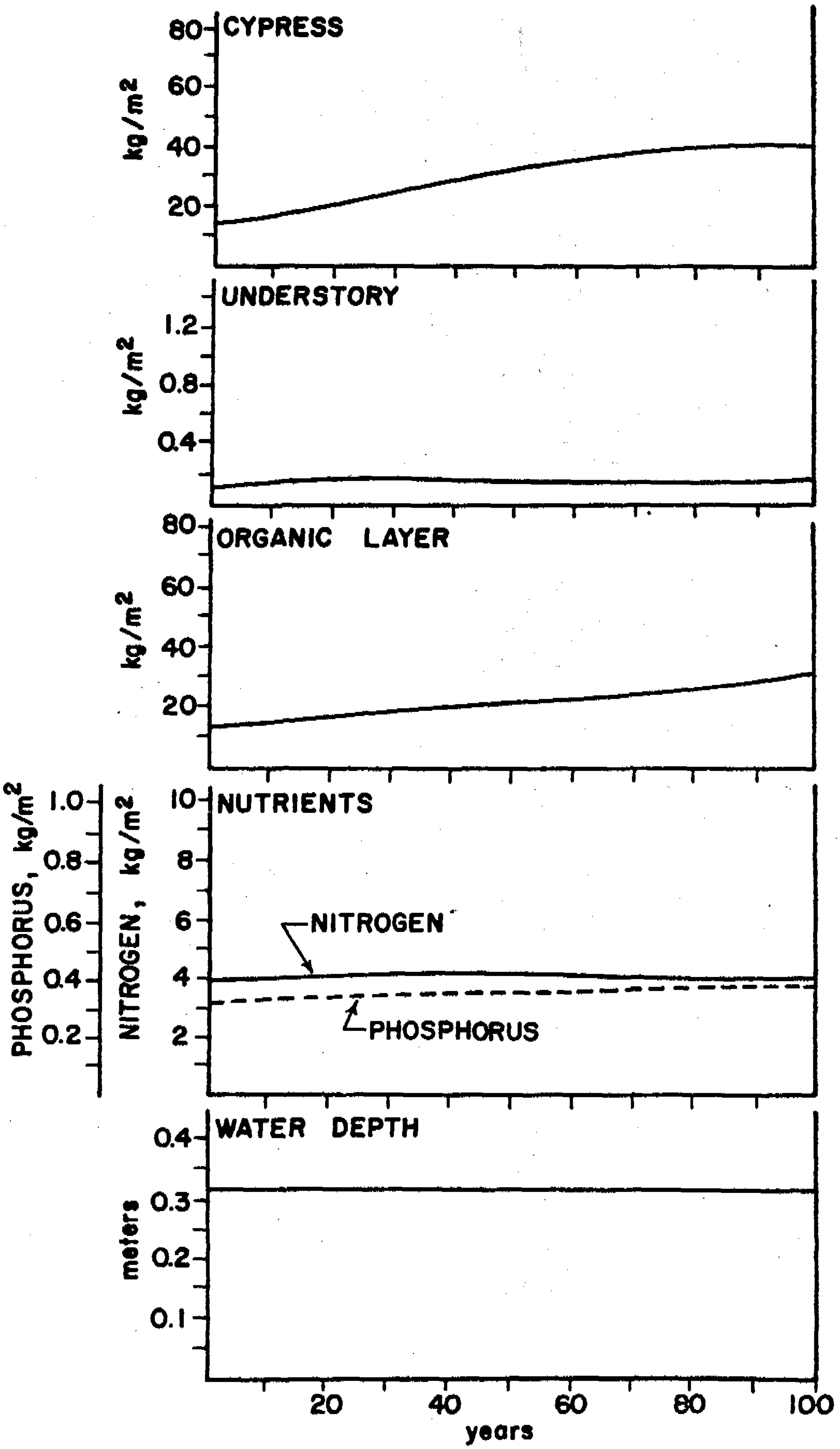

START
STOP
FLIP FLOP
HARVEST
FIRE INTENSITY
DEAD TREES
CYPRESS
SOLAR
PINELAND FIRE
FIRE
UNDER-STORY
WATER INFLOW
ORGANICS
P
N
NITROGEN LOSS
OXYGEN DIFFUSION
ET
WATER
PEAT
DECOMPOSITION
WET
DRY
N
P

Fig. 12.



CYPRESS
kg/m2
80
60
40
20
UNDERSTORY
1.2
0.8
0.4
ORGANIC LAYER
NUTRIENTS
NITROGEN
PHOSPHORUS
PHOSPHORUS, kg/m2
NITROGEN, kg/m2
1.0
0.6
0.2
10
8
6
4
2
WATER DEPTH
meters
0.3
0.1
100
years

Fig. 13.

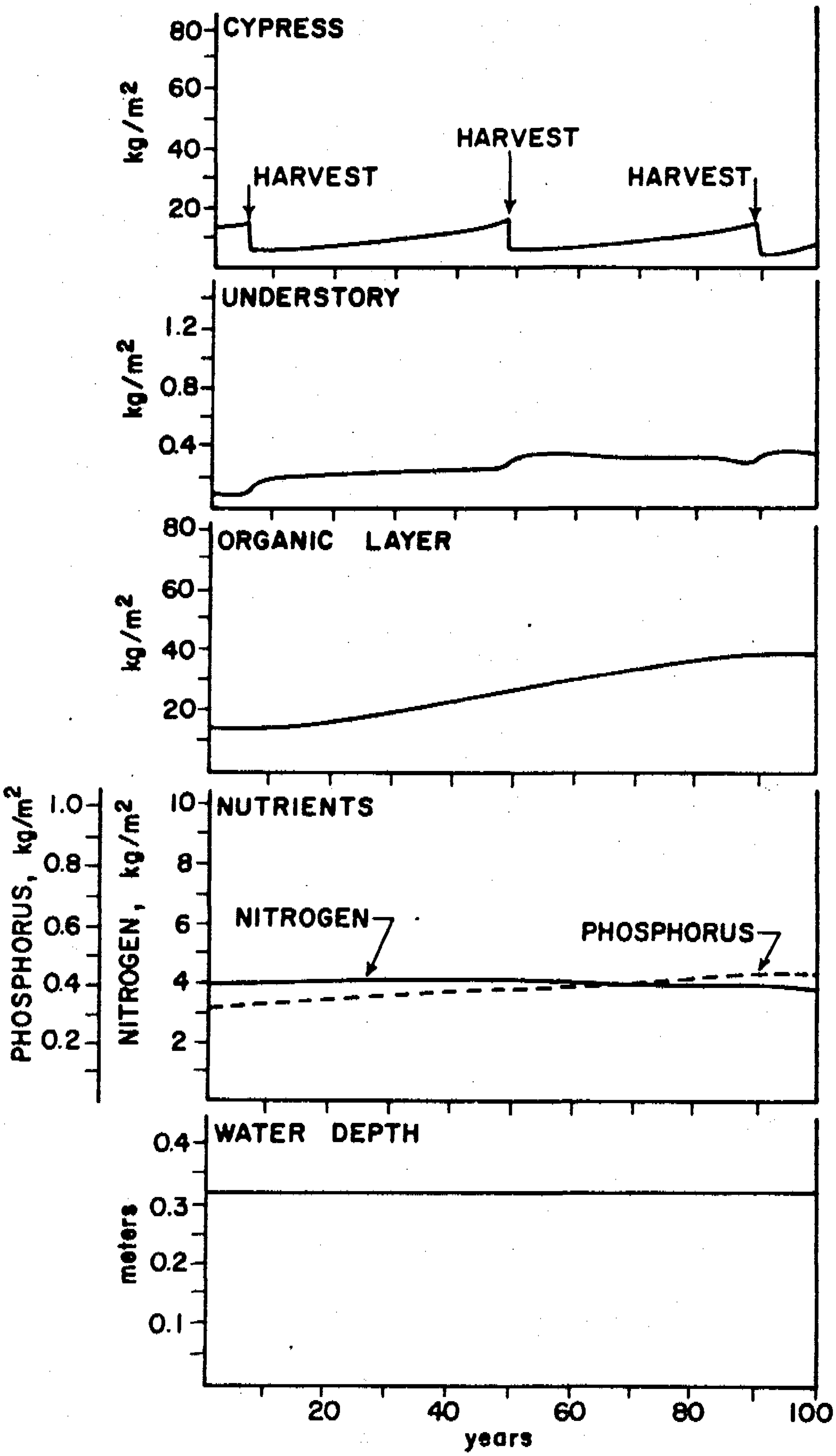
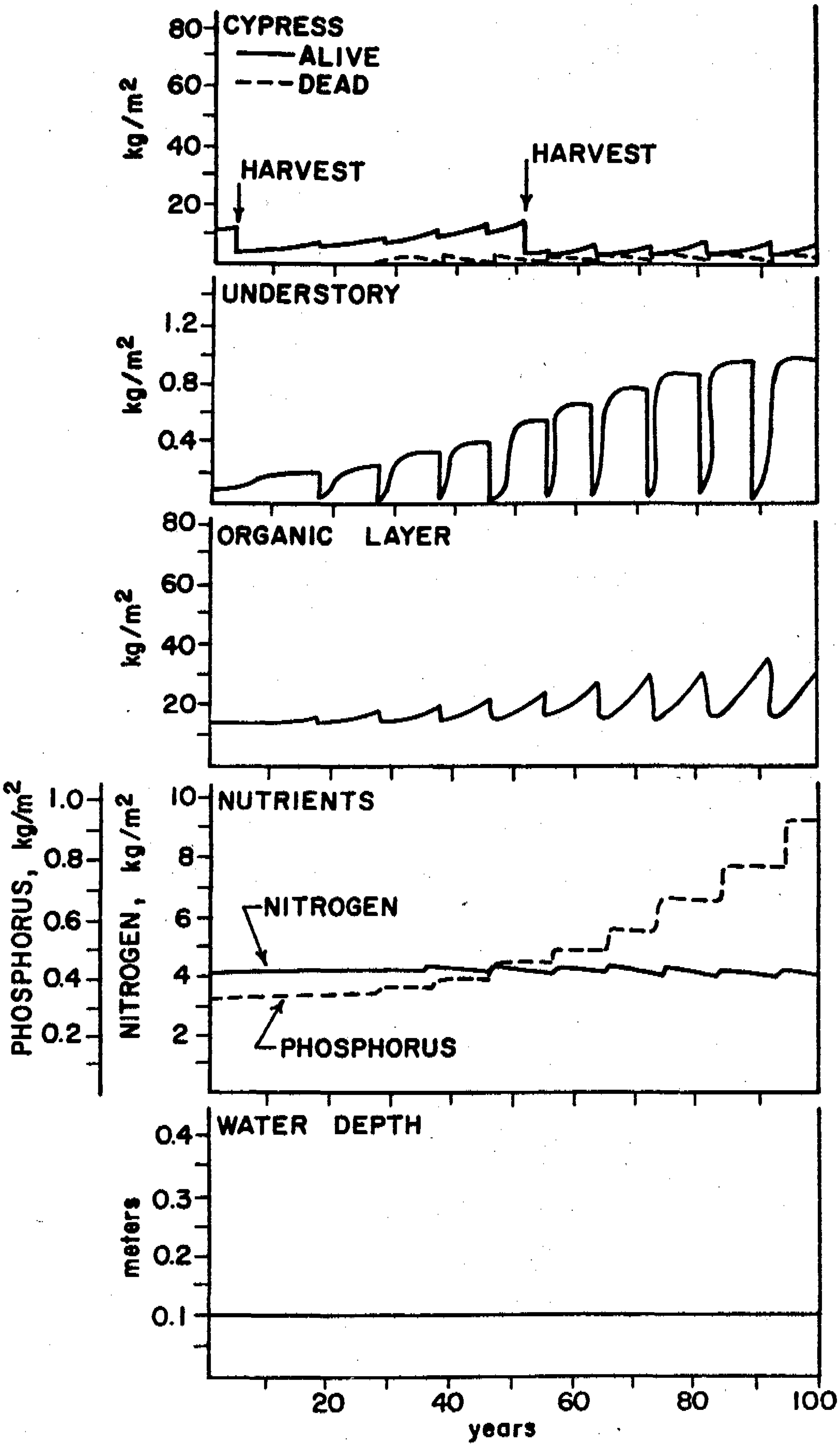

CYPRESS
kg/m2
HARVEST
HARVEST
HARVEST
UNDERSTORY
kg/m2
ORGANIC LAYER
kg/m2
NUTRIENTS
PHOSPHORUS, kg/m2
NITROGEN, kg/m2
NITROGEN
PHOSPHORUS
WATER DEPTH
meters
years

Fig. 14.



CYPRESS
ALIVE
DEAD
kg/m²
HARVEST
HARVEST
UNDERSTORY
kg/m²
ORGANIC LAYER
kg/m²
NUTRIENTS
PHOSPHORUS, kg/m²
NITROGEN, kg/m²
NITROGEN
PHOSPHORUS
WATER DEPTH
meters
years

Fig. 15.

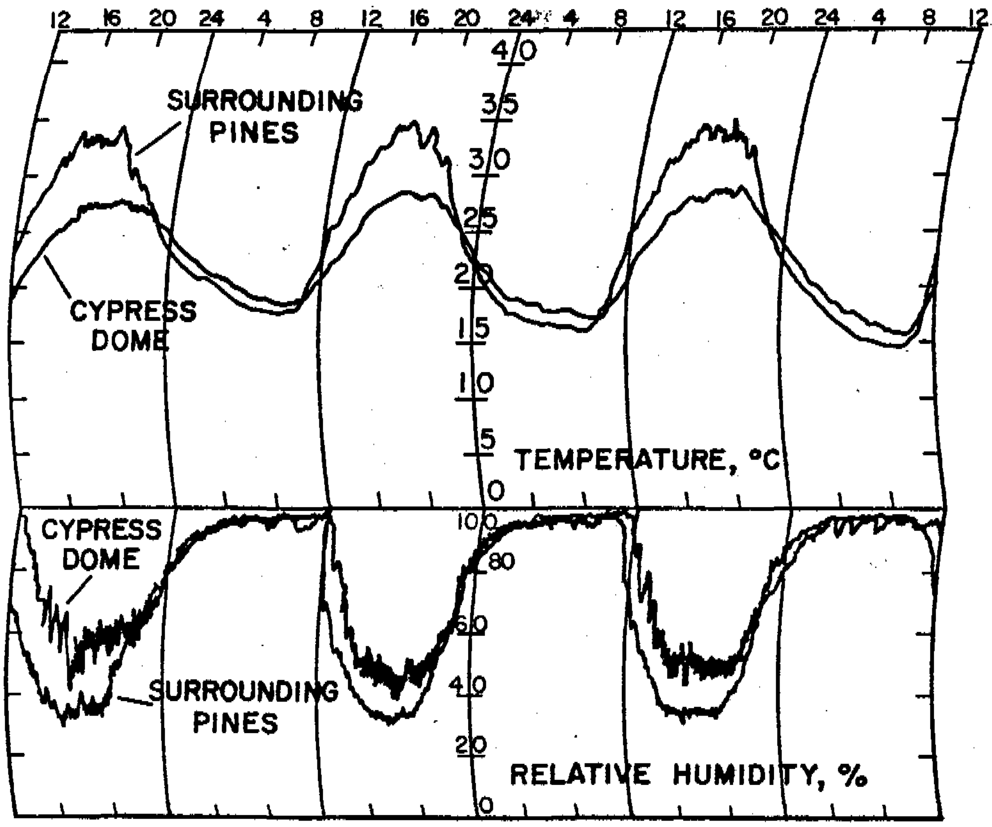
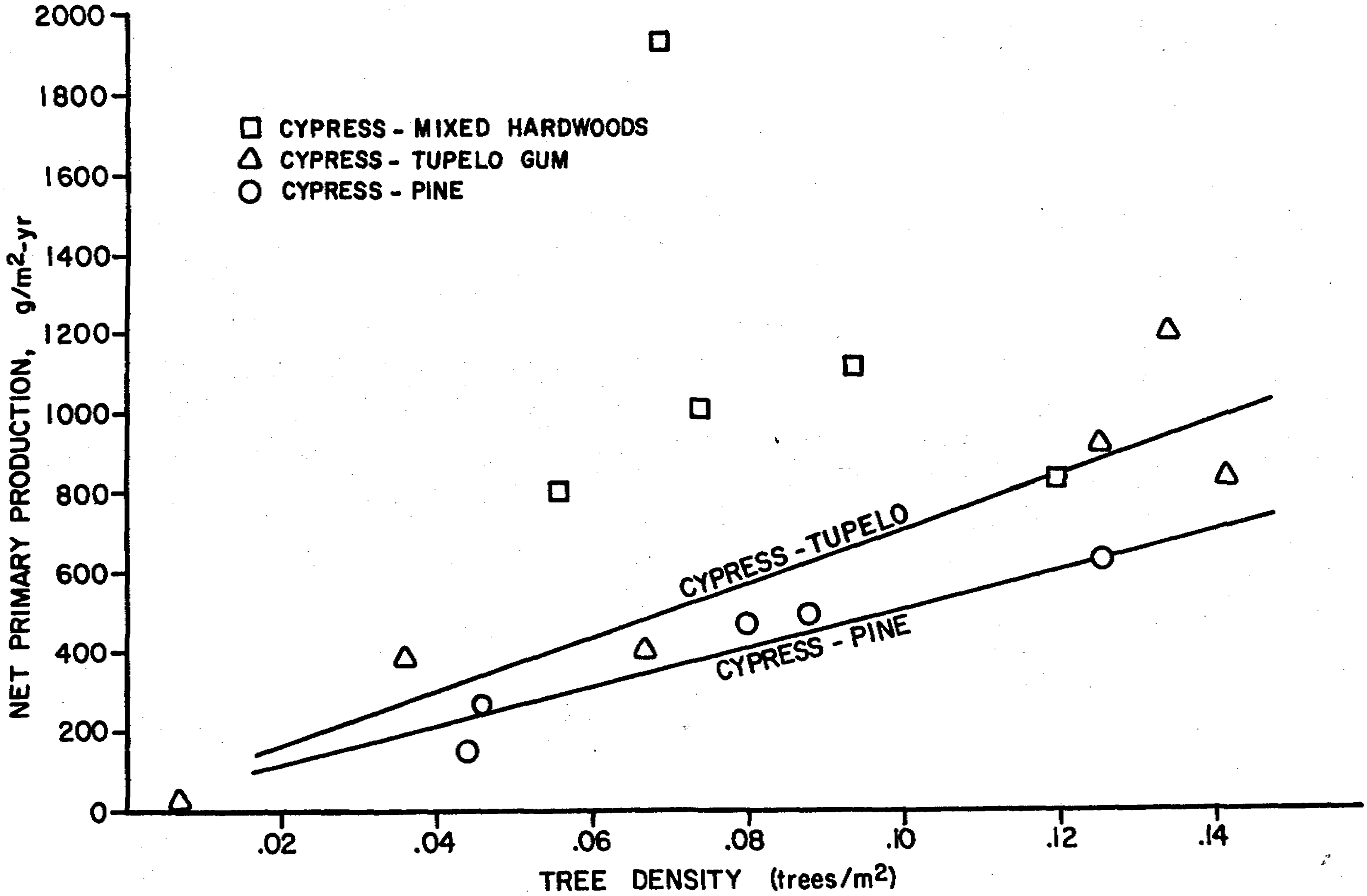

12
16
20
24
4
8
12
16
20
24
4
8
12
16
20
24
4
8
12
40
35
30
25
20
15
10
5
0
SURROUNDING
PINES
CYPRESS
DOME
TEMPERATURE, °C
100
80
60
40
20
0
CYPRESS
DOME
SURROUNDING
PINES
RELATIVE HUMIDITY, %

Fig. 16.



CYPRESS - MIXED HARDWOODS
CYPRESS - TUPELO GUM
CYPRESS - PINE
CYPRESS - TUPELO
CYPRESS - PINE
NET PRIMARY PRODUCTION, g/m2-yr
TREE DENSITY (trees/m2)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
.02
.04
.06
.08
.10
.12
.14

Fig. 17.

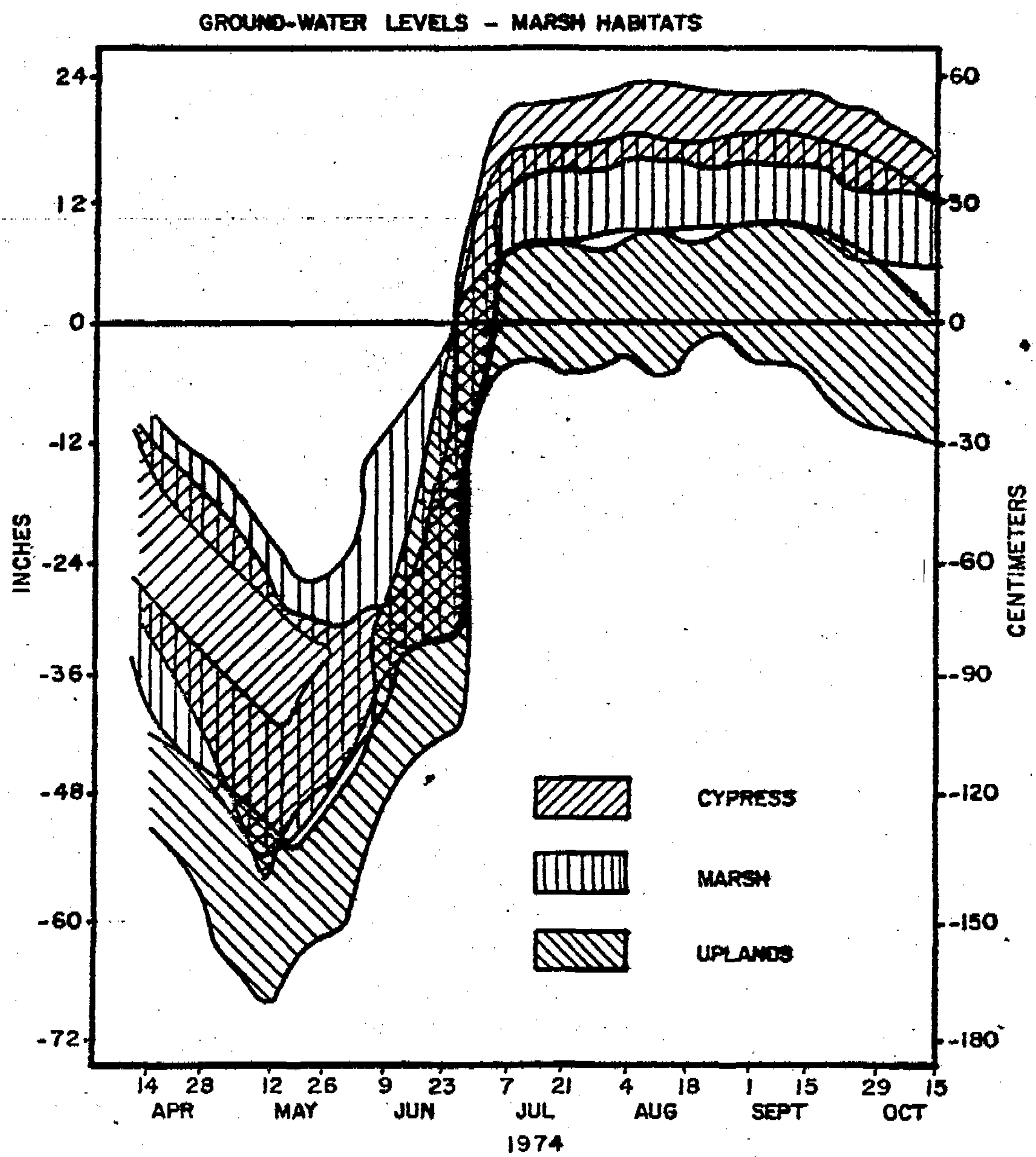

GROUND-WATER LEVELS - MARSH HABITATS
24
12
0
-12
-24
-36
-48
-60
-72
INCHES
60
30
0
-30
-60
-90
-120
-150
-180
CENTIMETERS
CYPRESS
MARSH
UPLANDS
14 28 12 26 9 23 7 21 4 18 1 15 29 15
APR MAY JUN JUL AUG SEPT OCT
1974

Fig. 18.

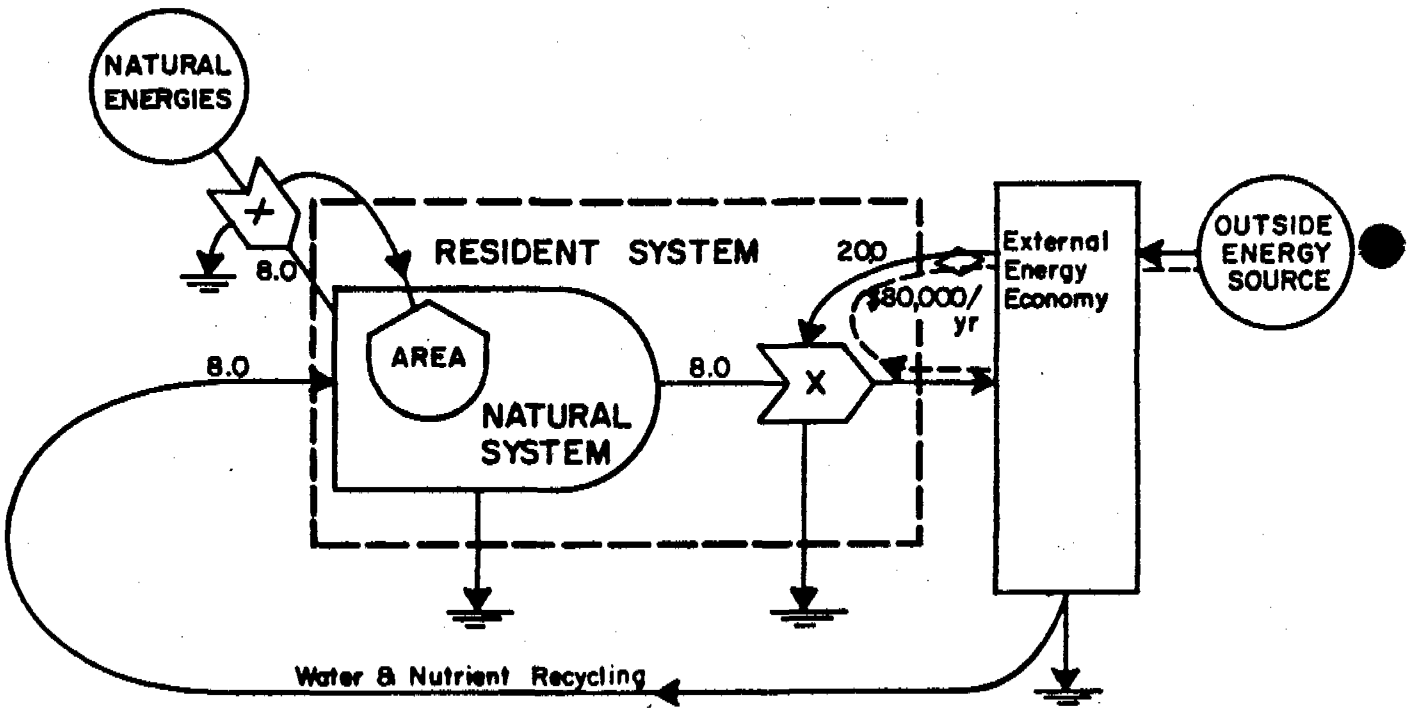

NATURAL ENERGIES
8.0
RESIDENT SYSTEM
200
$80,000/ yr
External Energy Economy
OUTSIDE ENERGY SOURCE
8.0
AREA
NATURAL SYSTEM
8.0
Water & Nutrient Recycling

Fig. 19a.

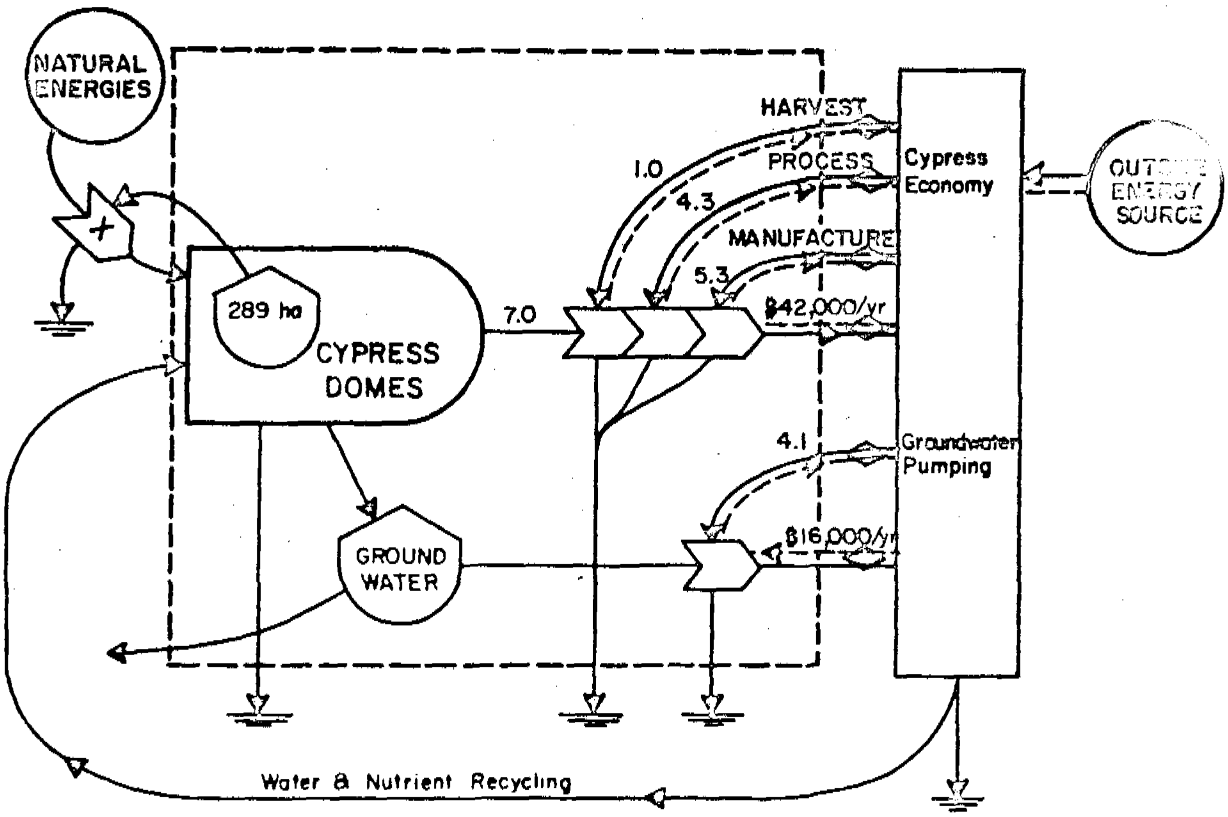

NATURAL ENERGIES
289 ha
CYPRESS DOMES
GROUND WATER
7.0
HARVEST
PROCESS
MANUFACTURE
1.0
4.3
5.3
$42,000/yr
4.1
$16,000/yr
Cypress Economy
Groundwater Pumping
Water & Nutrient Recycling

Fig. 19b.

THE AGRICULTURAL REUSE OF MUNICIPAL WASTEWATER IN BRAUNSCHWEIG, GERMANY

by

CORD TIETJEN*

In the eighteen-nineties, a combined sewer system 100 km in length was built in Braunschweig, ending in a confusing system of dams and ditches on a sewage farm of 350 ha, north of Braunschweig.

Braunschweig was one of those German towns which followed the example in England where in about 1843, at Edinburgh, Ashburton, or Devon, the first sewage fields were established for the twofold purpose of keeping the rivers clean and manuring the soil. The successful operation became an example for many other towns and also industries (Table 1, (1).

The procedure has been praised here and blamed there. The fact that underlies the secret of its successes and its failures is how properly the system had been designed and how well it was managed. A prevailing cause of failure is the increasing imbalance between growing population density and limited extension of the sewage fields. This leads to a decrease of the portion of land applied wastewater in final disposal.

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The sewage farm of Braunschweig made available 350 ha for a population of 115 000, that gives a relation of about 330 inhabitants/ha of farmland. It was not until 30 years after the sewage farm had been established that everyone recognized that this ratio of inhabitants to ha of sewage field could not be kept in proper balance: the population and the quantity of sewage grew continuously, but the sewage farm did not. Because of the inappropriate sewerage system combined with an automatic overflow, a great quantity of the collected water did not reach the sewage fields but was discharged into the river. Although in all new districts of the City the water was collected by a separate sewage system, this did not bring much relief.

The land treatment of sewage was a success with regard to purification and all sanitary standards were met. Therefore, a project came under discussion to enlarge the sewage farm many times, to collect and treat the waste-water from a large area, including many communities and three towns (Braunschweig, Wolfenbüttel, and Salzgitter) that together had about twice the population of Braunschweig.

Because the war, things went backward instead of ahead.

After the war, the new beginning met a situation characterized by lack of food, lack of fertilizer, a great surplus of sewage, and 50 years of experience about the effect on crop production of sewage application as manure.

- 3 -

The great demand for food: the technical progress in equipment development for sprinkler irrigation: and finally, very important, the encouraging results of bacteriological investigations by Popp et al. in sprinkler irrigation of wastewater on farm land near the city of Wolfsburg: these were favorable arguments for the proposal to found the Sewage Utilization Association of Braunschweig on September 1, 1954, by the union of the City of Braunschweig and about 350 farmers, with a total area of 4 200 ha including 3 000 ha of irrigated cropland, north of Braunschweig and north of the sewage farm with surface flooding (2,3).

THE SEWAGE UTILIZATION ASSOCIATION OF WOLFSBURG

Wolfsburg was a village before the Volkswagen factory was established there. In 1939, the sewage utilization association was founded to serve 15 000 inhabitants. Today the sewage flow is 16 500 m^3 per day from a population of about 100 000. The extension of the treatment facilities for the wastewater from 210 000 inhabitants has been started.

Members of the association are the city of Wolfsburg and about 200 farmers with 1 500 ha of spray irrigated fields including 310 ha of woodland.

Wolfsburg and Braunschweig have the same climate and the same farming conditions; the distance between both cities is only 25 km. Knowledge and experience of sewage disposal by utilization for crop production in a well organized spray irrigation operation could be transfered without difficulty from one site to the other. The favorable results of hygienic investigations and official supervision were an important basis for the foundation of the Braunschweig association (4,5).

THE SEWAGE UTILIZATION ASSOCIATION OF BRAUNSCHWEIG

The area of the SUA was divided into four districts with four pumping stations and storage basins, and a total capacity of 15 000 m^3. Reserve capacity was provided for by earth basins of 14,8 ha, and sludge basins of 5,6 ha with a depth of 1 m.

The four districts were constructed and outfitted one after the other: the first was completed in 1957, the last in 1965; learning by experience was possible. The ratio of population to irrigation fields, both kinds: spray irrigation and surface flooding, thus shifted to 240 000 inhabitants and 3 350 ha, that is 72 inhabitants to 1 ha. Including industry, in 1965 there was a 383 000 population equivalent (p.e.), the ratio was 114 p.e./ha. This ratio, of course, is submitted to change, i. e. in 1974 another 3 000 p.e. joined to the system.

The Association is headed by a committee of three farmers and two representatives of the city. The landowners, communities, and city administration are represented in a commission of 24 members. The manager of the Association is an agronomist; at his disposal are an office, a workshop, five irrigation specialists with assistants, and four pump operators.

Contributions to the budget come from the city, about 75 %, and from the farmers. The board of the water resources and the chamber of agriculture are controlling and advisory bodies. State control is exercized by the president of the administration district.

SEWAGE, SOIL, CLIMATE

The quantity of sewage handled per day is 45 000 m^3: of these 32 000 m^3 are delivered in a concrete gravity pipeline 1 000 - 800 mm in diameter to the area of the SUA, the rest is distributed on the old sewage farm.

By sprinkler irrigation, 300 mm of sewage are spread every year on the fields in six applications of 50 mm, three in summer and three in winter. That is the average: more exactly, amounts of sewage applied to various crops are as follows:

Potatoes	2 applications of 30 mm
Winter grain, spring barley	3 applications of 50 mm
Oats	4 applications of 50 mm
Spring wheat, sugar beet	5 applications of 50 mm.

Soil and climate are favorable for a supplementary water supply. Except for a more clayey strip on both sides of the River Oker, the whole area is characterized by an extremely uniform layer, 20 - 40 m thick, of fine to coarse to gravelly sand without stones, a sand of very low fertility with less than 1 % of organic matter. The groundwater level lies between 1,75 and 3,50 m. Damage by wind erosion occurs every spring and fall. There are almost no elevations, the surface is level, but the altitude decreases from south to north by 9 m. This smooth grade of about 0,05 % facilitates the transport of the sewage in a gravity line, and also the control of the groundwater flow.

The average mean value of the temperature is 8,5 ^{o}C; in the growth season from April to September it is 13,9 ^{o}C. The corresponding values for precipitation are 655 mm per year, and 393 mm in the growth season.

The balance of precipitation and evaporation is a positive value of 125 mm per year, but a negative value of 44 mm in the growth period, April to September. This explains the success of supplementary water supply, if it is applied according to the demand of the crop. This explains also the farmers' active interest in this water source, for they have experienced years with a still greater shortage in rainfall and know its catastrophic effect on plant growth and yields.

Results of field experiments show the effect of sewage sprinkler irrigation on plant growth and yields. An experimental field was established and data from all crops grown in the area of the SUA are available, Table 2. Sugar beets and potatoes show a remarkable increase of yield from sewage application in dry years when the natural water supply is unsufficient. To small grains, however, the sewage is beneficial in every year. This indicates that not only the water but also the sewage nutrients are useful. With the annual application rate of 300 mm, the quantity of nutrients applied per ha is: 135 kg N, 21 kg P, 78 kg K (Table 3). According to experience, spring grain makes the best use of the sewage. This influences the ratio of crops grown in the irrigation area: the portion of spring grain increases from 10 % in 1957 to 31 % in 1970, whereas winter grain decreases from 36 to 20 %, Table 4.

DITCHES, WATER QUALITY, WIND, AND HEDGES

A remarkable feature is the control of percolating water. Control is possible because of the uniform flow of the groundwater to the north and northeast, in the direction of the River Oker. However, a drainage scheme had to be worked out carefully. Some areas needed subsurface drainage by pipes. Very remarkable is the system of open ditches with a total length of about 22 km, most of them flowing a while parallel to the bank of the river before they discharge into it. They relieve the bank of catchment water and they are very effective in completing the purification of the drainage effluent. Special attention is drawn to the low contents of substances that are standard indicators for the presence of pollutants. The contents of nitrogen compounds, phosphate, sulfate, chloride, and biochemical oxygen demand lie within the critical limits. The ability of the sandy soil to hold fast the sewage nutrients and place them at the plants' disposal is evident. The same can be affirmed with regard to the pollution of the water table: investigations comparing groundwater inside and outside the irrigation area show this clearly, Table 5, (6).

The quality of water in the ditches reaching the inlets of the rivers Oker and Erse meet sanitary standards. It has been stated by the public medical board: "The discharge of the Oker Ditch at Neubrück ... is in its quality very definitely better than discharge of unobjectionably operating mechanical-biological treatment plants" (Popp, Oct. 10, 1971).

With regard to salmonella, L. Popp published results of investigations of the ditch water, (7). In 1965, 44 samples were taken, one per week. Salmonella were present in 16 samples, or 36 %. Comparatively, in 319 samples taken in 1962 - 1965 from the effluent of 45 sewage treatment plants, salmonella were found 142 times, that is in 45 % of samples. Salmonella typhosa was present in four effluents from treatment plants, but never in the ditch water.

Enterovirus were found in 5 of 157 samples from ditch water, while from 40 samples taken out of the outfall ditch of sewage treatment plants 13 were found positive, or 33 %.

An argument against sewage sprinkler irrigation is the additional spraying effect of the wind, which increases the danger of infection and contamination. Systematic investigations were carried out with different positions and ranges, and the wind velocity was measured. As a criterion of infectivity, the number of E. coli was determined from a spray that hit a 65 cm^2-disk with a culture medium, for 1 min. The number of E. coli/min decreased with the distance from the sprinkler. Higher wind velocity carried sewage spray and E. coli on farther, but the sectors covered by the sprinkler became narrower, thus decreasing in a sense the possibility of exposure.

In general, the danger of infection by sewage spray can be judged as small, but should not be neglected. Men, animals, vegetables, and fruits should not be exposed to sewage and sewage spray. This stresses the importance of shelter hedges, which had always been a special concern of the farmers as means against wind erosion. Hedges have been planted by the Union and the farmers, with a width up to 6 m, and on a total area of 60 ha. They shelter the highway and roads against sewage spray, and decrease the strength of the wind.

COSTS

A comparison of the costs of different procedures of sewage disposal is always difficult and limited to comparable conditions. To get a calculation base for the contribution of the City to the budget of the Association, an approximate comparison was given in 1971. In the average of five sewage treatment plants of communities with about the same population equivalent as Braunschweig, the total costs were 18 % higher than the land treatment at Braunschweig. Perhaps more important than monetary affairs might be a comparison of the degree of purification (BOD) achieved; this was 100 % by land treatment, but only 87 % in the average of the five treatment plants operating with an activated sludge process or digestion or both.

Today, the problem is to compensate for the steady increase of labor costs. After twenty years of operation with inflexible pressed-steel pipes of 6 m-pieces, fittings and couplings, medium sized rainers, and a very labor intensive moving of the lay-out, a solution is now expected in a mechanized sprinkling system. Various rain machines have been tested, with the common feature of a large size sector sprinkler. The sprinkler may be on a sledge and is pulled by the plastic pipeline which is coiled on a drum. Or the sprinkler is fixed on a carriage which pulls itself along the water hose and is driven by a hydraulic piston engine. Labor requirements for these modern procedures is about 1/4 of the original method, but investment requirement is 2,5 as much. However, at present labor wages, the breach even point in costs is reached, so that the modern methods will be the more economical ones in the future.

In addition, land consolidation is helpful and necessary, in order to adjust ways and roads, size and shape of the fields, the subsurface pressure line with hydrants and junctions, subsurface and ditch drainage, hedges and woodland, and even land use and the cropping system, according to the most efficient use of labor and equipment. Since 1955, 50 km of roads have been constructed, but up to now only part of the large project could be achieved.

CONCLUSION

Final and complete sewage disposal is a difficult task for sanitary engineers. Aspects of environmental protection must be observed. Remarkable efficiency is obtainable by land treatment of sewage in a cooperative program of sanitary engineering, water resources policy, and agriculture, if soil and climatic conditions are favorable and the farmer's economic situation can be improved.

This has been demonstrated by the Braunschweig and the Wolfsburg Sewage Utilization Associations since 1957 and 1939, respectively.

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TABLE 1

LAND APPLICATION OF WASTEWATER IN GERMANY

Year	Number of Facilities	Irrigated Fields, ha Total	Irrigated Fields, ha Average	Application mm/Year
1914	32	14 656	458	3900
1932	69	30 843	447	3185
1955+	98	11 444	117	1174

+Without German Democratic Republic

TABLE 2

CROP YIELDS, FIELD EXPERIMENTS AT NEUBRÜCK - ERSEHOF

Year	Crop	CWB[+] mm	Yields dt/ha No sewage	Yields dt/ha Sewage	Increase dt/ha	Increase %
1959	spring grain	- 307	0	29,8	29,8	
	potato	- 211	54,0	201,0	147,0	272
1960	winter rye	- 70	25,4	32,9	7,5	29,5
	potato	- 108	253	302	49	19,4
1961	winter rye	+ 102	12,6	32,8	10,2	88,8
	sugar beet	+ 29	449	455	6	1,3
1962	spring grain	- 39	32,3	43,4	11,1	34,4
	sugar beet	- 5	142	315	173	122
1963	potato	- 71	235	324	89	37,9
1964	sugar beet	- 99	259	545	286	110
	winter rye	- 142	16,0	27,0	11,0	68,8
1965	potato	+ 55	318	324	6	1,9
	spring barley	+ 77	24,4	41,3	16,9	69,3
1966	winter rye	- 10	22,5	30,2	7,7	34,2
1967	spring grain	+ 6	31,4	35,2	3,8	12,2
	potato	+ 15	320	312	- 8	2,5
1968	winter barley	- 96	20,8	29,3	8,5	40,8
	winter rye	- 96	24,6	36,5	11,9	48,3

[+] The climatic water balance (CWB) was calculated for each crop over the period (months) of greatest water demand i. e.:

winter grain	April to June
spring grain	May to July
potato	June to July
sugar beet	July to September

TABLE 3

NUTRIENTS IN THE SEWAGE

Mean of 240 Samples 1969 to 1971

Nutrient	mg/Litre
K	26
P	7
Na	180
Mg	24
N_{total}	45
NH_4-N	33
NO_2-N	0,1
NO_3-N	0,1

TABLE 4

CROP GROWING, PERCENTAGE OF AREA UNDER CULTIVATION

Crop	Total Area	District 1 Only			
	1968	1957	1960	1965	1970
Winter grain	17	36	23	20	2o
Spring grain	28	10	19	28	31
Potato	12	24	19	14	10
Sugar beet	19	12	19	19	18
Asparagus a.o.	12	8	10	8	11
Grassland	12	10	10	11	10

TABLE 5

WATER QUALITY DATA BRAUNSCHWEIG SEWAGE UTILIZATION ASSOCIATION 1973/74

Mean / Deviation   mg/Litre   n = Number of Samples

	Sewage	Oker River	Erse River	Groundwater Inside	Groundwater Outside	Drainage Effluent	Oker Ditch Neubrück Beginning	Oker Ditch Neubrück End
				Of The Irrigation Area				
	n = 16	n = 12	n = 12	n = 252	n = 58	n = 11	n = 11	n = 12
pH	7,1	7,2 0,3	7,1 0,2	6,7	7,0	7,0 0,2	7,0 0,1	7,1 0,3
E C mmho/cm	1,11	0,98 0,11	1,91 0,33	1,04	1,35	0,98 0,18	0,96 0,11	0,98 0,13
BOD	192	8,1 4,9	17 10	1,6	1,7	1,6 0,7	33 35	14 13
$KMnO_4$	274	28 8	43 32	17	34	14 4	55 47	22 11
NO_2-N	0,1	0,2 0,2	0,6 0,6	0,1	0,08	0,01 0,02	0,5 0,6	0,2 0,2
NO_3-N	0,2	8,4 3,0	7,0 3,1	30	8,7	26 4,9	13 8,7	14 6,0
NH_4-N	49	7,0 2,0	14,2 6,4	2,8	2,9	2,1 0,6	11 9,9	4,6 3,0
P_{total}	13	0,9 0,3	0,7 0,6	0,5	0,4	0,2 0,3	4,3 4,6	1,6 2,6
K	32	11 1,3	55 33	33	85	16 1,5	19 5	11 1,4
Na	77	69 23	98 30	57	50	67 12	68 17	66 12
Cl	128	153 34	454 112	133	158	138 13	131 13	137 12
SO_4	134	202 52	286 62	203	237	191 19	197 33	178 65
Zn_{total}	0,9	0,6 0,2	0,5 0,5	0,4	0,7	0,1 0,09	0,3 0,3	0,3 0,1
Cd_{total}	0,02	0,01 0,007	0,02 0,01	0,01	0,02	0,01 0,009	0,008 0,008	0,009 0,009
Cu_{total}	0,15	0,03 0,02	0,04 0,02	0,06	0,05	0,03 0,02	0,05 0,02	0,04 0,02
Pb_{total}	0,04	0,02 0,009	0,03 0,04	0,07	0,04	0,03 0,06	0,02 0,02	0,02 0,03
Fe_{total}	2,0	1,2 1,0	0,8 0,6	12	8,3	0,4 0,5	1,8 2,2	1,2 1,6
Mn_{total}	0,3	0,4 0,3	0,9 0,5	1,7	2,1	0,4 0,08	0,6 0,2	1,1 0,2
Co_{total}	0,2	0,12 0,07	0,27 0,18	0,14	0,19	0,14 0,08	0,11 0,07	0,12 0,08

Sewage: Pumping Station No. 2; spray collection

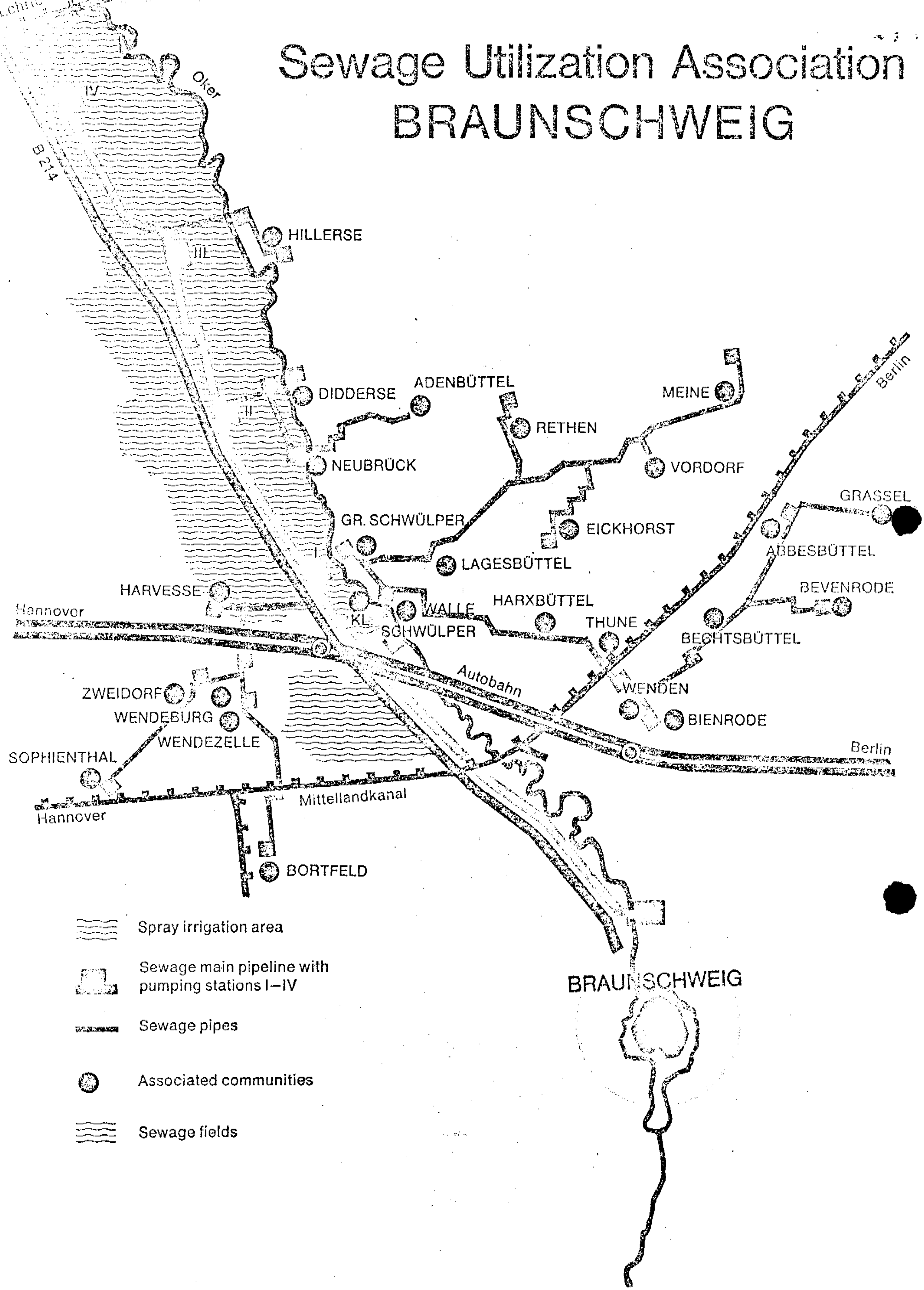
Oker and Erse Rivers: Samples taken before the rivers reach the irrigation area

Groundwater: 21 observation wells in District No. 1, and 5 wells beyond the rivers groundwater table 0,5 - 3,5 m

Drainage Effluent: District No. 1 irrigation fields

Oker Ditch Neubrück: Length 2680 m, parallel to the Oker River in a distance of 200 m

Sewage Utilization Association BRAUNSCHWEIG



WASTEWATER RECYCLING: A PERSPECTIVE ON RESEARCH NEEDS

By

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INTRODUCTION

Each of us recognizes the research needs and priorities related to our own discipline and have pursued them individually or collectively for many years. Our perspective of the problem is shaped by personal bias and mine will be no exception. As an ecologist by training, I am forced to take a rather broad view of ecological systems and also forced to think of man's place in ecosystems. As a member of an energy intensive society I am a voracious consumer of fossil-fuel and as a cog in the machinery of our culture am wasteful of material resources. I have, however, developed a great respect for the powerfulness of natural systems to eliminate species that don't play by the rules of the ecological game. I am a strong believer in the second law of thermodynamics and also believe that when I run out of food or those things needed to produce food that I will quickly become a member of an endangered species list. In recent years I have become increasingly aware of the possibility that I may be put on such a list and this has profoundly shaped my biases. Furthermore, I am conservative when it comes to placing too much reliance on future technological breakthroughs to solve our urgent food and energy problems. I would rather place greater emphasis on more efficient use of energy, materials and technologies available to us right now. These are my personal views and some would disagree. Yet I think that in any discussion of research priorities it is important to frame opinions within the context of ones own biases and perspectives. I would first like to summarize results of an earlier workshop that represents a group attempt at establishing research needs and then interject my own perspective. Hopefully, out of an ensemble of complementary biases will come objectivity.

IDENTIFIED RESEARCH NEEDS IN THE UNITED STATES

In the United States it has been only recently that the scientific community has made a coordinated effort to develop a comprehensive list of research needs and priorities relating to the general topic of municipal wastewater recycling. The U. S. Environmental Protection Agency in 1972 assembled a coordinating committee consisting of representatives from their own office and the U. S. Department of Agriculture. A subcommittee of this group was appointed to develop and implement procedures to harness the resources available within the federal government and universities in addressing the many problems associated with land application of wastewater and sludges. An output of this subcommittee was to be a detailed problem identification and identification of the alternative ways to solve the problem. A Research Needs workshop held in Champaign-Urbana, Illinois was an early spin off of their efforts (U.S.E.P.A., U.S.D.A., NASULGC, 1973).

Shortly after this workshop and after consultation with EPA, directors of Water Resources Research Institutes for the Great Lakes Region proposed a follow up working session to further refine and adopt research needs to conform to problems unique to the North Central part of the U. S. This meeting was in Chicago on March 19-21, 1974 and the proceedings have been published (GLUMORBA, 1974). Directors of Water Institutes from at least two other regions have met and will publish their proceedings in the near future.

Unfortunately, the proceedings of such workshops do not enjoy world-wide distribution so I will attempt to extract from the Chicago workshop, at least, findings I think are relevant to this meeting. This list will be subdivided according to discipline although it is important to recognize that wastewater management problems are interdisciplinary in nature.

Social Aspects

Studies of the structural variables which influence public response to wastewater recycling need be initiated. These variables would include such items as the size of the project, size of the communities being served, and level of public participation in the project.

Studies of individual responses to wastewater recycling projects need to be initiated. Included would be assessment of individual perceptions be they good or bad and the role of opposition groups.

Relocation of people resulting from large projects is a problem. Similar problems and studies related to reservoir construction need be confirmed or disconfirmed in the wastewater recycling context. Secondary impacts on people remaining nearby need by assessed. The most important social research need focused on the problem of population growth and its impact on the generation of waste and its mismanagement and on the resource depletion associated with current practices.

Political-Legal Aspects

There was a general consensus that the research needs to make wastewater recycling politically acceptable, legally sound, and institutionally manageable should have more immediate attention than research requirements in other areas where much more is known about the associated problems. Included should be: case studies to examine the conditions which made this waste management technique acceptable in some areas but totally unacceptable in others; research to determine what kinds of land-use regulations are necessary and desirable to avoid unacceptable environmental degradation; research to examine and evaluate the kinds of incentives which are now present or should be considered to make wastewater recycling acceptable; research to determine the optimum multi-jurisdictional "mix" of political groups to make this management technique politically acceptable and institutionally manageable; research should be undertaken to understand better the underlying causes and to explore the kinds of recommendations that might be made to foster a climate conducive to cooperation and mutual trust; state enabling legislation, administrative codes, rules and regulations need to be examined to determine the authority of the receiving jurisdiction to regulate land disposal. Research is needed to evaluate new concepts and methods to determine the types of controls necessary to preserve a quality environment consistent with the necessity to dispose of wastes in an aesthetic and cost-effective manner.

Economic Aspects

The topic of assessment of the scope of project impacts received a high priority rating. It would require assessment of the reduction in damage resulting from the improvement in water quality associated with land treatment activities. Changes in insecticide and fertilizer sales as crop varieties are changed in the project area, are indirect effects of the project which need extensive research. Impacts of changes in land use and land values including potential capital gains resulting from alternative uses need to be assessed. These indirect impacts may be as important as the direct impacts for assessing the economic consequences of the land utilization system.

Another economic research topic given a high priority was an economic evaluation of improvement in product markets. For example, new uses of the organic matter, nutrients, and water in the effluent need to be carefully explored.

Biological and Chemical Aspects

The high priority research needs in this area was considered to be associated with evaluating the public health problems associated with wastewater recycling. Included would have to be further studies on pathogenic organisms, toxic metals, nitrogen and nitrates, and developing protocols for site selection and monitoring programs.

Hydrological and Physical Aspects

Research needs in this area should be directed at the goals of optimizing the hydraulic load while maintaining a viable plant cover and in meeting water quality standards for surface or ground waters. It was agreed that water balance studies have received adequate attention but deep percolation and surface runoff need to be studied further.

Engineering and Management Aspects

The highest priority need identified in this discipline was to document the mangement of existing facilities. Considered should be equipment, operational features, personnel needs, and dollar and energy costs. It was pointed out that engineering systems for wastewater recycling are, at present, afterthoughts in reaction to the development of our cities. The planning profession should be encouraged to develop techniques to anticipate the wastewater development needs predictively in the future rather than to react as we have been forced to do today.

Environmental Aspects

The topic of research needs related to environmental aspects of recycling municipal wastewater focused primarily on the human health related problems that might be associated with such recycling, both with respect to the immediate on-site location and to pheripheral off-site locations.

THE GLOBAL PERSPECTIVE

I would now like to supplement the above list with a discussion that hopefully puts wastewater management into broader perspective. Backing away from the end of a sewage treatment plant effluent pipe is necessary if we are to gain the perspective needed to manage our wastes within the long-term constraints of the biosphere. Too often we become so involved with engineering optimal designs for some small segment of our complex technological society that we totally loose sight of the direction that many of these technologies are taking us. Take for example the complex engineering designs for the plumbing which is located downstream from a technology all of us take for granted, the flush toilet. This technology was no doubt prompted by the need for convenience, and at the time of its invention, the problem of how to get rid of human waste to avoid problems of disease was paramount. Our modern sewer system of pipes, valves, lift stations, vacuum filters, etc. are nothing more than a sophisticated response to an 18th century problem.[1] True, disease potential is still a problem and will continue to be, but backing away from the end of the pipe in the 20th century might reveal problems of equal or greater importance.

As we move further and further away from the end of this pipe in the United States, we see a complex of more and more pipes but soon they all blend into one large pipe, sucking enormous quantities of finite mineral resources at one end, powered by a pump consuming oil and coal at a rate of thousands of billions of K cal per year, and discarding a mixture of water and minerals out the other end of the pipe to a place where we can't get them back. Simply stated we have so closely geared our wastewater technology to the 18th century goal of getting rid of wastes that we have lost sight of the 21st century problem of depletion of available mineral and energy resources.

An example of one such resource threatened by this absurd practice is the element, phosphorus. The biogeochemical cycling of this element with and without man is shown in Figure 1. Total known and potential reserves of the world amount to approximately 5.2 billion metric tons, 41% being in the U. S. (Johnson, 1974). About 76% of all phosphates refined in the U. S. are channeled into fertilizer production and 7% into detergents and soaps. An unknown but undoubtedly very small fraction of it is ever reused. At current and projected per capita consumption rates of this element coupled with projected population increases we could deplete this resource as soon as the year 2075 (Johnson, 1974). Independent

[1] For a more thorough discussion of this topic the reader is referred to the excellent paper by Love (1975).

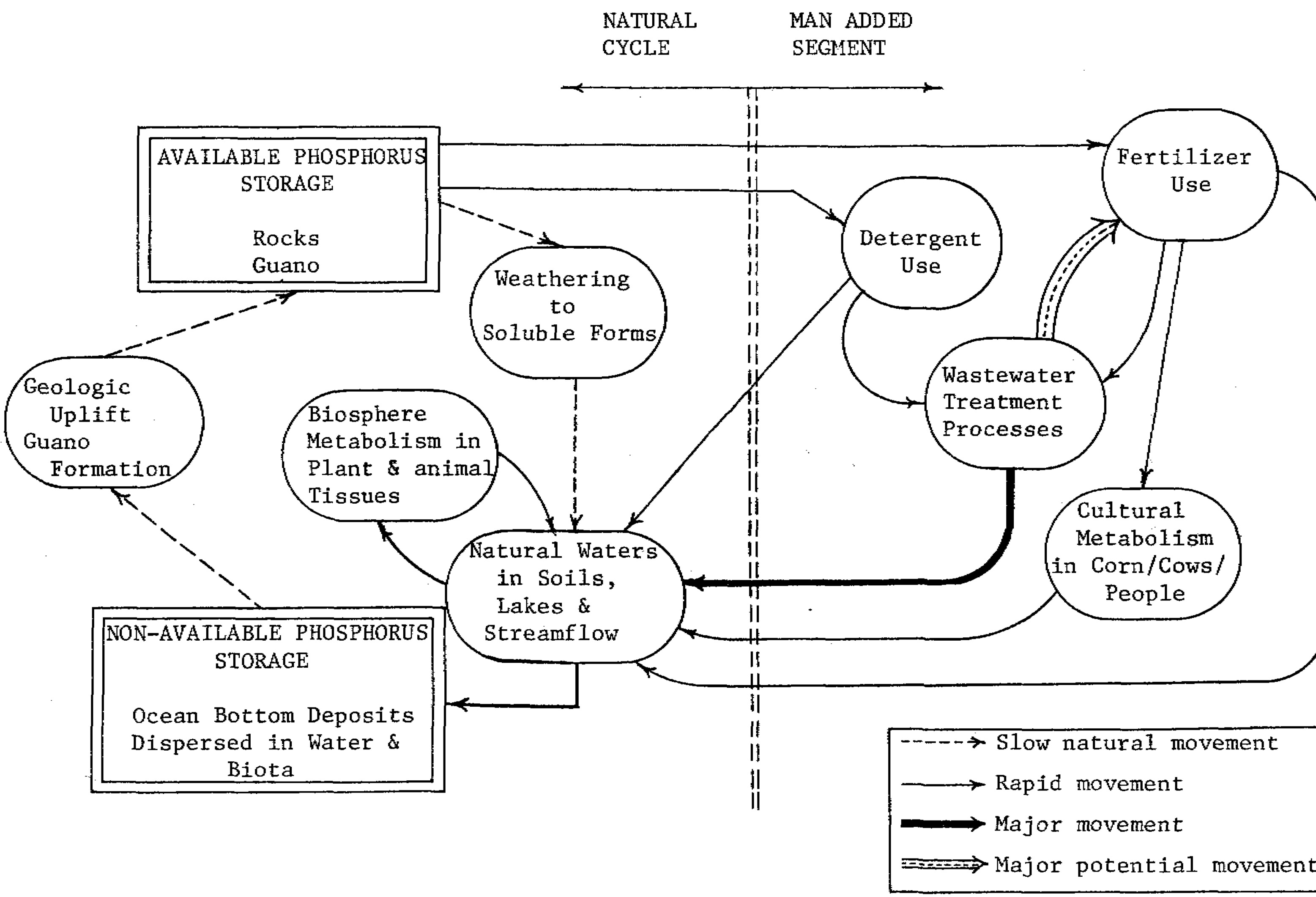

Figure 1. The phosphorus cycle illustrating natural and human induced features. *Note the rapid movement to non-available storage induced by human activity and the potential recycling that could be accomplished by terrestrial application of wastewater. (After Bahr, Cole and Stevens, 1972)*

projections by Mesarovic (1973) looking on the global scale are even less optomistic. Thus, it is vividly obvious that there is no alternative within the long-term constraints of our finite mineral and energy resources than to do anything but recycle. To quote Dr. Howard Tanner (GLUMORBA, 1974) in reference to recycling he states:

> *"It will not be redirected for the sake of a clean environment. It will be redirected as a closed loop because it will be economically necessary. It cannot be done by an adjustment to existing concepts but rather must be grasped as a totally different objective -- to renovate and reuse in place of dispose of."*

To restate my biases mentioned earlier, I am confident in the ability of natural systems to apply negative feedback control on such wasteful practices as our management of phosphorus. Economic necessity for recycling as mentioned by Dr. Tanner is no doubt going to come about, but I do not have the confidence in our system of pricing goods and services to the point where I feel comfortable that economics will redirect our practices in time to avoid chaos.

It is still economical to waste and we have already incurred an irreversible loss of mineral resources. In addition, we are suffering an environmental side effect in the form of eutrophication (See Vollenweider, 1968 and Borgstrom, 1973).

Coming from a state surrounded by the Great Lakes I am often asked about the condition of Lake Erie. Quite simply stated it is extremely productive -- but this productivity is not channeled into human food resources. The nutrient loadings are extremely high and originate largely from controllable municipal and industrial wastewater effluents. This can be seen in Table 1, in a breakdown of nutrient sources to the heavily populated western basin of the lake. Of the 16,724 thousand Kg/year of

Table 1. Phosphorus loadings to the western basin of Lake Erie (after Bahr, 1972).

Source		Phosphorus Loading Thousands of Kg/yr
Lake Huron	Total	2,807
Detroit River (excluding Lake Huron)		
U.S. Municipal and Industrial		8,586
*U.S. Runoff		483
Canadian Municipal and Industrial		1,336
*Canadian Runoff		620
	Total	11,026
Maumee River		
Municipal and Industrial		1,563
*Runoff		806
	Total	2,370
Huron River		
Municipal and Industrial		116
*Runoff		75
	Total	191
Raisin River		
Municipal and Industrial		85
*Runoff		95
	Total	180
Portage River		
Municipal and Industrial		70
*Runoff		78
	Total	148
	GRAND TOTAL	16,724

*Includes geological leaching and all other non-point sources phosphorus entering the western basin of this lake an estimated 80% of the inputs represent controllable sources. If only half of this amount were directed to agricultural land it would satisfy the yearly phosphate fertilizer requirements for 385,000 hectares (1,486 mi^2) of corn. Yet, in keeping with the 18th century goal of getting rid of waste and embracing non-recycling waste treatment technologies of the 1930's, millions (perhaps billions) of dollars in capital outlays and millions of dollars of annual operational costs are scheduled to be spent on activated sludge treatment plants. These facilities will remove carbon and will also employ chemical phosphorus precipitation techniques. The assumption being made is that phosphorus removal from sewage will limit primary productivity in Lake Erie and eutrophication problems will be eased. This assumption is probably true but what is the goal? It's obviously to reduce the rate of eutrophication and through disinfection, reduce the health hazards associated with the lake. These are admirable goals that few would disagree with. Do we not, however, have as a goal the conservation of mineral resources? And what about the goal of conservation of our energy resources? Sure these are important goals and we even have whole agencies devoted to maximizing these goals. But back to simple mathematics. I was taught that in a differential equation you cannot maximize more than one variable (goal) at a time. You can optimize several variables (goals) but not maximize them simultaneously.

In terms of wastewater management in a watershed we cannot for example simultaneously maximize the prevention of eutrophication through conventional chemical phosphorus precipitation and maximize the conservation of mineral resources. It is quite clear in this case that mineral resources would not be conserved since this scheme for wastewater treatment provides for no recycle of phosphorus back into the human food chain. If we maximize for energy conservation we would be forced into not treating sewage since this is a technology that requires energy. We would thus conserve energy at the expense of increasing the rate of lake eutrophication.

If we opt to maximize the conservation of mineral resources we might shut down our phosphorus mines in Florida, close our fertilizer factories and build a large network of pipes from the flush toilets of Detroit to the agricultural fields of Iowa and Illinois. We would have to install gigantic pumps to handle the tremendous quantity of water and energy costs would not be cheap. Moreover, we would have to make an all out effort to prevent the runoff of nutrients from our fields, adding to our energy expenditure. In this case, however, we would have clean lakes and streams in addition to an assured supply of nutrients. Could we afford the energy expenditure of achieving this goal? Closing the fertilizer industry and eliminating conventional sewage treatment plants would certainly represent an energy savings. But how much? Pumping sewage from Detroit to Iowa would certainly represent an energy cost, but how much? And if we tried to reduce the amount of water we had to pump to Iowa (a definite energy savings) how much energy would be required for example to overhaul our system of flush toilets and washing machines in order to use less water? I think these are relevant questions. Moreover, I think they are questions that need answers before any rational policy on wastewater management can be arrived at.

I believe that goals should reflect the desires of an informed public and that the scientific community has a responsibility to inform the public on the consequences of trying to achieve these goals. Yet, are there any published literature that can provide, in a quantitative sense, the consequences of trying to achieve various combinations of goals associated with managing wastewater? I am not aware of any.

At the risk of being accused of making invalid assumptions and extrapolations I would like to present some calculations that attempt to answer a few of the questions raised earlier. This exercise is intended to illustrate an approach, not to prove or disprove the value of a particular alternative. If nothing more, I hope this evaluation will generate discussion and more sophisticated inquiry since it is a critical research need.

ENERGY, WASTEWATER AND MINERAL FERTILIZERS

In 1972 we became involved in a study to evaluate the environmental impact of a series of wastewater management schemes proposed by the Detroit District of the U. S. Army Corps of Engineers (Bahr, 1972). The area encompassed the southeastern portion of Michigan (population = 5,000,000). Several basic wastewater management schemes were proposed: (1) advanced biological treatment; (2) physical-chemical treatment; and (3) land application of partially treated wastewater. Dollar costs for comparable levels of treatment for each were competitive but it was obvious that the land treatment alternative was the only scheme that involved nutrient recycling. Yet, a close look at electrical energy requirements revealed that transmission of wastewater to acceptable land areas represented considerable energy costs. The difficult question to answer in this regard was what would be the savings in energy due to the fact that the land application sites would not require inputs of fertilizer. The energy costs associated with acquiring raw materials and subsequent processing and transportation of fertilizers are certainly real but at the time of this study these data were not readily available. To be sure, any accurate energy assessment of a waste treatment alternative must take these energy savings into consideration. I will present a few rough estimates of these energy costs and savings and will present them along with the approach and assumptions made in arriving at them.

Energy for Fertilizers

The first comprehensive look at energy requirements for a model agricultural production unit was conducted by Pimentel, et al. (1973) in their input-output analysis of corn production. Employing various assumptions and drawing on numerous references that can be found in the original paper they calculated the caloric input to a one acre corn field. Of the total caloric input, 36% was from fertilizers; 940,800 K cal for nitrogen, 47,100 K cal for phosphorus and 68,000 K cal for potassium. These values were based on energy equivalents of 18,500 K cal/Kg N, 3,350 K cal/Kg K, and 2,310 K cal/Kg K, including mining and processing In the case of phosphorus, a more recent calculation by Johnson (1974) revealed a 30% higher energy value for the mining and processing of this element; 4,300 K cal/Kg P. Arriving at these values is an exaustive process and when the approach used is a direct one (summing only the identifiable energy costs) one generally comes up with an underestimate of the total energy input to a particular product. Additionally, the calculated energy equivalent for a particular good is probably a function of the "energy" put into the analysis itself.

Another approach to arriving at the energy required to produce a product and one that avoids some of the above problems is to use the method of Odum (1971). Here, he assumes that the price paid for a good is equivalent to the total work done in the economy to produce that good. In this case, money flows countercurrent to fossil-fuel energy and on a national scale, a dollar will reflect a certain quality of fossil-fuel calories. We briefly explored this approach and used a value of 25,000 K cal of fossil-fuel equivalents per dollar (Odum, personal communication) to recalculate the energy cost of producing fertilizer. The cost of concentrated superphosphate (47% P_2O_5) fertilizer in 1970 was \$83 per metric ton (U.S.D.A., 1974) or 37¢/Kg expressed as P. At 25,000 K cal/\$ a Kg of phosphorus is thus equivalent to approximately 9,700 K cal. This value is about 190% higher than that given by Pimentel, _et al_. (1973), and 125% higher than that calculated by Johnson (1974). Assuming this calculation is valid for the phosphorus industry, there apparently are many hidden energy costs not included in even an exaustive evaluation.

The 1970 cost of K_2O of 56 per metric ton (U.S.D.A., 1974) amounts to 6.6¢ per Kg expressed as K or 1,690 K cal/Kg. This energy value is about 27% less than shown by Pimentel, _et al_. (1973). Arriving at a comparable cost for nitrogen fertilizer is complicated due to the changing nature of the feedstocks for synthesizing ammonia and the nature of the final fertilizer form. Using an approximate value of 15.4¢ per Kg for N in the urea form for 1970 obtained from extrapolating data from Harre, _et al_. (1973) gives an energy equivalent value of 3,860 K cal/Kg N. This is obviously a gross underestimate of the true value since direct consumption of natural gas and other fossil-fuel feedstocks represent a high percentage of the materials needed to produce ammonia. Easily accounted for feedstocks alone such as natural gas, naphtha, oil and coal input approximately 13.8 million K cal per metric ton of ammonia or about 16,760 K cal per Kg N (Calculated from values given by Harre, et al, 1974). This value quickly approaches the value of 18,500 K cal/Kg N used by Pimentel, et al. (1973).

For purposes of arriving at an estimate of the true energy value of different fertilizer components for comparative purposes I will adopt as a base, a value of 9,700 K cal/Kg for phosphorus arrived at using Odum's energy equivalent value of 25,000 K cal/dollar and 1970 prices. This value is 125% higher than the most exaustive direct calculation to date (Johnson, 1974) and thus may be considered as a conservative multiplier in arriving at energy equivalents for nitrogen and potassium based on direct calculations. Using the energy values of Pimentel, et al. (1973) for nitrogen and potassium and multiplying them by 2.25 to arrive at "hidden" energy costs makes nitrogen equivalent to 41,700 K cal/Kg N and potassium equivalent to 5,208 K cal/Kg K. Using 1970 fertilizer application rates of 125 Kg of nitrogen, 35 Kg of phosphorus and 67 Kg of potassium per hectare (U.S.D.A., 1971 as cited by Pimentel, et al., 1973) yields Table 2.

Table 2. Fertilizer energy inputs to corn production.*

Element	Energy Equivalent K cal/Kg	Application Rate Kg/ha	Totals K cal/ha
Nitrogen	41,700	125	5,212,500
Phosphorus	9,700	35	339,500
Potassium	5,208	67	348,900
		Total	5,900,900

*Modified from Pimentel, et al. (1973). See text for details. Energy costs do not include those for application of fertilizer to field.

The total fertilizer energy input to a one hectare corn field is calculated to be about 5,900,900 K cal, not including additional energy expenditures in applying the fertilizers. Using this energy value as a base for comparison one can develop a comparable set of energy values using secondary effluent as a nutrient source rather than manufactured mineral fertilizers.

Energy for Wastewater Irrigation

Values for the nutrient concentration in "typical" secondary effluent used in the following calculation are N = 40 mg/l (total), P = 10 mg/l, and K = 14 mg/l (Pound, 1973). To meet the application rates shown in Table 1 it will be necessary to apply a minimum of approximately 4,900 m^3/ha of secondary effluent. Due to an imbalance in nutrient ratios for wastewater it will be necessary to exceed application rates for nitrogen and phosphorus in order to meet the minimal requirements of 67 Kg/ha of potassium. The net nutrient loadings at 4,900 m^3/ha would be 197 Kg N, 49 Kg P, and 67 Kg K.

Published energy costs for wastewater treatment have largely focused on operational electrical power consumption and no comprehensive search has been made into other energy considerations such as energy costs for capital construction and energy requirements for synthesis, processing and transportation of chemicals. Smith (1973) gives an excellent review of electrical energy costs for unit processes in wastewater treatment. Using his values, secondary treatment of 4,900 m^3 of wastewater would use 1,112,000 K cal and tertiary treatment approximately 988,000 K cal. These values, however, are underestimates.

For purposes of energy comparisons let us assume that secondary effluent can be obtained at no cost. True, a considerable amount of energy has gone into producing a secondary effluent but it is also true that in most places in the U. S. it is simply wasted into the nearest surface stream. The energy costs in transforming "free" secondary effluent into a useful nutrient source for agriculture would not be unlike (conceptually at least) the energy costs of transforming "free" rock phosphorus or atmospheric nitrogen into mineral fertilizers. In the case of secondary effluent, these costs would largely be associated with energy costs of conveying the effluent to agricultural land.

Johnson (1974) estimated these energy expenditures to be approximately 14,000 Kw hr/million m^3 of effluent/Km for conveyance and 300,000 Kw hr/ million m^3 for irrigation. For a transmission distance of 1 Km this would total 58,800 K cal for conveyance and 1,260,000 K cal for irrigating 4,900 m^3 of secondary effluent. These values only consider operation energy costs and not energy costs for capital equipment and other materials and supplies. An estimate of the total energy costs for a system of conveyance and irrigation can be calculated using the dollar cost data of Pound (1973) and Odum's energy/price ratio. For a hypothetical spray irrigation system Pound calculates a cost of 19.7¢ per 1,000 gals. This includes capital costs of land, equipment, pumps, earthwork, etc. as well as operating costs. At 25,000 K cal/$, this system would require 6,450,000 K cal to convey and irrigate 4,900 m^3 of secondary effluent on one hectare of land. It is important to note that in this case, the land application site was located only 1.6 Km from the wastewater source.

Wastewater Application vs. Mineral Fertilizers

Using values arrived at above it is possible to develop comparative energy requirements for conventional mineral fertilization and for land application of wastewater. From Table 2 the energy cost for mining, processing and transporting N, P, and K to the farm amounted to 5,900,900 K cal per hectare. An additional 2,520,000 K cal per hectare would be required to apply the fertilizer to the fields based on the study of Johnson (1974). And if the one hectare corn field receives 30 cm of irrigation water per season, as many are, an additional 2,238,000 K cal per acre would be required. Thus, the total energy inputs for fertilization and irrigation based on the above values and assumptions would be approximately 10,625,000 K cal/ha.

Wastewater irrigation on the other hand would require approximately 6,425,000 K cal/ha to achieve a comparable nutrient and water loading; 4,200,000 K cal/ha less than with mineral fertilizers.

The above assumes that the distance from the wastewater source to the farm is only 1.61 Km. The economic evaluation by Pound (1973) showed that the capital costs amortized over 15 years resulted in a cost of approximately 10¢/1,000 gal or \$130 per 4,900 m^3. Of the \$130 per 4,900 m^3, 33% or \$42 per 4,900 m^3 was attributed to the cost of the 1.61 Km transmission line. Converted into energy equivalents each additional Km would thus require approximately 652,000 K cal over the life of the transmission line. Operating costs of 58,800 K cal/Km would raise the caloric input per Km of transmission line to 710,000 K cal/Km. Since the energy inputs to the wastewater irrigation system discussed earlier were 4,200,000 K cal less than a comparable field receiving mineral fertilizer, this amount of energy could be put into additional transmission line. In fact about 6 more Km or a total of 7.6 Km of transmission line could be used in the wastewater irrigation system before the caloric inputs of the wastewater irrigation system exceed that of conventional fertilization.

The calculations given above are at best, rough approximations and represent a quick synthesis of available data from other studies. A point to be made however is that wastewater irrigation does appear to represent a competitive energy alternative to conventional fertilization in some cases. This, of course, needs more detailed study.

In a period of our history where world food problems are critical, energy availability is questionable and vital plant nutrients are becoming more scarce, a more in-depth evaluation of the type presented above would appear to be a necessary prerequisite for any wise policy decision relating to the management of wastewater or fertilizers. I would consider such an evaluation as the highest priority research topic facing us at this time.

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A Facility for Research In Recycling Treated Wastewater

Robert C. Ball
Institute of Water Research
Michigan State University
East Lansing

Facility and Research supported by Rockefeller Foundation, Ford Foundation, Kresge Foundation, Environmental Protection Agency, Michigan Clean Water Bond Issue, and Michigan State University

A Facility for Research In
Recycling Treated Wastewater

One of the greatest environmental questions is what to do with vast quantities of municipal wastewater. Conventional methods of wastewater treatment can contribute importantly to wastewater clean up but the discharge of even these wastes has created eutrophication of lakes and degradation of waterways through the nutrient materials that they contain even after advanced treatment. We are also becoming aware of perhaps an even more important problem. Many products used by man contain elements that represent scarce and diminishing resources or compounds that have a high energy demand in their manufacture. In an economy faced with both material and energy shortages we cannot condone the manufacturing of products at high cost to both of these, use them once and then discard them. But this is exactly how we handle plant nutrients. We mine our increasingly limited supplies of phosphorus and use our scarce fossil energy in the production of nitrogen fertilizers in order to sustain the food production that feeds the millions in our cities only to waste these elements into the nearest stream. This gross mismanagement coupled with requirements for high water and air quality and the difficulties of removing nutrients to levels acceptable to discharge into natural waters has prompted an extensive search for alternate methods.

It is well known that nutrients present in wastewater can be turned into the production of harvestable food and fiber if applied in appropriate amounts to the land or to the water. The quantity and schedule of application of wastewater to agricultural lands or to aquaculture systems to achieve an optimal level of food or fiber production and, at the same time, achieve a high level of water renovation is not well understood at this time. The number and depth of research studies in this area has been very limited and there are many important questions vital to human health, energy and resource conservation that have not been answered. We believe that maximum efficiency of research on these questions can be obtained from an integrated study of recycling systems incorporating the facets of both terrestrial and aquatic ecosystems. The plans, estimates and designs for functioning reuse and recycling systems cannot be based on concepts alone but must be reduced to reasonably accurate estimates based on experience gained with working model systems. Such systems will require a coordinated multi-disciplinary effort of considerable magnitude.

PROJECT DESCRIPTION

The physical facility for the Michigan State University wastewater recycling project consists of four basic elements: (1) a conventional activated sludge sewage treatment plant; (2) a 4.5 mile transmission line; (3) a lake system; and (4) a land irrigation system. This system is schematically shown in Figure 1 and 2 and by airview in Figure 3. The following is a more detailed discussion of the facility.

Sewage Treatment Plant and Transmission Line

The East Lansing Sewage Treatment Plant services the City of East Lansing, Michigan State University with its 50,000 students and employees, and the adjoining community and represents the first element of our system. It is presently undergoing modification and enlargment to a capacity of 15,000,000 gallons per day. As an integral part of this modification the Michigan State

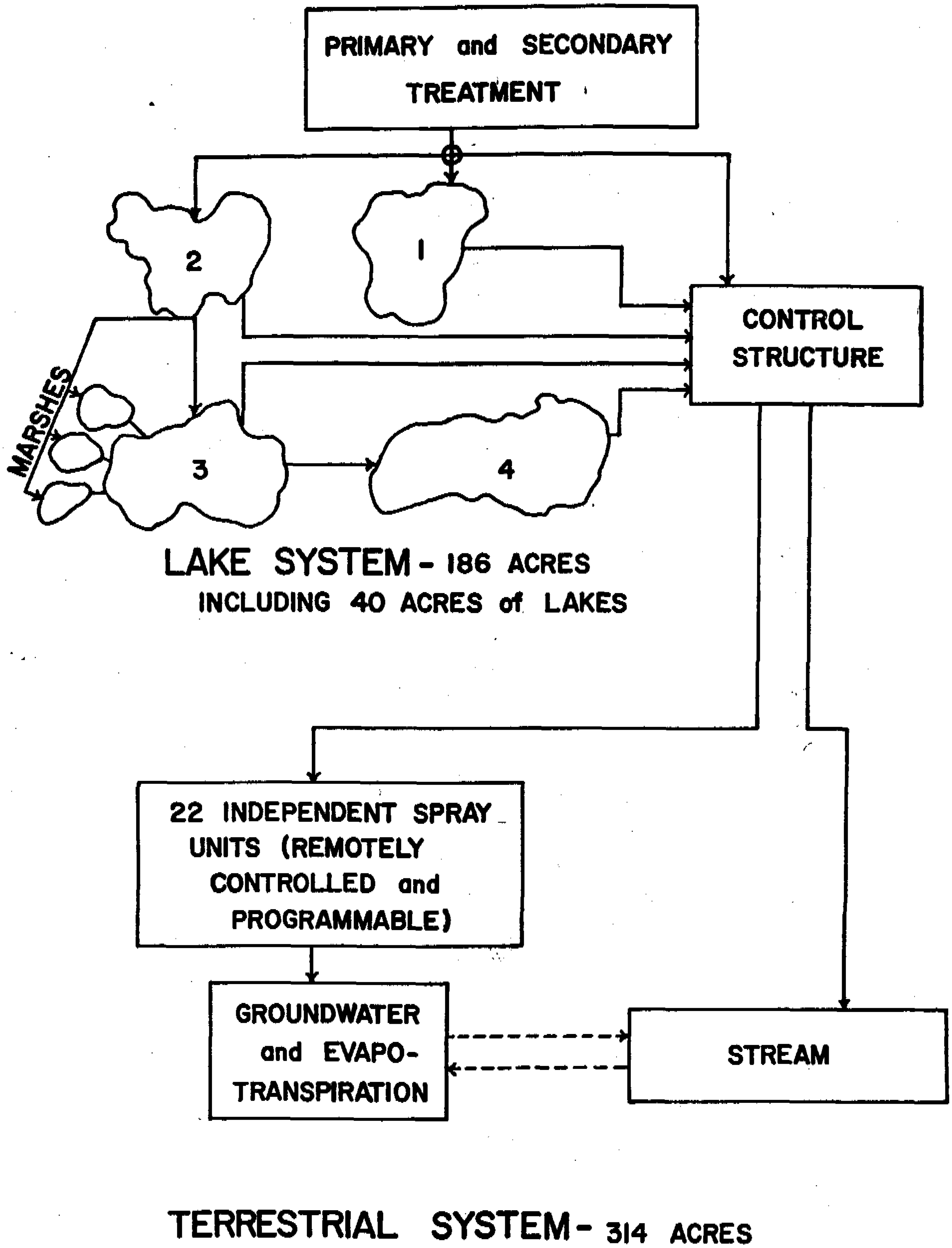

PRIMARY and SECONDARY TREATMENT
2
1
CONTROL STRUCTURE
MARSHES
3
4
LAKE SYSTEM - 186 ACRES
INCLUDING 40 ACRES of LAKES
22 INDEPENDENT SPRAY UNITS (REMOTELY CONTROLLED and PROGRAMMABLE)
GROUNDWATER and EVAPO-TRANSPIRATION
STREAM
TERRESTRIAL SYSTEM - 314 ACRES

Figure 1

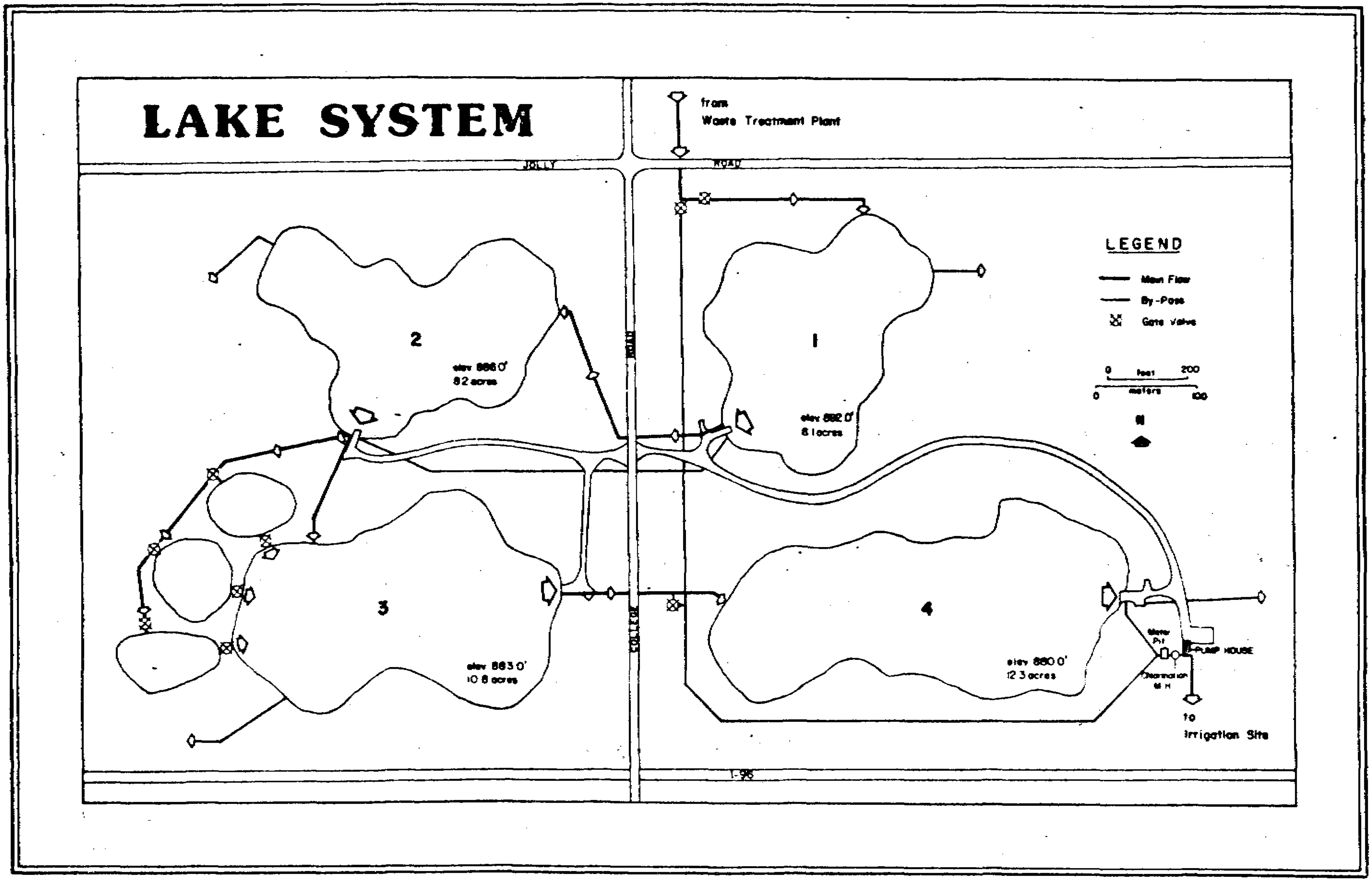
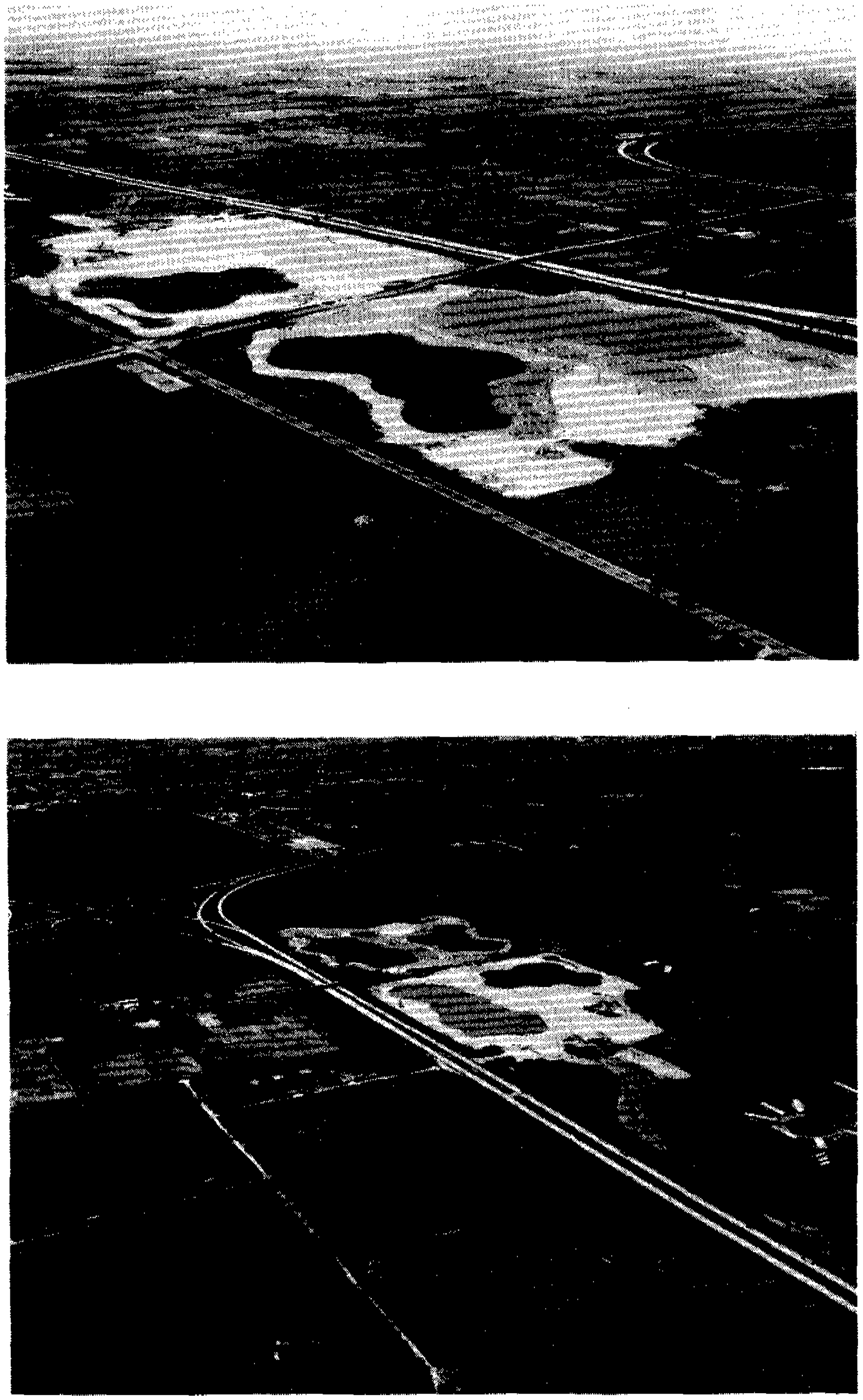

LAKE SYSTEM
from
Waste Treatment Plant
JOLLY
ROAD
LEGEND
Main Flow
By-Pass
Gate Valve
0 feet 200
0 meters 100
N
2
elev 886.0'
8.2 acres
1
elev 892.0'
8.1 acres
3
elev 883.0'
10.8 acres
4
elev 880.0'
12.3 acres
PUMP HOUSE
to
Irrigation Site
I-96

Figure 2



Aerial views of the completed Water Quality Management Project. The upper photo approaches from the north with the three marshes visible center right. The lower photo presents a southern approach, affording a panoramic view of the site and adjacent research areas utilized by other departments within the University.

FIGURE 3

University program will develop and operate a parallel unit within this plant that will have a capacity of 2,000,000 gallons per day. Our portion will differ from the total plant in that the effluent being received will not have pre-treatment for the partial chemical removal of phosphorus. The effluent (secondary or primary) from this subunit can be directed to a pumping station with a present capacity of 2,000,000 gallons per day and provisions for additional pumps to increase its capacity to 6,000,000 gallons per day. Flow will be transmitted through the second element of the system, a 21 inch concrete-asbestos pipeline which traverses 4.5 miles to the southern border of the Michigan State University campus and on to the 500 acre research site. Here it discharges into the first of four man-made lakes.

Lake System

The flow from the first lake is then by gravity through each of the other lakes and to a control building and pump house servicing the adjacent spray irrigation site. The lakes have a total surface area of 40 acres with the maximum depth of 8 feet at each outlet structure and a mean depth of 6 feet. This depth was chosen to maintain the entire bottom within the euphotic zone in order to encourage the growth of rooted aquatic plants. The bottom was also contoured in a uniform manner to permit mechanical harvesting. Each lake has a collection basin at the outlet so that water may be lowered to collect fish or other aquatic fauna in a very small area. This area is serviced by a ramp to allow access by both boats and trucks. Control of discharge and lake level is afforded by both sliding gate valves and slash boards. The interlake transfer system and connection to the irrigation site is so designed that effluent can be taken directly from the pipeline or water can be intercepted at the discharge of any of the lakes in the system or mixed from any combination of lakes. This feature will afford researchers with a wide range of water qualities to be applied and tested on the irrigation site.

Marsh System

Marsh systems with their high rates of internal nutrient cycling may prove to be an extremely efficient system for the uptake and conversion of wastewater nutrients into useable products. To test the possibilities and feasibility of this idea we constructed three, one acre marshes which are fed from the discharge of Lake 2 in the system. Return water from the marshes enters Lake 3. The basins of the marshes were constructed in a terrace design that resulted in three zones of depths of 18, 24 and 36 inches. It is estimated that this will allow us to create in these basins biota quite comparable with natural marshes of this area.

In the construction of the lake basins and marshes particular attention was given to the sealing of the basins to prevent loss of water through the bottom soils. They were sealed with native clay and percolation tests indicated a low permiability of 0.07 inches per day.

Land Irrigation System

The last element of the system is a terrestrial site located on 350 acres immediately south of the lake system. As with much of the soils of the glaciated parts of Michigan and the Great Lakes basin there is a large array of soil types ranging from heavy clay to light sandy soils. Within the terrestrial site are forested areas, a pine plantation, cultivated fields and fields that have allowed to regress into old-field plant associations. The land is gently rolling and the entire area is drained by the Fenton outlet which enters a tributary of the Red Cedar river which in turn flows into the Grand River, a tributary of Lake

Michigan. The irrigation site is bounded on three sides by an 800 foot buffer zone where we will not spray effluent at the present time but it is available for overland flow irrigation and research not requiring spraying of effluent.

Within the terrestrial area is a 145 acre spray irrigation site. Twenty-two individual valves, remotely controlled and programmable from the control building, direct the flow of water to the sets of surface irrigation pipe. Four, 700 foot tapered aluminum laterals extending north or south from a valve comprise a set. Water supply to each set is carried by a 21 inch underground pipe and the system is designed for winter operation. Spraying is by conventional Buckner 8600 sprinkler heads. The design of the individual spray valve systems is shown in Figure 4.

Within and surrounding both the lake and land sites we have drilled approximately 60 wells to monitor the level and quality of subsurface water. Depths vary from just a few feet into the glacial drift to over 200 feet, within the underlying sandstone aquifer. Approximately 1/3 of these wells will be equipped with automatic monitoring and sampling equipment as part of a major effort to characterize the subsurface hydrology in the region. All wells are cased, sealed and fully protected from external contamination. Surface water flow for the entire watershed in study will be measured by means of a network of weirs. Many of these are now installed. Climatological stations at selected points in the area provide the additional information to generate a detailed look at the entire hydrological cycle for the area.

NOVEMBER 1, 1974

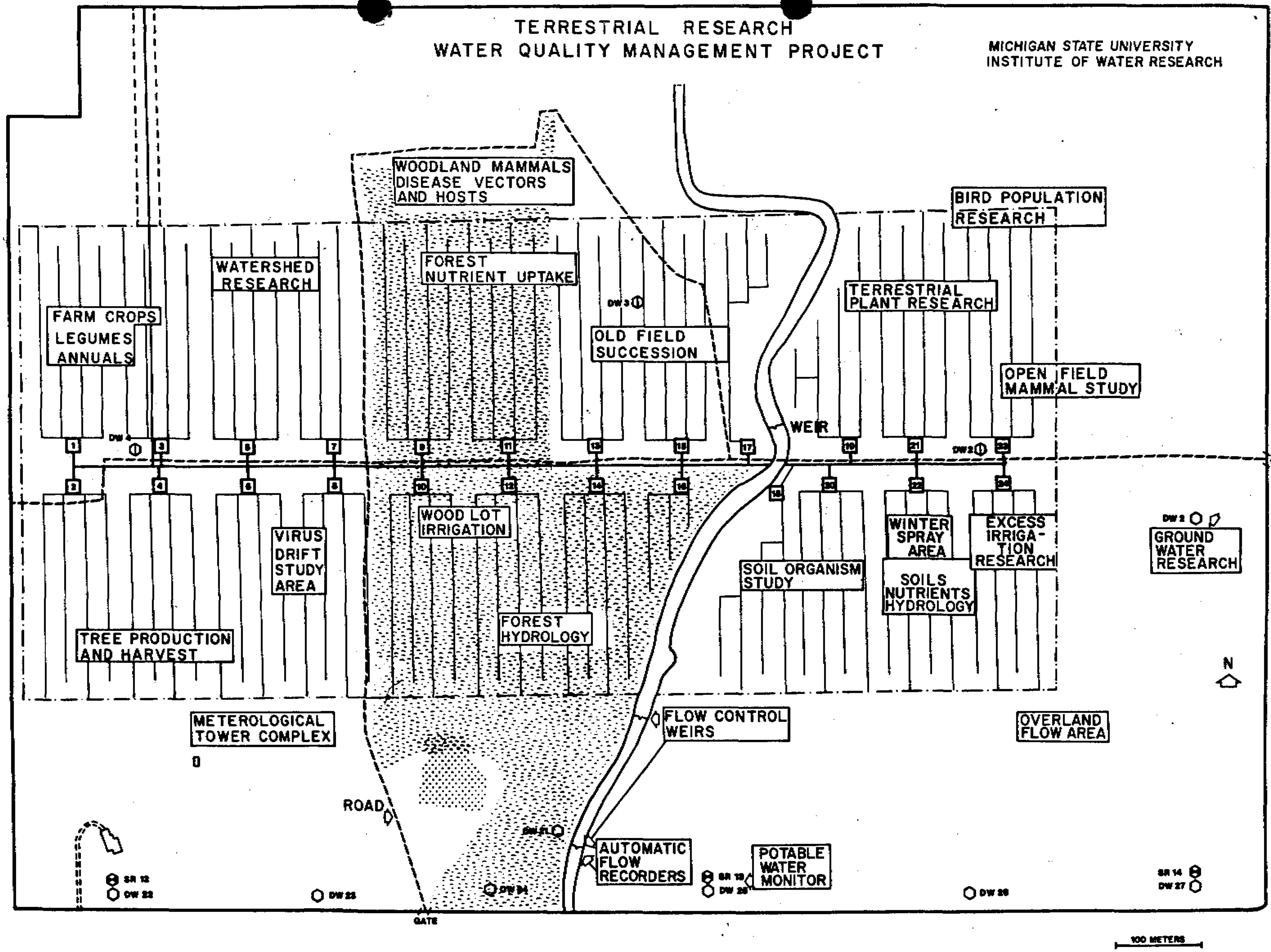


Figure 4

Total System Flexibility

The physical facility as it was designed affords maximum flexibility in the development of research involving an integrated land and lake management system for the restoration of water quality and the recycling of nutrients. We have the potential for hydraulically stressing any area of the terrestrial system with effluent far in excess of its capacity of handling it to the extreme of no water application above that of natural rainfall. Within this range we have the capability of spraying at a variety of qualities as selected by the investigator, and on any temporal pattern that lends itself to research in the development of a particular management strategy.

MONITORING

As the research on the many facets of wastewater renovation and nutrient recycling progresses there will be need for basic data common to many projects. In our judgment the most efficient and accurate means of having these data available is from a central laboratory staffed by highly trained and competent technicians directed by a senior scientist. The data base generated by these laboratories will be available to any research worker upon request.

In addition the directors of these units will be carrying out basic research in their specialty.

Hydrologic Studies

Studies are also underway at the terrestrial site to explain the hydrological response as the watershed receives spray irrigation with treated municipal wastewater. Inasmuch as major producing wells are situated nearby, the groundwater flow beneath the WQMP must also be monitored carefully to assess the impact of the spray irrigation and lake operations on the aquifer system. A comprehensive groundwater study has been underway for over two years with the ultimate objective of being able to predict and monitor the dispersive nature of water with varying degrees of quality in the flow regions of the aquifer.

A digital computer program has also been developed and implemented to make use of triangular finite elements. With the Galerkin method the space variables can be distinguished in the basic unsteady flow equation. Coupled with a central difference time step formulation, this can solve the resulting systems of algebraic equations. The computer model is designed to handle a variety of regional situations, including steady or unsteady and confined or unconfined flows, finite element inputs such as recharge, pumping and field properties. Numerical solutions show some of the limitations of the model. Applications to field situations emphasize flow analyses at the Water Quality Management site. A six year simulation of the hydrodynamic response of the aquifer is also being compiled with historical pumping data.

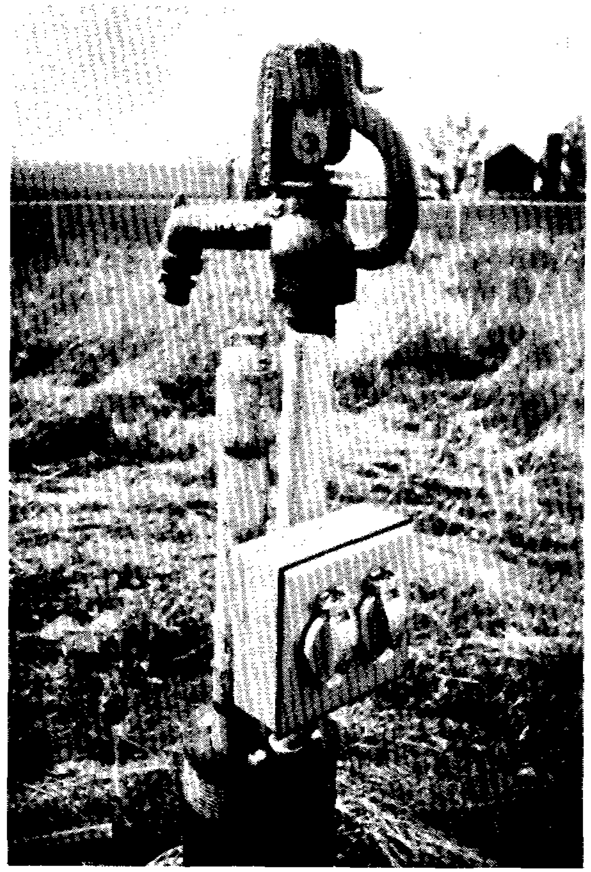
In another aspect of this project, flows are being analyzed in the glacial drift overlying the aquifer. Once these flows have been traced more accurately, the percolation of recharged water downward into the main aquifer can be estimated and its response studied. In addition, the unsteady flow model will be coupled with the convective-dispersion equations to predict water quality in the aquifer. In the future isoparametric elements in the flow model will be used to ease data input manipulation and reduce the required computer storage. At the same time the available numerical techniques will be reviewed to solve the convective-dispersion equations and determine which ones are best suited for the present system. Then comprehensive analyses of surface hydrology and runoff will be combined with subsurface flow at the spray irrigation site to explain the total movements of irrigation water.

The piezometric surface and water table conditions at the site are now being monitored so that future flow predictions can be correlated with field data. Six automatic water level recorders were installed on selected test wells for continuous monitoring. At specified time intervals, water levels in all of the test wells (Figure 5) are manually recorded by means of drop lines. Well borings have been analyzed and the coordinates of all the test wells in the system have been determined.

CHEMICAL AND PHYSICAL MONITORING PROGRAM

The Water Quality Management Project Laboratory is monitoring over 50 chemical, physical and biological parameters as the effluent passes through various stages from the treatment plant through the lake and irrigation site. These parameters and the sampling timetable are presented in Table I. The average concentrations and ranges of selected chemical parameters for the system is presented in Table II. This analytical program will provide a data base for all scientists conducting research on the project.

Daily 24 hour composite samples are collected to represent raw, primary, and secondary effluent at the East Lansing Sewage Treatment Plant. At the site of the Water Quality Management Project, 24 hour composite samples are also collected from the influent to each lake and the final effluent concentration from Lake 4. The aquatic plants and sediments are also sampled periodically. From these data the percentage of elements for each parameter



Microbiological and hydrological sampling covers all phases of the water flow system. Above: Deep rock well, showing connection for permanent installation and frost free valve. Below: Inlet pipe to lake at low stage prior to filling.

FIGURE 5

can be ascertained to facilitate tracing their translocation into either the sediments or the aquatic plants.

Possible groundwater contamination at both the lakes and spray irrigation sites is monitored by monthly analyses of well water. Forty-one drift wells, 14 shallow rock and 4 deep rock wells have been positioned throughout the study area. All wells are 4 inches in diameter, have a three foot copper screen point and sanitary seals to prevent bacteriological contamination. The drift wells, the shallowest of the three, are positioned in the glacial drift between 40 and 60 feet deep. Samples are obtained by pressurizing the drift wells and forcing the water through a plastic pipe which extends to the bottom of the well. Both shallow and deep rock wells extend into the aquifer which provides the water supply for the University. The shallow rock wells are approximately 85 feet deep on the average, and the deep rock wells average about 180 feet. All are equipped with submersible pumps for sampling. The most severe constraint in planning the sampling program form these wells was to guard against contamination, particularly from potential sources of viruses. Comparing post-operational data with background levels should detect contamination from the lake or spray irrigation water.

Surface runoff in the study area is channeled into Felton Drain. Although it now flows only in the spring and summer, this will probably increase significantly when spray irrigation begins. Therefore, monthly samples will also be analyzed to determine the chemical characteristics of this water. Effluent from Lake 4 can be discharged from an experimental stream into the Red Cedar River via Herron Creek. At present this creek, like Felton Drain, has an intermittant flow, but operational flow levels will also be monitored.

After the effluent has been sprayed on the irrigation site, analyses will be conducted of water collected in soil section infiltrometers and plant tissue. These data will indicate how much of the remaining nutrients is absorbed by the soil and terrestrial systems after the water has gone through the lake system.

Collecting and processing of chemical parameters is automated as much possible, Figure 6

Microbiological and Viral Monitoring

At the East Lansing plant the wastewater treatment does not remove all of the pathogens from the sewage, especially the viruses which are difficult to destroy even by chlorinating the treated water. Forty percent of the samples still contained viruses after the effluent was chlorinated at the East Lansing plant. They remained in water that was discharged into the Red Cedar River just downstream from the Kalamazoo Street bridge. Viruses also remained in forty-four percent of the samples of river water taken as far as 500 feet downstream from the chlorinated effluent. The samples ran as high as 70 percent when the effluent was not chlorinated.

The WQMP microbiological and viral research program is designed to find methods of preventing public health hazards when municipal wastewater is eliminated and/or reused. The primary objectives of the program are to:

1. Measure the pathogens, bacteria and viruses in the East Lansing wastewater in the WQMP lakes and on the land after spray irrigation.

2. Determine the rate and efficiency of removing these pathogens during processing by the East Lansing wastewater treatment plant and as the water passes through the WQMP wastewater renovation system.

3. Monitor the water from the wells drilled around the Water Quality Management lakes to detect contamination of the aquifer.

TABLE I

The Michigan State University Water Quality Management Project chemical, biological, and physical monitoring program experimental analyses design.

	Chemical, Biological or Physical Parameter	Sampling Frequency						Analyses Per Year
		STP	Lake Water	Lake Sediments	Campus & Test Wells	Felton and Herron Creek	Soil Samples	
1.	Temperature	cont	cont	4Y	MIS	MIS	2Y	1032
2.	pH	cont	cont	4Y	MIS	MIS	2Y	1032
3.	Dissolved Oxygen	cont	cont	NSR	MIS	MIS	NSR	828
4.	Specific Conductance	cont	cont	4Y	MIS	MIS	NSR	924
5.	Turbidity	cont	cont	NSR	MIS	MIS	NSR	828
6.	Light Penetration	cont	cont	NSR	NSR	NSR	NSR	-0-
7.	Redox Potential	cont	cont	4Y	MIS	MIS	2Y	1032
8.	Ammonia	D24C	2D12C	4Y	MG	M168C	2Y	5777
9.	Nitrate	D24C	2D12C	4Y	MG	M168C	2Y	5777
10.	Nitrite	D24C	2D12C	4Y	MG	M168C	2Y	5777
11.	Kjeldahl Nitrogen	D24C	2D12C	4Y	MG	M168C	2Y	5777
12.	Ortho phosphate	D24C	2D12C	4Y	MG	M168C	NSR	5669
13.	Total Inorganic Phosphorus	D24C	2D12C	4Y	MG	M168C	NSR	5669
14.	Total Phosphorus	D24C	2D12C	4Y	MG	M168C	2Y	5777
15.	Chloride	D24C	2D12C	4Y	MG	M168C	2Y	5777
16.	COD	D24C	2D12C	4Y	MG	M168C	2Y	5777
17.	Silicates	W168C	W168C	NSR	MG	M168C	NSR	1244
18.	Hardness	D24C	2D12C	NSR	MG	M168C	NSR	5573
19.	Cyanide	W168C	W168C	NSR	MG	M168C	NSR	1244
20.	Sulfide	W168C	W168C	4Y	MG	M168C	NSR	1340
21.	Alkalinity	D24C	D24C	NSR	MG	M168C	NSR	2748
22.	Phenol	W168C	W168C	NSR	MG	M168C	NSR	1244
23.	Dichromate	W168C	W168C	NSR	MG	M168C	NSR	1244
24.	Fluoride	W168C	W168C	NSR	MG	M168C	2Y	1352
25.	Sulfate	W168C	W168C	4Y	MG	M168C	2Y	1448
26.	Boron	D24C	2D12C	4Y	MG	M168C	2Y	5777

Table I (cont'd)

27. Total Carbon	D24C	2D12C	4Y	MG	M168C	2Y	5777
28. Total Filterable Carbon	D24C	2D12C	NSR	MG	M168C	NSR	5573
29. Filterable Organic Carbon	D24C	2D12C	NSR	MG	M168C	NSR	5573
30. Total Organic Carbon	D24C	2D12C	4Y	MG	M168C	2Y	5777
31. BOD_5	D24C	2D12C	4Y	MG	M168C	NSR	5669
32. Suspended Solids	D24C	2D12C	NSR	MG	M168C	NSR	5573
33. Settleable Solids	D24C	2D12C	NSR	MG	M168C	NSR	5573
34. Dissolved Solids	D24C	2D12C	NSR	MG	M168C	NSR	5573
35. Hexane Extractables	D24C	2D12C	4Y	MG	M168C	2Y	5777
36. Aluminum	W168C	W168C	4YDC	MG	M168C	2Y	1400
37. Arsenic	W168C	W168C	4YDC	MG	M168C	2Y	1400
38. Cadmium	W168C	W168C	4YDC	MG	M168C	2Y	1400
39. Calcium	W168C	W168C	4YDC	MG	M168C	2Y	1400
40. Chromium	W168C	W168C	4YDC	MG	M168C	2Y	1400
41. Cobalt	W168C	W168C	4YDC	MG	M168C	2Y	1400
42. Copper	W168C	W168C	4YDC	MG	M168C	2Y	1400
43. Iron	W168C	W168C	4YDC	MG	M168C	2Y	1400
44. Lead	W168C	W168C	4YDC	MG	M168C	2Y	1400
45. Magnesium	W168C	W168C	4YDC	MG	M168C	2Y	1400
46. Manganese	W168C	W168C	4YDC	MG	M168C	2Y	1400
47. Mercury	W168C	W168C	4YDC	MG	M168C	2Y	1400
48. Nickel	W168C	W168C	4YDC	MG	M168C	2Y	1400
49. Potassium	W168C	W168C	4YDC	MG	M168C	2Y	1400
50. Sodium	W168C	W168C	4YDC	MG	M168C	2Y	1400
51. Residual Chlorine	D24C	SAR	NSR	NSR	SAR	NSR	365

KEY

Type of Sampling

C = Composite
G = Grab
DC = Core Sample

SAR = Sample as required
NSR = No sample required
IS = *In situ* analysis

Frequency of Sampling

CONT = Continuous
H = Hourly
D = Daily

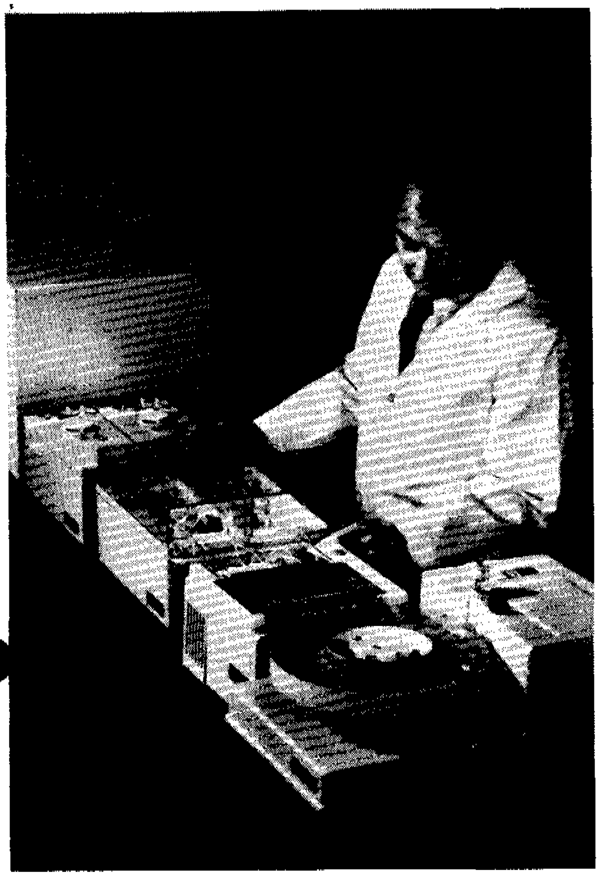
W = Weekly
M = Monthly
Y = Yearly

Intergers preceeding the frequency code letter designate the numbers of samples taken within that period. Interger preceeding the letter C indicates the length of the sample is composited.

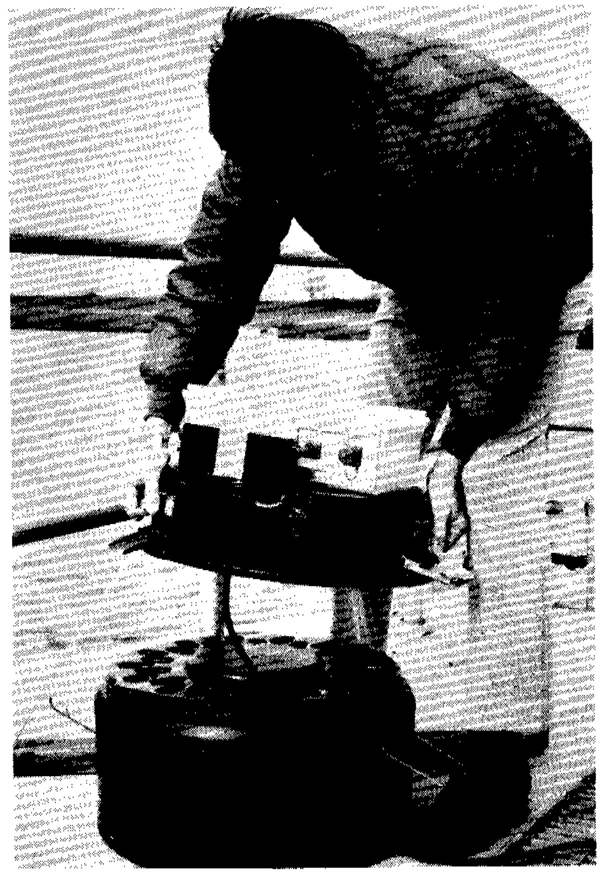
TABLE II

Average concentrations (ppm) and ranges (within parenthesis) of selected chemical parameters in East Lansing Wastewater and the WQMP lake system during the period of October, 1973 - March, 1975.

Chemical Parameter	East Lansing Wastewater			WQMP Lake System			
	Raw	Primary	Secondary	Lake 1	Lake 2	Lake 3	Lake 4
Total Phosphorus mg/l-P	7.0 (3.6-9.5)	5.0 (2.6-10.5)	2.6 (0.5-9.1)	1.91 (0.86-3.23)	1.34 (0.57-2.62)	1.37 (0.55-3.35)	0.54 (0.22-1.27)
Soluble Phosphorus mg/l-P	3.0 (2.7-5.7)	1.1 (2.1-3.8)	1.1 (0.3-7.9)	1.49 (0.55-2.66)	1.24 (0.57-2.62)	1.06 (0.51-2.32)	0.34 (0.12-0.80)
Ammonia Nitrogen mg/l-N	9.3 (4.1-32)	16 (8.6-25)	9.7 (5.2-22)	4.87 (0.36-9.7)	4.91 (0.26-10.6)	3.77 (0.27-8.1)	3.36 (0.10-8.3)
Nitrite Nitrogen mg/l-N	0.005 (<0.005-0.03)	0.25 (<0.005-0.13)	0.25 (0.07-0.90)	0.15 (0.006-0.33)	0.09 (0.03-0.18)	0.16 (0.02-0.15)	0.06 (0.20-0.09)
Nitrate Nitrogen mg/l-N	0.54 (0.16-3.1)	0.2 (0.09-2.33)	1.07 (0.16-7.0)	1.64 (0.06-12.3)	1.64 (0.06-10.9)	1.02 (0.10-1.72)	0.77 (0.10-1.25)
Kjeldahl Nitrogen mg/l-N	25.3 (4.4-38)	26.3 (18.7-45)	12.7 (8.5-28)	9.75 (1.16-21)	9.45 (3.30-15)	8.53 (4.50-15)	5.73 (2.0-14)
Total Carbon mg/l-C	183 (67-202)	171 (55-215)	120 (60-227)	55 (27-80)	47 (24-69)	43 (24-60)	31 (10-46)
Total Organic Carbon mg/l-C	73 (43-105)	50 (38-97)	30 (12-111)	14 (6-48)	8.6 (0-11)	9 (4-13)	7 (3-20)
Boron mg/l-B	0.33 (0.49-0.19)	0.31 (0.35-0.29)	0.33 (0.42-0.21)	0.33 (0.41-0.26)	0.25 (0.30-0.20)	0.25 (0.31-0.23)	0.25 (0.29-0.19)
Calcium mg/l-Ca	108 (95-125)	110 (85-125)	113 (90-129)	49 (39-71)	46 (30-70)	45 (34-68)	33 (15-51)
Sodium mg/l-Na	103 (58-295)	110 (59-295)	119 (63-300)	82 (68-111)	79 (49-108)	78 (60-108)	59 (16-79)
Magnesium mg/l-Mg	25 (20-29)	26 (20-30)	24 (20-28)	20 (14-32)	19 (13-32)	19 (14-32)	12 (4-20)
Manganese mg/l-Mn	0.16 (0.10-0.39)	--- ---	0.09 (0.03-0.18)	0.05 (<0.05-0.10)	0.05 (<0.05-0.09)	<0.05 (<0.03-<0.05)	<0.05 (<0.03-<0.05)



A Technicon Auto Analyzer II system (above) with the SOLID prep Sampler II (Below) is used to analyze solid as well as liquid samples for many chemical parameters.



The Isco flow proportioned wastewater sampler is being used to collect a representive composite sample of the lake effluent.

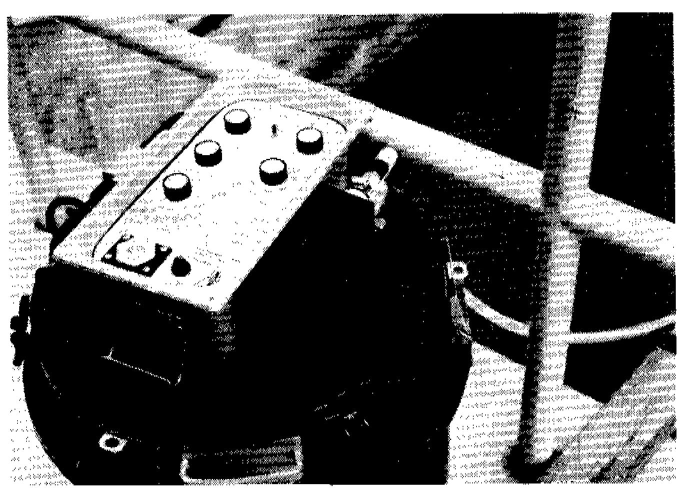


FIGURE 6

4. Monitor the purity of the University's water supply.

Analyses for viruses will be accomplished with methods that have been used at the East Lansing wastewater plant for many years to isolate pathogenic Salmonella and Shigella. Studies at the original and present water treatment plant have authenticated the value of one method for isolating viruses in wastewater. Pad samplers, 4 inch squares of absorbent cotton, are placed between two layers of cheese cloth and are held in place by sewing the three layers together. They will be used on all flowing water to trap bacteria and virus particles. After the pad accumulates and concentrates the bacteria and viruses, they can be isolated with standard methods. Careful concentration and strict culture procedures are required to isolate the complex viruses. The fluid in the pad is expressed and approximately 100 ml is concentrated by ultracentrifugation. The resulting "sediment" is then suspended in approximately 3 ml of the supernatant fluid in about a 1:95 volume concentration. After the bacteria are eliminated with antibiotics, the sample is centrifuged at slow speeds. If the bacterial sterility controls then are negative, the sample is introduced on cultures of African green monkey kidney cells. The isolated viruses are subsequently passed into secondary cultures to be identified by serological methods if that is necessary.

Water samples from the monitoring wells are collected in gallon volumes and passed through the continuous flow ultracentrifuge to remove the viruses. Specific polyethylene imines are added to the water sample to enchance survival of the infective virus particles. After concentration, the samples are tested for sterility and introduced into the cell cultures as described previously. If samples of water from the monitoring wells contain viruses, the quantity will be determined by plaque counting. These methods are routinely used in this laboratory for the isolation of viruses from sewage and water.

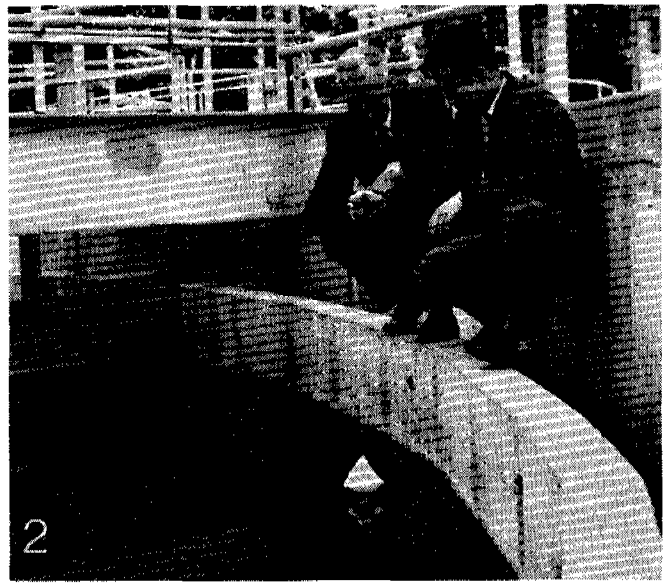
More enteric bacteria and viruses are in wastewater in the late summer and early fall. Therefore, more water samples are collected during these periods. Pad samples (Figure 7) will be taken at various stages of treatment in the East Lansing wastewater plant as well as from the inflow and outflow of the WQMP lakes to compare the recovery of pathogens. In both systems more effort will be exerted to recover and analyze viruses than the pathogenic bacteria which are more readily destroyed.

Meteorology

As an adjunct service to a number of other research projects which would include virus dissemination through wind drift, evapo-transpiration from forests, old fields, cultivated plantations, and bare fields, studies involving lake circulation and material transport, studies of basic phytoplankton and macrophyte productivity, crop production, and other as yet unidentified areas of research we have installed a 150 foot meteorological tower that, when completed, will be the base for a large array of transducers that will sense the record meteorological events such as solar energy, wind direction, velocity, and duration, air and soil temperatures, soil moisture, and other parameters that can be collected automatically through transducers. These data will go directly to a minicomputer with a facility for immediate read-out and automatic transferral to the major computer for storage, analysis and retrieval when needed

Data Management

A data management system is being implemented to handle the large volume of data that is generated. This system is designed to: (1) store, retrieve,



Carefully controlled health hazards and basic microbial research are combined in the sampling of wastewater; (1) plastic spheres for bacteria and algae, (2) cotton pads for viruses. After concentration, the microbes are examined by means of the (3) transmission and (4) scanning electron microscopes.

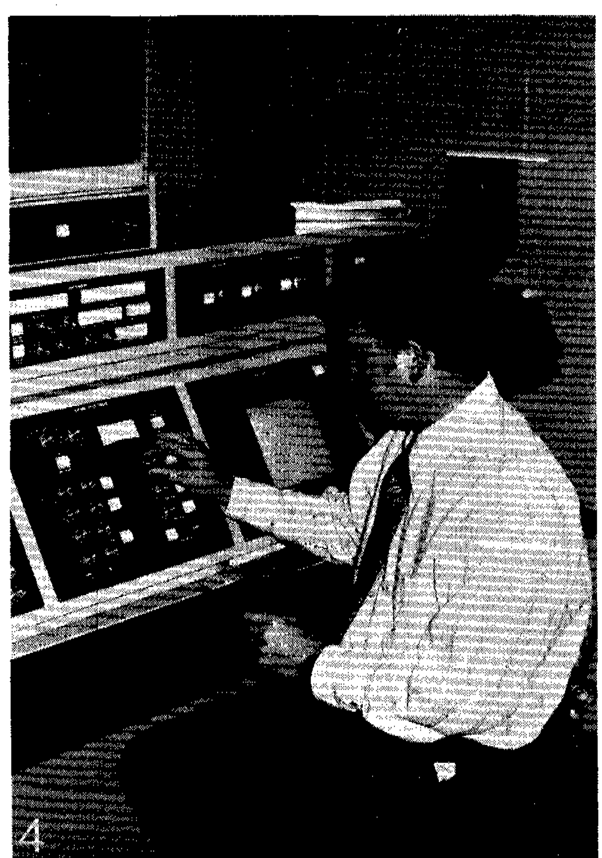


FIGURE 7

prepare, manipulate and display all data, (2) tranfer data from the producer to all authorized users, and (3) prevent its loss, destruction or unauthorized use. The data are stored on the Michigan State University CDC 6500 computer under the mnemonics and code acquisition numbering of the STORET data management system whenever it can be used.

The minicomputer-based system is composed of a central site, which houses the minicomputer, and up to eight satellite stations. Under minicomputer control, up to twenty-four parameters can be monitored at each of these stations. Data is transmitted back to the central site over standard telephone wires. Data derived from this system will be used to make on-line management decisions and to provide much of the data base required to develop a better understanding of the physical, chemical, and biological interactions associated with the ecosystem.

RESEARCH MPC

The project site on the Michigan State University campus was designed to encourage maximum cooperative research by scientists from such diverse areas as limnology, botany, crop and soil sciences, economics, engineering, entomology, fisheries, forestry, horticulture , hydrology, geology, sociology, and zoology and chemistry.

The research can characterize the dynamics of wastewater constituents in an integrated system of wastewater treatment and nutrient recyling. First, the magnitude and direction of the biotic and abiotic factors are being identified to determine how they affect the movement of phosphorus, nitrogen and carbon in aquatic and terrestrial systems. The movement of these nutrients, especially phosphorus, is being monitored through several significant subunits of the WQMP.

Municipal Wastewater Effluent for
Forage Crop Production to Feed Livestock

The objectives of this research program are threefold. First, to compare annual crops with perennial forage crops that produce the high yields needed to feed livestock over several years without having to be re-established. When the crops are irrigated with high levels of sewage effluent they can be harvested under varying time frequencies to obtain the maximum biomass per acre. Second, to determine how soils and plants fix minerals and the fate of heavy metals when wastewater effluent is applied on perennial forage crops and annual crops. And, third, to estimate the in vitro disgestibility as an indicator of in vivo disgestibility to secure maximum biomass and adsorption of nutrients.

The field plots were established on two acres and irrigated from early May to late November with 1, 2, and 3 inches of wastewater effluent each week. Table III gives the estimated soil loading per acre with an application of one inch of secondary effluent from the East Lansing plant. The soil was categorized as a uniform Miami loam by taking forty-five samples in 1 foot increments to a depth of 10 feet.

The eight perennial legumes and eight perennial grasses were established in August, 1973, by seeding with a precision planter. In one area rye was sown in early September to serve as a winter cover crop. The annual crops - two varities of hybrid corn, one forage sorghum, and one sorghum sudangrass - were established in mid-May with a no-till planter after the rye was treated with Paraquat herbicide to kill the top growth (Table IV).

Wastewater effluent was first available on July 16, 1973. Then effluent spray levels of 1, 2 and 3 inches per week were started and continued for 14

TABLE III

Estimated soil loadings per acre on application of one inch of East Lansing Sewage Treatment Plant secondary effluent.

Chemical Parameter	Concentration in Secondary Effluent (mg/l)	Grams per Acre inch	Pounds per Acre inch	Pounds per Acre Per Year**
Organic Nitrogen	2.2	227	0.5	36
Nitrate Nitrogen	3.07	317	0.7	50
Nitrite Nitrogen	0.25	26	0.06	4.1
Ammonia Nitrogen	9.70	1261	2.77	200
Soluble Phosphorus	1.1	143	0.3	23
Total Phosphorus	4.9	637	1.4	101
Total Carbon	150	15,450	34	2,445
Total Organic Carbon	30	3,090	6.8	489
Dissolved Organic Carbon	20	2,060	4.5	326
Suspended Solids	63	6,489	14.3	1,027
Volatile Solids	25	2,575	5.7	408
Chlorides	261	26,883	59.1	4,254
Iron*	0.81	83	0.18	13.2
Manganese	0.09	9	0.02	1.5
Zinc	0.19	20	0.04	3.1
Nickel	0.11	11	0.025	1.8
Copper	0.06	6	0.013	1.0
Mercury	0.00005	0.005	0.00001	0.0008

* Iron is being added for chemical phosphorus removal at the East Lansing Sewage Treatment Plant.

** At a rate of two inches per week between March and November (36 weeks).

TABLE IV

Plants Irrigated with Municipal Wastewater Effluent For Forage Crop Production
(Planted August, 1973, Harvested in 1974)

PERRENIALS

Grasses

Smooth bromegrass (*Bromus inermis* Leyss) cultivar Sac (southern)
Smooth bromegrass (*Bromus inermis* Leyss) Canadian source (northern)
Orchardgrass (*Dactylis glomerata* L.) cultivar Nordstern
Tall fescue (*Festuca arundinacea* Schred.) cultivar Ky. 31
Timothy (*Phleum pratense* Leyss) cultivar Verdant
Kentucky bluegrass (*Poa pratensis* Leyss) cultivar Park
Creeping foxtail (*Alopecurus arundinaceus* Poir) cultivar Garrison
Reed canarygrass (*Phalaris arundinacea* L.) Commercial

Legumes

Alfalfa (*Medicago sativa* L.) cultivar Saranac
Alfalfa (*Medicago sativa* L.) cultivar Agate (*Phytophthora* resistant)
Alfalfa (*Medicago sativa* L.) cultivar Vernal
Alfalfa (*Medicago sativa* L.) cultivar 520
Alfalfa (*Medicago sativa* L.) cultivar Iroquois
Alfalfa (*Medicago sativa* L.) cultivar Ramsey
Birdsfoot trefoil (*Lotus corniculatus* L.) cultivar Viking
Birdsfoot trefoil (*Lctus corniculatus* L.) cultivar Carrol
Red clover (*Trifolium pratense*) cultivar Arlington

ANNUALS

(planted each spring starting 1974, harvested the same year)

Corn (*Zea mays* L.) cultivar Funk G-4444
Corn (*Zea mays* L.) cultivar Mich. 560-3X
Sudangrass (*Sorghum sudanense* P. Stapf) cultivar Piper
Sorghum-sudangrass hybrid (*Sorghum bicolor* L. Moench x *S. sudanense* P. Stapf) cultivar Pioneer 908
Forage sorghum (*Sorghum bicolor* L. Moench) cultivar Pioneer 931

weeks until October 21 when the final plots were harvested and the soil was sampled. The untilled soil absorbed the effluent rapidly. One inch was absorbed in an hour without any runoff even on plots that received three inches per week in three applications of one inch each on Monday, Wednesday and Friday.

Yields of the first annual grass crop and three harvests of perennial grasses were lower than expected, probably because in the 14-week irrigation period only approximately 27, 54, and 91 pounds of nitrogen were applied per acre at the 1, 2, and 3 inch levels of effluent, respectively Figure 8. At least 150 pounds of nitrogen per acre are necessary for a good yield of perennial grasses and annual grass crops such as corn. Even at the high rate of effluent spray with 91 lb of nitrogen per acre, annual and perennial grasses were deficient by about 60 lbs per acre. However, the legumes yielded well and showed no symptoms of mineral or nitrogen deficiency. Apparently they fixed enough nitrogen symbiotically from the air to obtain their optimum requirement of around 200 pounds per acre since the effluent was deficient in applied nitrogen.

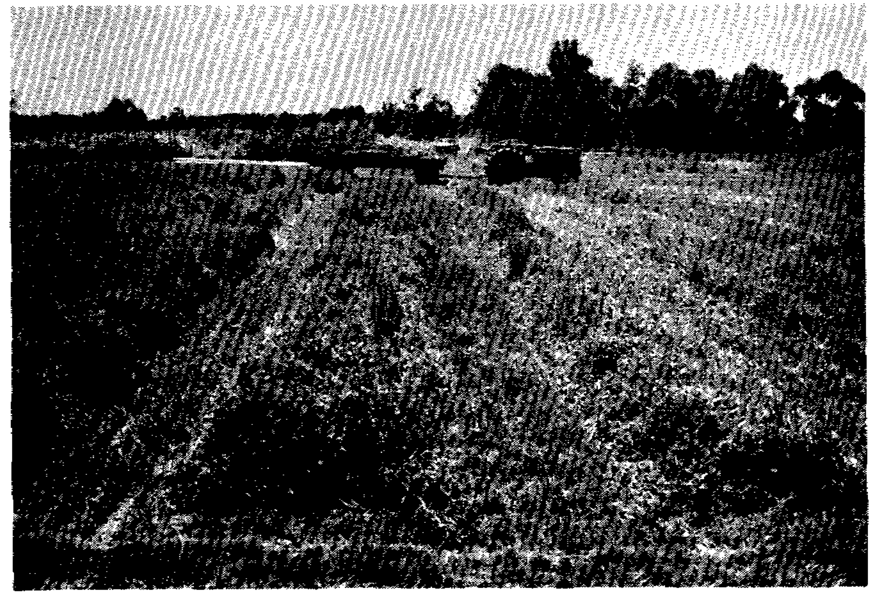
The soil was sampled in 45 locations in one foot increments to a depth of 10 feet for soil profile data on; pH, conductivity, extractable P, K, Ca, Mg, Na, Cl, NO_3, N, Ca, Cd, Co, Cu, Fe, Mn, Ni, Pb, andfZn, plus Kjeldahl N and total C. Samples of plants were analyzed for these elements with: micro-Kjeldahl, emission spectrographic, atomic absorption, ion electrode and colorimetric analyses. Certain elements such as: Cl, Cd, Co, Ni, and Pb are determined in plant tissues only if spot checks show them to be a potential problem. Samples were collected in the fall of 1973 for baseline data and in 1974 after one year of cropping. The first year's samples have been analyzed, and the 1974 samples are being analyzed now. Plant samples have been ground up and are being analyzed for nitrogen and minerals and for in vitro digestibility.

In 1975 the same annual crops are again being planted. And soybeans have been added because the other legumes performed so well in 1974. The first effluent was applied in mid-April to be continued for 26 weeks. Approximately 70, 140, and 210 pounds of nitrogen are being added per acre at 1, 2, and 3 inch levels in 1975. This should generate differential yields of the annual and perennial grass crops.

Wood Production

Within the terrestrial system is an area supporting a mixed hardwood forest typical of most woodlots in southern Michigan. Here, the survival and growth of the trees will be documented under different spray application regimes and the penetration, uptake of nutrients, and loss from the system will be measured by a variety of techniques. Partially covered by the spray irrigation system is a pine plantation which will serve as contrast for the deciduous forest system. Plans for additional research include the planting of trees that can be used for intensive cropping on a short term rotation. It is presumed that these will be used for pulp wood and their complete removal and utilization will serve to take the accumulated nitrogen and phosphorus that they have incorporated during their growth completely out of the system.

Incorporated as a by-product of the forest research will be a study not only of the nutrients removed from the system but also a study of the return and internal cycling of these materials in the form of leaf fall to the forest soils. This offers the possibility of the selection and management



Twenty varieties of forage and grain crops are being tested by Dr. Milo Tesar for their tolerance and response to nutrient rich water.

of tree types for maximum binding and holding of those nutrients that we desire to either remove from the system or have tied up in such a manner that they will not enter the potable water aquifer. Percolation through the litter and soil of the forst floor is being qualitatively and quantitatively evaluated (Figure 9).

Old Field - Plant Ecosystems

Preliminary work over the past several years has indicated that the natural diversity of an old-field community (Figure 10) may be one of the most effective and efficient units for capturing nutrients from the wastewaters sprayed on the land. There is also evidence from these studies that spraying them with nutrient rich effluent will appreciably change the composition of the plant community. The efficiency of these plant communities in trapping nutrients and the role of grazers, especially insects, in transporting nutrient material out of the study system will be a topic of continuing research.

Prior to any application of wastewater the entire terrestrial site was analyzed in detail for existing plant arrays and samples collected where feasible for future reference.

Following the first application of wastewater a research and analysis program has been undertaken throughout those areas receiving water to monitor changes in growth patterns, species composition, and associated environmental changes. This survey included both the open field areas as well as the forested area and the tree planation zone.

Soil Research

Any system such as the one proposed at Michigan State University will be dependent to an important degree on the: (1) selection of a site with soils compatable with the concept of total waste management and recycling; and

Installation of porous cup tension lysimeter on hardwood irrigation plots. Samples of water percolating through the forest soil can be removed and tested for water quality changes.

FIGURE 9

Drs. Frank Reed and S. N. Stephenson observe spray head action of the irrigation process, the first step in the terrestrial ecology study on the effects of wastewater application on natural ecosystems.

FIGURE 10

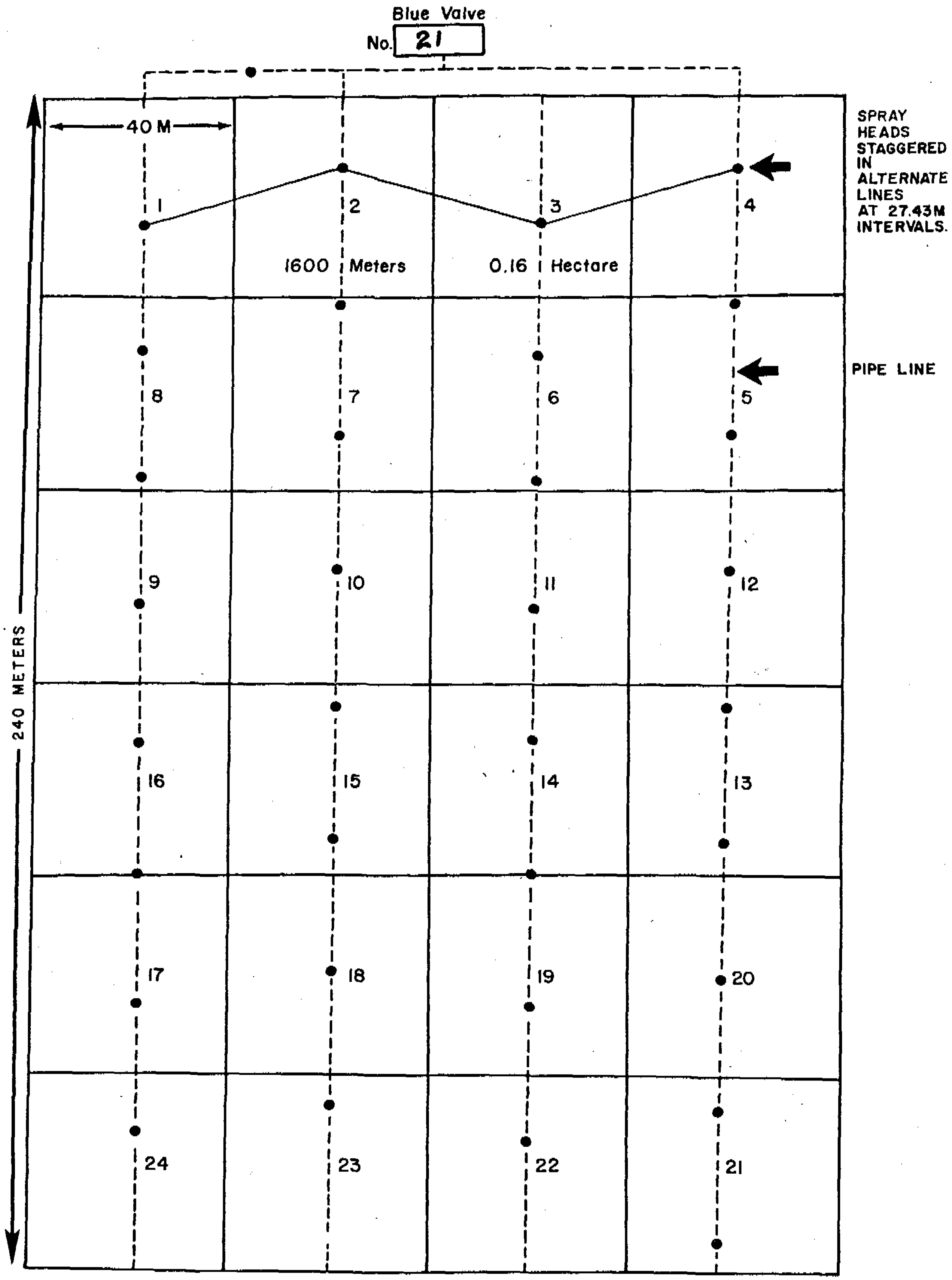
(2) management of the soil complex in such a manner as to accomplish the mission of the project over a long period (approaching steady state); and (3) a subsurface geology that both serves as a filter and does not present a barrier to water movement downward from the water application site.

Detailed studies will be carried out on both the short and long range effects on soil texture and composition of heavy application of wastewater. This will involve studies of the retention of heavy metals in the soil mantle and development of strategies that will allow the heavy application of nitrogen in its several forms and yet allow the transformation and uptake of the nitrogen into forms that will either be held in the crops or be driven off to the atmosphere in the form of gaseous nitrogen.

The site selected has a great variety of soils characteristic of the Great Lakes drainage basin thus affording the opportunity for in-depth research on wastewater application to diverse soil types. Figure 11 shows the arrangement of a single value unit which controls four spray lines of 240 meters each and a total of 32 spray heads.

Mammals

The effects of a spray irrigation system on the wild animals of an area is not well known. Other studies have shown that the lush vegetation of wastewater irrigation plots often serves as an attractant to wildlife. To document any changes that might result from the spray operation we have undertaken a thorough study of trapping, marking, and identifying ranges of the common animal inhabitants of the land site. At this point in the project we have no conclusive data on changes in the animal population with the exception of the invasion of the area by a small herd of whitetailed deer. Deer had been sighted on the area before any activities associated


Blue Valve
No. 21
40 M
1
2
3
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1600 Meters
0.16 Hectare
SPRAY HEADS STAGGERED IN ALTERNATE LINES AT 27.43M INTERVALS.
8
7
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PIPE LINE
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24
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22
21
240 METERS

with this project began but the planting of farm crops and irrigating them with nutrient rich water has proven to be a magnet that has drawn deer to the research site used for farm crops. In this instance six varieties of alfalfa were planted and the deer selected out one of these varieties and completely destroyed the research plot. Such information on deer as well as other animals will be useful in advising and predicting the problems associated with the production of farm crops in areas that are the natural habitat of wild animals.

Arthropods and Phytopathic Nematodes

We have been obtaining pre-operational baseline data that could be used as point of reference in planning studies to determine the longterm effects of wastewater irrigation on the haematophagous insects, soil microarthropods and phytopathic nematodes in the project site. Dog heartworm is endemic and common in the area and California encephalitis is known from the area although it is very rare. Both of these diseases are transmitted by mosquitoes of a species known to occur in the area. Any irrigation system has the potential for increasing the mosquitoe and biting pest population of the immediate area, and we belive that was essential that we establish population densities prior to any spray operation and again following a period of spray regime. Nine species of mammals were trapped and blood samples taken for serological survey for the presence of California encephalities virus antibodies. Limited serological evidence indicate that there may exist an enzootic focus of California encephalitis virus activity on the project site and that an efficient mosquitoe vector, Aedes triseriatus, is indiginess to the locality. We are presently undertaking a more intensive and critical study that will be required to determine the public health significance of this finding and its relationship to the future operational plans of the project.

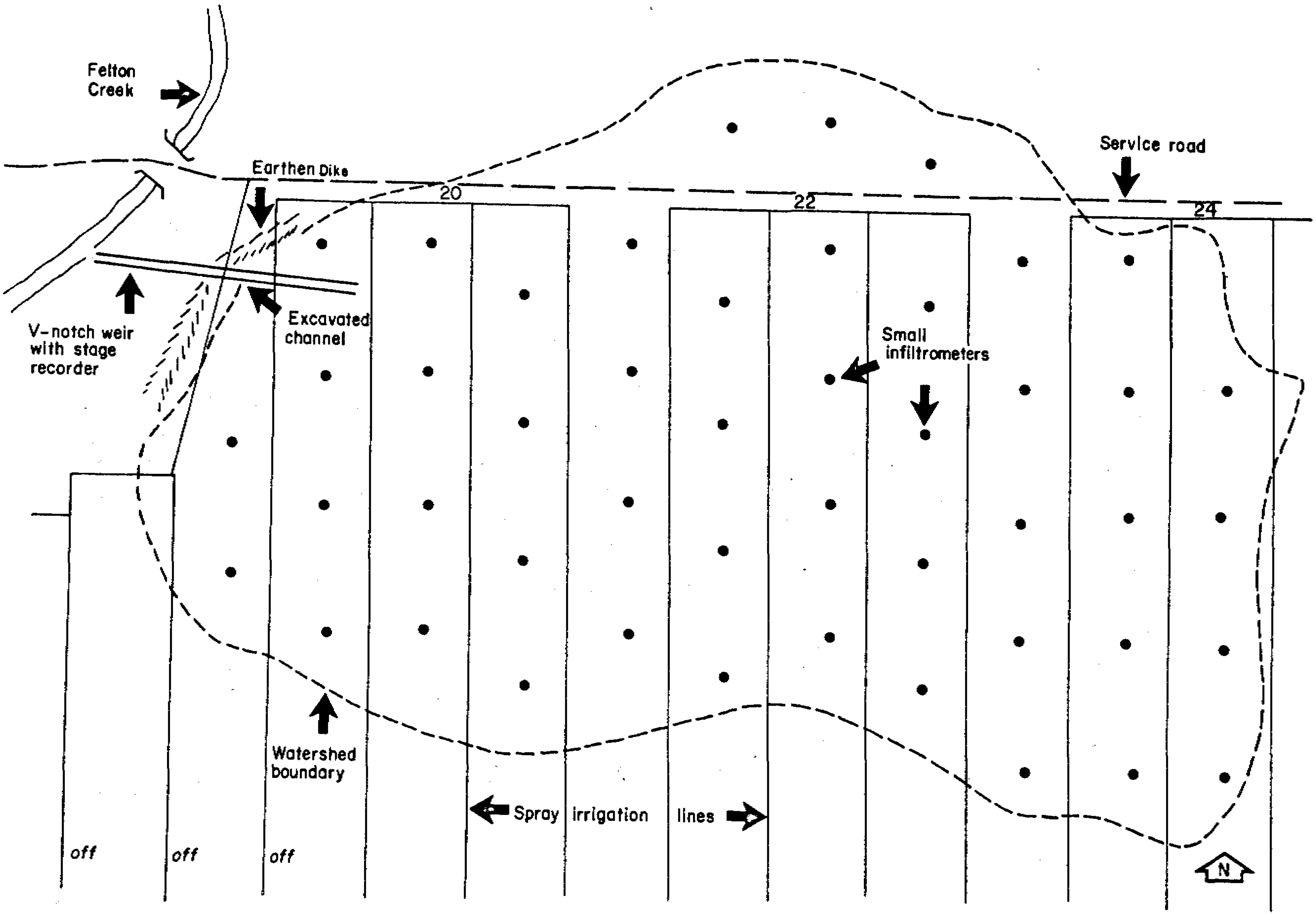
From a public relations standpoint it is essential that a spray and lake water recycling project not only avoid increasing the potential for health problems but also to avoid fostering or harboring a nuisance such as a horde of mosquitoes.

A survey of the nematodes on the project areas that were slated to be used for cultivated crops was undertaken and the root-lesion, rootknot and cyst nematodes were analyzed for population densities and frequencies of occurrence prior to spray operation. Soil-borne nematodes have been identified as economic problems in Michigan agriculture and we are exploring the possibility that these could become a serious threat to irrigated crops using nutrient-rich water.

To enable us to evaluate changes in soil composition and texture and assess the nutrient impact of litter resulting from wastewater irrigation a survey of the soil fauna was undertaken prior to the operation of the plant. The soil-dwelling fauna was characterized by relatively large numbers of nematodes, Collembola, oribatid and predatory mites. A variety of insects and other soil organisms were found in the soil and estimates made of their distribution and densities.

Preliminary analysis of the data showed that soil type plays only a minor role in the distribution of Collembola and Diploda species but that cover type and concurrent mositure and temperature levels seemed to restrict some species to certain areas. The projected use of these collections and data is not well defined at this time but we have made an overall attempt to categorize and classify the plants and organisms as well as the physical features of the site prior to any perturbations caused by application of wastewater to the land.

The possibility and methodology of operating a combination lake and land wastewater treatment system in a region at 43° N latitude offers a variety of challenges. In this climate the lakes have a frozen cover from 70-90 days and depending on the type of soil and cover, there is about the same period that the surface of the ground is frozen. We are presently developing management strategies that will allow us to run the lake system throughout the year and to test the changes in efficiency of the system with the changing seasons. To test the efficiency and feasibility of winter spray operation we modified one area of the land spray distribution system to allow detailed evaluation of the effects of spraying at temperatures below 0° C. The spray lines in this area were leveled to allow rapid draining following the spray application, and the major automatic valves were insulated against the low temperatures that would be encountered. The entire area is shown in Figure 12.and small infiltrometers were placed throughout the entire sub-watershed basin. The outlet drain of the spray-land unit was equipped with a measuring V-notch weir and a provision made for an insulated automatic water sampler installation. This unit was in operation during the major cold period of the winter when temperatures reached as low as -14° C. The data on this sub-watershed has not been analyzed completely but there is every indication that we can proceed into an extensive research program into the operation of a land recycling system with the complete confidence of being able to operate during the winter months.



DESIGN OF THE WINTER SPRAY RESEARCH AREA

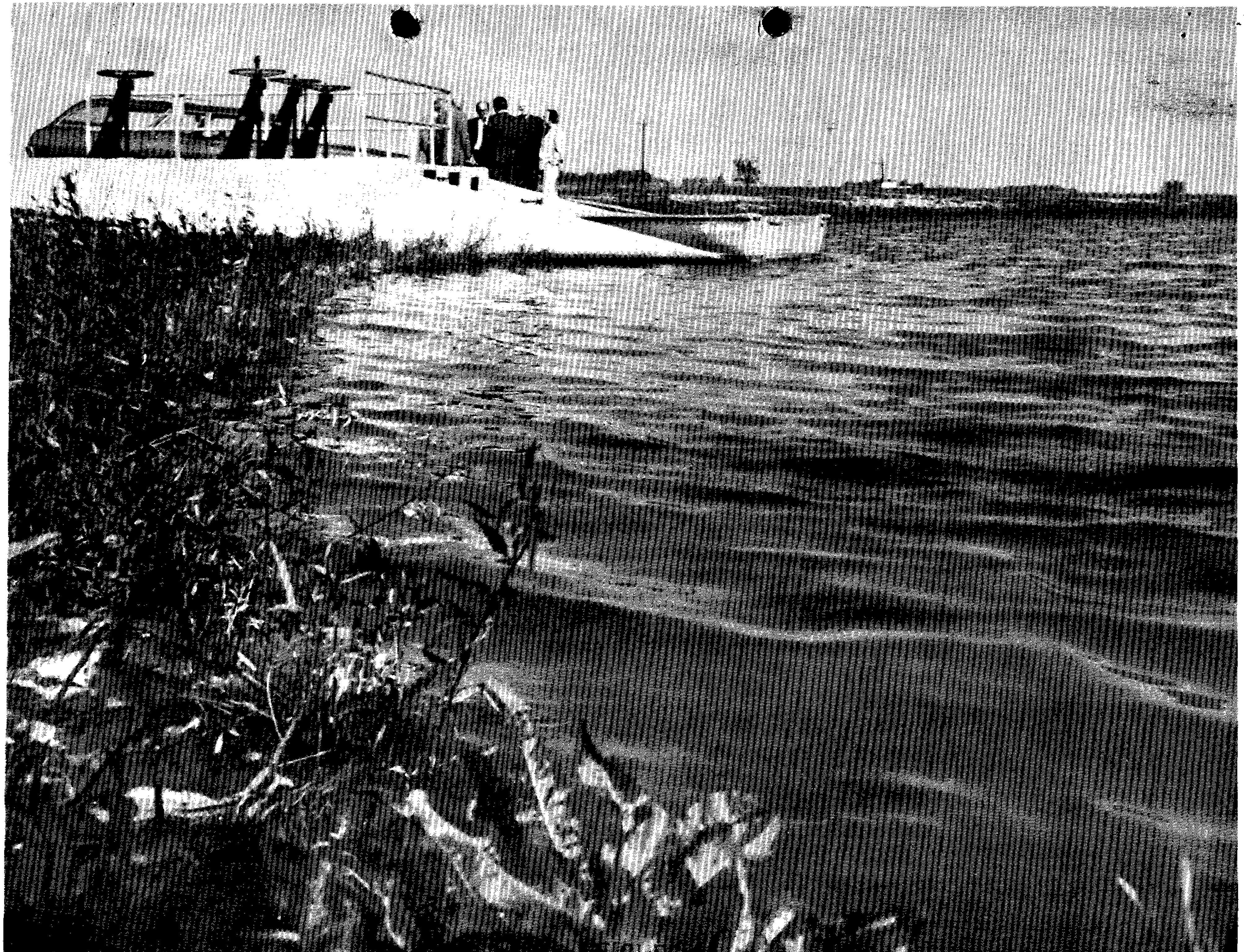
Figure 12

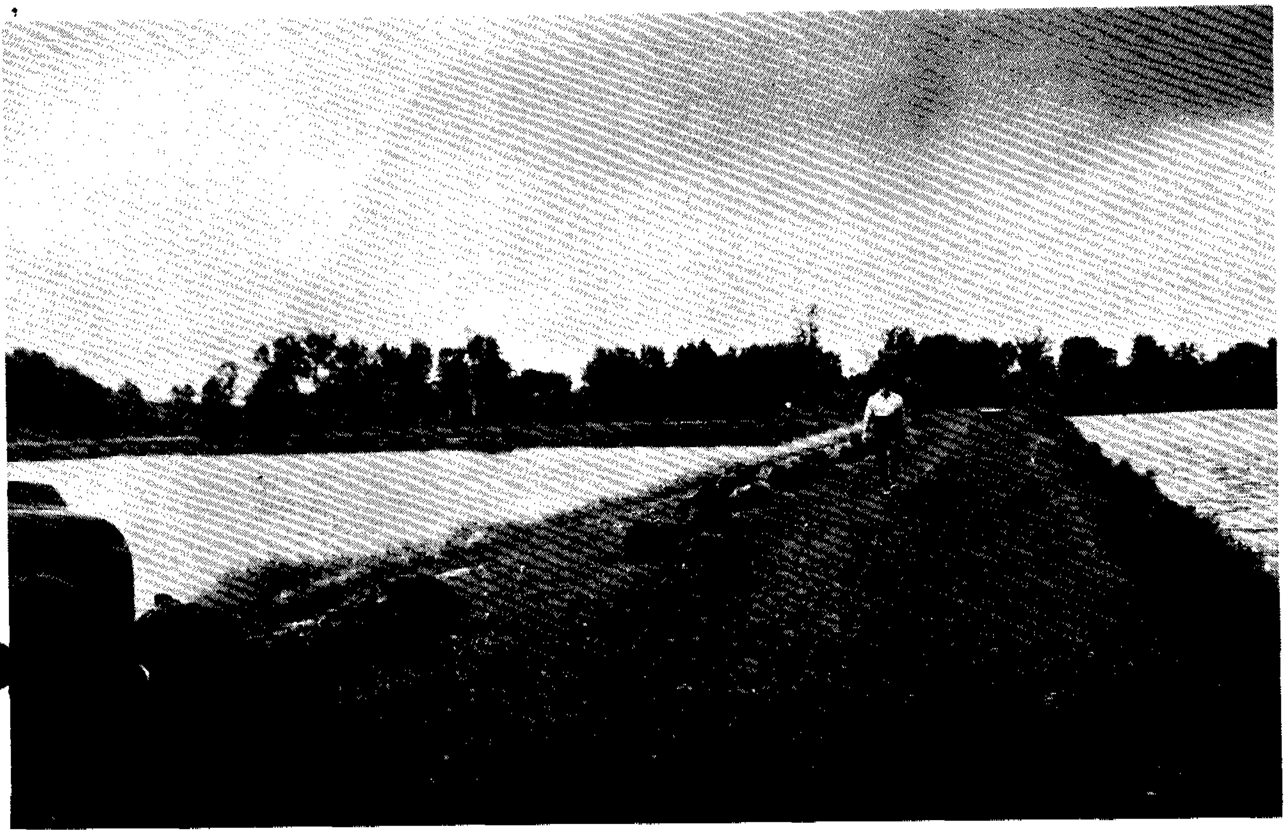
Lake Management Research

We believe that the most unique feature of the Water Quality Management Project is the wide versatility of the system which includes three major points at which we can control and manage the system. These are (1) the waste treatment plant that can operate under full research control, (2) the multiple lake (Figure 12) system and its accompanying manifold distribution system which enables us to take water of any desired quality to the field site, and (3) the spray irrigation site with its almost limitless capacity for variability and research modeling.

Under the a priori assumption that, due to the ease removing and processing, we would concentrate on the production of higher aquatic plants in the three lower lakes of the series rather than algal production.

Aquatic vascular plants can be the dominant primary producers in aerobic wastewater ponds in Michigan during the June-October season. As such, they produce oxygen to maintain the aerobic nature of the environment, absorb inorganic and organic components of the waste, reduce turbidity by stabilizing the sediments, serve as a substrate for periphyton that contributes to waste processing, and represent an easily harvestable resource, the removal of which promotes the efficiency (in terms of BOD, nutrient, metal, pesticide, etc. removal) and longevity (less rapid sedimentation and filling) of the pond system. Since the dispersal of these plants into newly constructed pond systems occurs at a low rate relative to other organisms, an initial objective of this project has been to "seed" in those species (Figure 13) that are known from our previous work to be adapted to such systems (*Potamogeton foliosus*, *Elodea canadensis*, *Ceratophyllum demersum*, and *Typha latifolia*), and additionally, species that are major weed problems





Planting of the lakes to higher aquatic vegetation and (below) the luxuriant growth resulting from this planting.

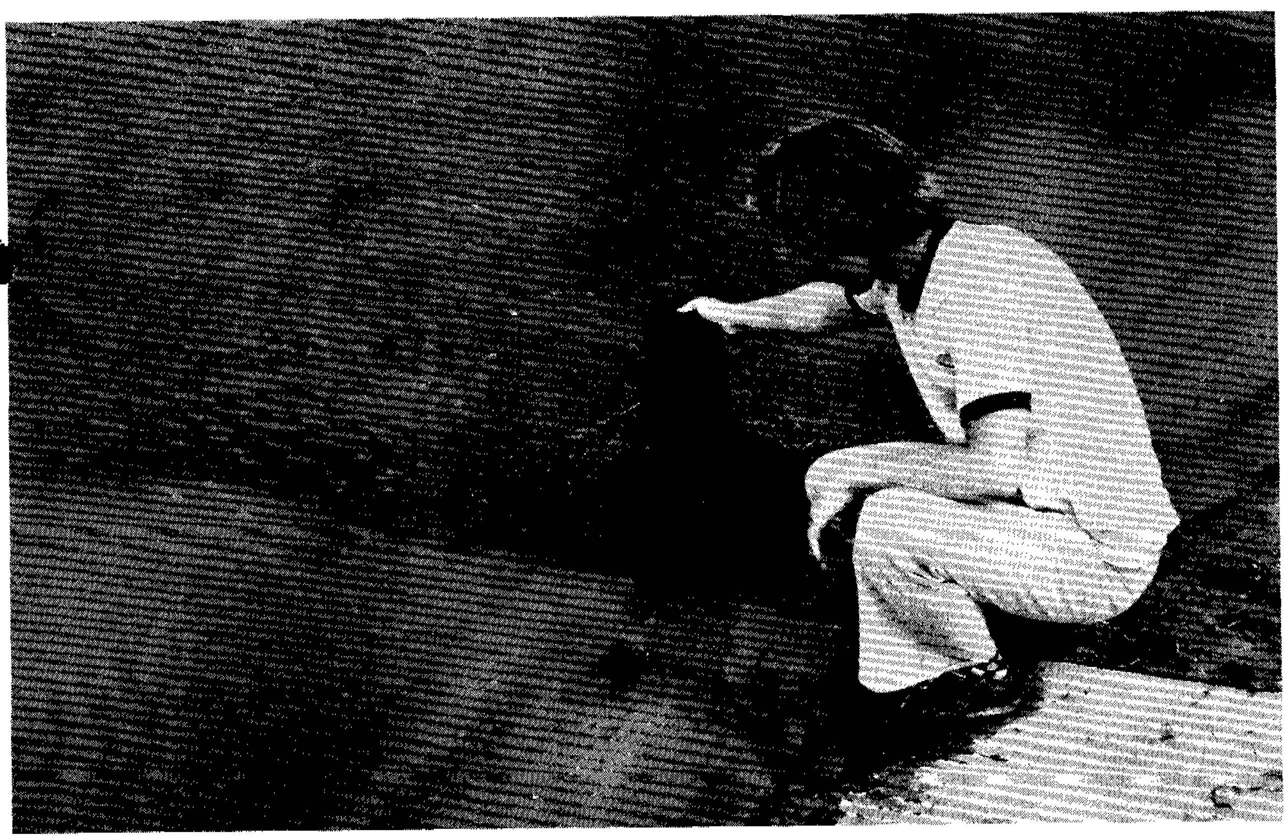


FIGURE 13

in eutrophic lakes of the region (*Elodea nuttallii*, *Najas flexilis*, *Myriophyllum spicatum*, and other species of footnote 1, Table V).

It is too early in the experiment to give a judgment on the survival and reproduction of the introduced plants. But from general observation, the lower three lakes are being rapidly and densely populated with the macrophytes. It is anticipated that following the dropping of the seeds late in the summer that the lakes will be harvested by a mechanical harvester and the plants evaluated for biomass production and nutrient removal. It is anticipated that within the foreseeable future these aquatic plants, if they lend themselves to adequate volume of harvest, will be removed from the ponds and, along with a harvest of crayfish and forage fish, be processed into pellitized animal food. The value of these as a concentrated food source will be evaluated in a fish rearing station that is in the late stages of planning and should be in operation by the latter part of this summer.

The marshes of the system have been planted to typical marsh plants, but because of the sterile nature of the clay sub-soil have been rather slow to colonize the area. They are presently beginning to show increasing growth rate and spreading and it is anticipated that the invasion of the marshes will be complete at the end of this growing season.

The lakes are producing vast numbers of zooplankton which we are able to harvest through a combination light and pump trap. These are going to be of extremely high nutritional value in fish culture and it is anticipated that with a very minimum of constant effort that the volume necessary to run a sizeable fish cultural operation from this source of food alone can be realized.

Table 1. Quantities (weights in kilograms) of vascular hydrophytes[1] transplanted to the newly constructed lakes of the MSU Water Quality Management Project during October-November, 1973.

Site	Species[2]	Fresh Weight	Dry Weight	Ash Weight	Ash-Free Dry Weight	Dry Weight per Ha.	No. Seeds[3] per m^2	No. Rhizomes[4] per Marsh
Lake 1	Not Planted							
Lake 2	_Potamogeton foliosus_	302	42	13	29	13	150	
	Elodea canadensis	359	26	8	18	8		
	Najas flexilis- Elodea nuttallii	306	26	9	17	8		
	Myriophyllum spicatum	276	30	7	23	9		
Lake 3	_Potamogeton foliosus_	60	13	4	9	3	25	
	Elodea canadensis	144	17	4	13	4		
Lake 4	_Potamogeton foliosus_	66	15	4	11	3	26	
	Elodea canadensis	148	17	4	13	3		
Marsh A	_Potamogeton foliosus_	12	2.7	0.8	1.9	7	58	
	Typha latifolia	8	1.7	0.4	1.3	4		608
Marsh B	_Potamogeton foliosus_	13	2.8	0.8	2.0	7	60	
	Typha latifolia	7	1.7	0.4	1.3	4		597
Marsh C	_Potamogeton foliosus_	12	2.8	0.8	2.0	7	60	
	Typha latifolia	7	1.6	0.3	1.3	4		593

[1]Other species known to be introduced with tabled species as occasionally shoots: _Ceratophyllum demersum_, _Chara_ sp., _Heteranthera dubia_, _Lemna minor_, _Lemna trisulca_, _Myriophyllum_ sp., _Najas marina_, _Potamogeton berchtoldii_, _P. crispus_, _P. gramineus_, _P. illinoensis_, _P. pectinatus_ in Lake 2 and _Najas marina_, _Potamogeton berchtoldii_ and _P. pectinatus_ in Lakes 3 and 4 and in the marshes.

[2]Harvested vegetation broadcast over water surface, except for _Typha latifolia_. Sources of collection: _P. foliosus_, Carson City waste stabilization pond, Montcalm Co., Mi; _E. canadensis_, catchment pond Lansing municipal airport, Ingham Co., Mi; _N. flexilis-E. nuttallii_, Lake Lansing, Ingham Co., Mi; _M. spicatum_, Lake Chemung, Livingston Co., Mi; _T. latifolia_, Game Food Nurseries, Oshkosh, Wisconsin.

[3]No seeds observed for _E. canadensis_ and _M. spicatum_; _N. flexilis_ with seed, numbers not estimated.

[4]Recently harvested rhizome tips 10-20 cm in length planted 5-10 cm into clay of level 1 in Marshes A and C and level 2 of Marsh B.

Fish Production, Fish Cultural, and Recreation

The acceptance of a wastewater treatment system utilizing lakes would find instant acceptance as part of a community if it could be considered as a recreational unit, part of a green belt zone, and a fish producing unit. We believe that the lakes that we have developed have all of the attributes of being a very acceptable part of a community structure and that at least one and probably two of the lakes can be opened to sport fishing at some later date. To this end we have planted the fourth lakes in the series with a forage fish, a pan fish, and a sport fish. This combination of fishes has been shown to be biologically compatable with eutrophic waters and produce a highly productive sport fishery. Future plans call for one of the lakes to be used exclusively for the production of commercial fish that can be harvested either by fish-out procedures or through other means of annual harvest.

SUMMARY

We have designed and built a research, demonstration, and teaching facility to explore the potential of alternate methods of wastewater treatment and nutrient and water recycling that we believe has great flexibility to seek answers to the vast number of unanswered problems concerning land and crop treatments of waste and the utilization of lake ecosystems for cleansing water and recycling of nutrients. We are most fortunate in attaining our goal of having the facility located on the campus of a major university. We believe that the success of such a complex project depends largely on the availability of the expertise of a large number of disciplines working in concert to determine the technical and economic feasibility of this concept of water management. We have involved nearly every major unit of a large university in the project. We believe that the confidence of the Rockefeller, Kresge, and Ford Foundations in providing the strong and initial support for the project and that the support of the State of Michigan and the Federal Environmental Protection Agency will be fully justified by the research findings that is starting to flow from this facility.

In addition to research into design and management criteria for the successful operation of this type of wastewater treatment, the WQMP has inspired a multitude of innovative ancillary research projects. Those factors which interact to control aquatic fertility will be evaluated as well as hydrophonics and high-rate fish culture. The terrestrial research will enhance food and fiber production through the use of wastewater while basic land resources are protected and improved. Other research areas include the economic and social evaluation of this form of waste recycling adjacent to a large urban population. For this the maximum public recreation potential of the WQMP will be assessed.

The Relationship Between Environmental Factors and Viruses in the Inductions of Fish Tumors

Prepared for

The International Conference on the
Renovation and Recycling of Wastewater
Through Aquatic and Terrestrial Systems

Bellagio Study and Conference Center

Bellagio, Italy

July 16-21, 1975

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It is estimated that 85% of the cancer problems in man are environmentally induced (directly or indirectly) by the effects of the air, food, and water he consumes. However, only in a few cases that mainly include tumors in industrial workers (i.e. vinyl chloride) has it been possible to identify the oncogenic compounds. As a result of our rapidly mushrooming technological society, thousands of new compounds are annually being released into our environment. Few of these compounds have been screened for carcinogenic activity (i.e. 5,000 up to 1972) [1] which represents a small number of the tens of thousands, perhaps hundreds of thousands of chemicals which have extensive environmental contact with man, and ought to be screened for potential carcinogenic activity.

Very likely, in lieu of the above, the incidence of neoplasia in man could increase because of new carcinogens, increased levels of carcinogens already in the environment, or the combined effects of the same which might reveal their effects decades after their introduction. Furthermore, in man, environmental carcinogens have a tendency to express themselves some 30 years post-exposure. It is apparent that there is an urgent need to detect and identify those factors in the environment which could be contributors to the etiology of cancer in man.

At present, the screening of compounds for carcinogenic potential is progressing at a rapidly accelerated rate due to utilization of technological and conceptual advances in a number of areas in biology. Carcinogen screening, however, is largely confined to testing individual compounds in a battery of in vivo and in vitro assays. Some of these assays have been found to be relatively insensitive to the detection of known carcinogens and do not facilitate testing from an environmental approach, in which the whole ecosystem and its combined effect on an animal during its life would have to be considered.

Viruses, chemicals, and radiation are recognized carcinogens whose (2) increasingly apparent, however, that a number of diverse factors play a role in tumor induction (i.e. genetic background, hormones, immune surveillance, and/or combinations of the above). Cancer therefore, must be considered to be a multifactoral syndrome, being the net result of a complex milieu of biologic events whose expression is largely dependent to the animal's life exposure and response to his environment. With regards to the problems of environmental carcinogen screening, it is particularly relevant to note that duplication of the spectrum of combined environmental insults to which man is exposed over his life time technically and conceptually cannot be duplicated in a laboratory setting. It is increasingly apparent that there is an urgent need for new model systems for evaluation of the environment, particularly with regards to carcinogens.

With regards to waste water re-utilization and water re-use for human consumption, it has been documented that our aquatic environments are fouled with chemicals and classes of chemicals, which are of known or are of suspect carcinogenic properties [3,4,5,6,7,8]. Several of these are known to act in concert with oncogenic viruses to induce neoplasia [9,10,11,12,13]. The subject of chemical induction of oncogenic viruses has been excellently reviewed by Casto and DiPaolo[14] and in a series of papers in a monograph edited by Tso and DiPaolo[15]. Similarily, there are numerous examples of the combined effects of chemicals (co-carcinogens) inducing neoplasia, of which neither alone could induce[16]. It would seem a logical hypothesis, therefore, to suspect that alteration of an animal's environment by a battery of extrensic environmental factors might result in the induction of oncogenic viruses. Although knowledge of such mechanisms is obscure, they might very well play an extremely important role in the etiology of cancer.

It is apparent that a sentinel system for the early detection and identification of water-born environmental carcinogens is urgently needed. The following briefly discusses the potential utility of monitoring aquatic organisms and their associated neoplasms as indicators of water-born environmental carcinogens.

The potential value that fish and other poikilotherms might have for the detection of carcinogens is perhaps best exemplified by the aflatoxin story. In the early 1960's, trout hatcheries in the Pacific Northwest were plagued by epizootics of hepatomas, which were traced to the feed. It was soon determined that the hepatomas were caused by contamination of feed with the common fungus (Aspergillus flavus) which produced aflatoxin. On the molar basis, aflatoxins are by far the most active hepatocarcinogens known. Of the animal systems tested, rainbow trout were the most susceptible; tumors could be induced with 0.05 ppb aflatoxin[17]. Recently there has been considerable speculation that the prevalence of hepatocarcinomas in the Orient might be associated with high aflatoxin levels in native diets[3]. It is apparent that fish have already made a major contribution to the cancer program.

Another example of the potential utility of monitoring aquatic organisms to detect environmental carcinogens are investigations linking industrial discharges and the occurrence of cancer in red algae (Porphyra tenera) in Japan, where it is cultivated for commercial purposes. Algal cancers were found in several locations on the shoreline of Japan, although a very high incidence of algal concerns were found in the vicinity of an industrial city. Mud samples from this area induced cancerous growths in Porphyra within three weeks under laboratory conditons. Chemical analysis of the mud and subsequent laboratory testing revealed the inducing agents to be polycyclic aromatic hydrocarbons including benzanthrone, dibenzanthrone, and 2-chloroanthraquinone [18,19,20,21]. Thus, it was possible to pinpoint the source and identity of cancer-causing pollution to the coal industry by monitoring the environment.

In order to utilize fish and other aquatic organisms as sentinel animals for the detection of environmental carcinogens, we first must know what neoplasms occur naturally in the species in question. Pathology reference standards of tissues from normal and tumor bearing specimens together with heavy metal and organic analysis from a variety of geographical localities (polluted and non-polluted) are needed. Supplementary laboratory and epizootiological data (i.e., transmission-transplantation characteristics, electron microscopy, cell culture, anatomial location of lesions, etc.) are also needed to make a valid approach to the occurrence of neoplasia in polluted and non-polluted environments.

As an approach to this problem over 6,000 questionnaires were circulated by this investigator to fisheries personnel and biologists around the world inquiring as to the occurrence of neoplasms in fish and other poikilotherms. In response to the above, preserved specimens, photographs, field data, case reports, etc. were received from widely separated geographical locations (from Alaska to South Africa).

The responses to this inquiry were both exciting and frightening. First, it is apparent the occurrence of neoplasia in fish and other poikilotherms is no unusual phenomena, and a number of "new" neoplasms were identified which may have value as reference models for human cancer.

What is frightening were the numerous reports of high incidences of neoplasms in poikilotherms inhabiting heavily polluted waters in North America. These reports stimulate the following thought provoking questions: Do these reports represent the presence of carcinogens in the environment? Could they reflect an interaction of different chemicals in the environment?

Oncogenic viruses and chemicals? Oncogenic viruses alone?

It is apparent that intensive studies to determine the incidence, etiology, geographical distribution, etc. of tumors in animals in aquatic environments are urgently needed. There is practical value in these studies as they re-emphasize the need for closer surveillance of wildlife populations as possible indicators for the presence of carcinogens.

When epizootics of neoplasms are discovered, particularly those inhabitating polluted environments, there are many confounding considerations which must be given as to their significance with regards to the environment. For example, during the course of this author's investigations, an epizootic of a papilloma (7%) was discovered in white suckers (Catostomus commersoni) inhabiting a moderately polluted watershed. A majority of the tumors were found on the head region, however, these tumors were also often found on the fins and flank. Prior to the discovery of this papilloma, thousands of white suckers from a number of geographical regions had been examined with no detection of neoplasms.

One consideration, based on the above observations, was whether the neoplasms were a reflection of carcinogens in the watershed. Electron microscopy studies of the tumors revealed the presence of C-type virus particles, similar to those reported in a number of animal neoplasms[22]. Cell culture transformation and laboratory transmission trials were, however, refractory. In response to the questionnaires and our own netting studies the same neoplasm was diagnosed in white suckers inhabiting widely separated geographical regions (i.e. 3,000 miles) some of which were populations inhabiting pristine (non-polluted) watersheds. In view of the widespread geographical distribution of the syndrome and its association with a virus, the tumor was considered to be a naturally occurring neoplasm of the species and was presumably of viral etiology.

Later, during the course of netting studies in a heavily polluted basin on the Great Lakes (receiving industrial and domestic wastes) a 26.6% incidence of the papilloma was found in white suckers. In this basin, 100% of the white suckers exhibiting the syndrome had neoplasms exclusively associated with the lips. Furthermore, and inordinately high proportion of the white sucker catch exhibited the syndrome "clustering" compared to other areas surveyed at varying distances from the industrial basin. Another interesting observation regarding the anatomical location and clustering recorded, was that the tumors on the fish in the waters receiving the industrial and domestic effluents were larger than those in the surrounding areas.

With regards to the anatomical predilection for the lips of the syndrome, no behavioral pattern of the species (i.e. courtship or spawning activities) have been observed which would facilitate mechanical transmission of an infectious agent (i.e. virus) or transplantation of tumor cells to this anatomical site. Another comfounding factor with regards to a mechanical horizontal transmission by contact is the observation that specimens usually had neoplasms associated with only one lip. Considering that the opposite lip would be in almost constant contact with the tumor, it seems unlikely that the neoplasm is solely due to an extremely efficient infectious process. Similarily, laboratory transmission trials have been, to date, refractory.

The white sucker is a bottom-dwelling fish, spending its entire life feeding and resting on the bottom. These activities could expose their mouth parts (lips) to carcinogens which may accumulate in bottom sediments or they might cause mechanical abrasions to this region facilitating transmission of virus, both of which alone or in concert might give rise to neoplasm. The anatomical shift in location of the syndrome together with the clustering recorded strongly suggest that extrensic environmental factors (carcinogens) in associationship with C-Type virus play a role in the etiology of the syndrome.

Another example of an epizootic of tumors in feral fish which mav have an environmental etiology are epizootics of gonadal tumors (Sertoli cell) in Great Lake goldfish (Cassarius auratus), carp (Cyprinus carpio), and goldfish X carp hybrids.[23] Clustering has been found with the syndrome and laboratory investigations to elucidate the etiology of the syndrome has failed to give evidence of an infectious agent or process (i.e. contagion, transplantation, and electron microscopy). With regards to the clustering recorded, the role of an extrensic environmental factor or factors in the etiology of the syndrome is supported by a review of museum collections made in the 1950's in which no tumors were found; whereas fish collected from the same locations this past year a tumor incidence greater than 90% in the same age-sex group was recorded. These observations strongly suggest that since 1950, a chemical or battery of extrensic environmental factors has been discharged into these waters which have oncogenic potential or the abililty to induce oncogenic virus expression resulting in tumor formation.

With regards to the apparent propensities of a species in a particular zoogeographical realm to develop a particular neoplasm it must be recognized that this may be a function of genetic variation. Such variation might provide the correct setting for the action of various extrinsic agents (i.e., virus) in the etiology of a particular neoplasm. However, it is apparent that extrinsic environmental factors may so damage animal development that the biologic variation produced is responsible for neoplasia. The effects may be direct, subtle, or delayed. Its expression may depend on a unique concert of genetic, viral, and environmental factors, without which the neoplasm may never have had the opportunity for expression. Somewhere within this melieu, may lie a spectrum of biologic variation whose existence is unproven, whose nature is unknown, and whose effect on the host undetermined. It is apparent that the emergence of detectable neoplasms is the culmination of a complex series of anatomical and

and biochemical events. Although this process may occur within a well defined environment (i.e. laboratory transplantation studies) alterations in the host environment may have profound consequences in the development of neoplasms.

At present, a catalog of neoplasms in fish is slowly being accumulated. Incidence data collected and compared among various populations around the world would do a great deal to correlate particular types of neoplasms to extrensic environmental factors. Presently, the actual incidence of neoplasms, is largely unknown and reports will undoubtedly vary (as they do in human medicine) with the source of material (i.e. age), location of study (i.e. species density, geographical characteristics, etc.), competence of autopsy, etc.

It is apparent, however, that fish and other poikilotherms have consierable utility as sentinel animals for the early detection and eventual identification of environmental carcinogens. By the process of biological magnification, fish and other aquatic animals are exposed to environmental factors several thousand times greater than those in water. As the animal species which inhabits our heavily polluted aquatic environments, they are exposed to water-born pollutants which far exceed levels to which other animals are exposed. In light of the narrow biologic spectrum to which carcinogens are screened (particularly with regards to possible synergistic roles which various chemicals and viruses might play in the induction of neoplasms) monitoring for neoplasia in wildlife exposed directly to a multitude of environmental pollutants has obvious merit.

Recent technical and conceptual advances in virology, molecular biology, genetics, and biochemistry have expanded knowledge of neoplasia enormously. In order to fully utilize these advances, there is a need for animal models to reproduce neoplasia seen in man. There are numerous biologic similarities which are common to all vertebrates. Numerous too, are the dissimilarities which distinguish species and genera within this class of animals. Of medical significance are those differences which confer biologic advantage or disadvantage to the host. Fish and other poikilotherms might provide a unique role in the development of new model systems for elucidating the basic neoplastic processes.

Of course the question exists, do these neoplasms represent a public health threat. Although this consideration may seem "far out" one must bear in mind the successful transmission of Rous sarcoma virus into poikilotherms,[24] the replication of viruses associated with adenocarcinoma of frogs in cells of mammalian origin,[25] the replication of human viruses in fish cell lines,[26] and the roles that snakes are believed to play in the epizootiology of equine encephalitis[27]. It is apparent, that the possible roles of fish as possible reservoirs of human viruses has been sorely neglected.

With regards to the future, it is proposed that a global system of monitoring neoplasms in selected species of aquatic animals which have widespread geographical distribution be initiated. Presently, one of the most productive areas in human cancer research have been similar studies with regards to the occurrence of human cancers. It would seem likely, therefore, that a system of monotirong fauna and flora in our environment is as relevant or perhaps more relevant than monitoring human populations with regards to environmental carcinogens. Although this hypothesis might sound pragmatic today, it may well be paradoxical tomorrow.

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28. Supported by grants from the Canadian National Cancer Institute, Environment Canada, Canadian National Sportsmen's Show, Anna Fuller Fund, and the Ontario Department of Natural Resources. Dr. Sonstegard is a Research Scholar of the Canadian National Cancer Institute.

BIOLOGICAL RECYCLING OF DISSOLVED NUTRIENTS IN TREATED DOMESTIC WASTEWATERS USING HYDROPONIC AND AQUACULTURAL METHODS

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INTRODUCTION

A multidisciplinary research program is directed toward gathering the basic knowledge and technological expertise needed to develop potentially widely usable systems for fresh water aquaculture and hydroponics. A major aim is to find efficient, economically viable mechanisms for the biological capture of nutrient materials in the water streams processed by small to medium size sewage waste treatment plants. The combination of aquacultural and hydroponic techniques with wastewater treatment, in the absence of toxic industrial wastes, has great appeal because it seems likely to offer solutions to a range of important problems. It probably can:

(1) Provide a realistic basis for the development of fresh water aquaculture as a substantial industry within the United States and in other technologically advanced countries. Among major reasons why such development has not taken place to date in most advanced countries are: (a) the lack of suitable geographic sites in many areas--sites which waste treatment plants may often be able to provide; (b) the lack of adequately predictable, reliable flows of water of consistently high enough quality to permit mass culture of useful organisms--a lack which waste treatment plants generally should be able to remedy; and (c) the lack of environmentally

acceptable mechanisms for disposing of the wastes produced by large scale aquaculture operations--another lack readily remediable by waste treatment plants.

(2) Assist substantially with the economics of the operation and maintenance of many small to medium size waste treatment plants and their districts. This can be done via the production of salable products of many kinds, especially food products.

(3) These economic contributions can also probably be associated with significant energy savings, since the products grown will require little, if any, fertilization. Irrigation, waste removal, and waste treatment can all be taken care of within the culture systems at small energy cost. All four of these processes are very costly in terms of energy in traditional forms of agriculture [cf. Table 1, p. 444 in Pimentel, et al., "Food production and the energy crisis," Science 182: 443-449 (1973)].

(4) Assist to limited, but still useful, extents with the improvement of the quality of waters discharged from waste treatment plants into natural bodies of water.

The research program is a cooperative venture between the IEEB and the Las Virgenes Municipal Water District (LVMWD), Calabasas, California. The laboratory portion of the program is carried out in IEEB facilities at UCLA, the on-site portions on the grounds of the Tapia Park treatment plant of the LVMWD in Malibu, about 45 minutes drive west of UCLA.

The project involves research work and technology development and testing in five areas:

1) Evaluation of potential culture species

2) Design of culture systems

3) Systems development and analysis

4) Water quality

5) Product quality

In addition, there is a Project Management component.

The specific goal of the project is to develop wastewater based fresh water aquacultural and hydroponic intensive culture systems for economically useful organisms that can be used with any of the many thousand small to medium-sized wastewater treatment plants in the world which do not have to handle substantial amounts of toxic industrial wastes.

The program is presently supported by UCLA, the LVMWD, and the Rockefeller Foundation (grant no. RF 74070).

BACKGROUND

There have been many discussions of the potential of aquaculture for an important role in world food production via the utilization of nutrients in wastewaters. Many systems are currently under investigation for their potential to relate to the inexpensive production of food. Various countries have developed systems which are specifically workable in their own societies.

In the United States and elsewhere research is being conducted on systems with potential application to large segments of the world. A review of these projects shows that they are considering systems either with unique capacity in food production or for wastewater benefaction, but rarely for a combination of the two. As results both of the locations of the institutions and of the interests of the investigators, these groups have tended to ignore some of the most direct systems, namely fresh water aquaculture and hydroponics.

Research in the United States has largely concentrated on use of sewage to enrich marine environments so as to force the growth of algae and plankton (Ryther-Goldman Program, Woods Hole, Massachusetts). These organisms then act as sources of food for other marine species in the food chain, such as oysters and scallops. Considerable attention is also being given to marine plants which have economic value, such as Irish moss and kelp. This research has provided valuable foundations in terms of providing designs for systems with general applicability to the problems we are studying. However, this approach is limited in that it is tied to a marine location, and is restricted to certain climatic regions. The designs of these systems necessitate great dilution of the nutrients carried in the (usually fresh) wastewater stream. These systems also involve the contamination, to some degree, of previously unpolluted marine habitats.

The development of fresh water systems has the advantage of extending the already partly developed marine technology to many inland locations. It also provides for the use of more concentrated forms of sewage, allows for more efficient use of the heat usually concentrated in wastewater, and greatly broadens the numbers of locations and species with which wastewater aquaculture can be practiced.

OBJECTIVES

The overall objective of the program is to try to develop a widely adaptable, flexible array of hydroponic and aquacultural methods based upon maximum utilization of dissolved nutrient materials in wastewaters. Ideally, all of the systems would grow entirely upon the nutrients in the wastewater stream. One of the central research problems involved is the determination of how close to ideality it is possible to go.

A variety of alternate strategies are being assessed. These include: (a) hydroponic culture of selected higher plants, either in single or multiple species culture, both in parallel and in series sequences; and (b) various artificial food chains, beginning with appropriate algae and including selected herbivorous and carnivorous animals. The food chain alternatives include different types of sequential arrays (monospecific cultures feeding one another in sequence) and different mixed arrays (polyculture artificial ecosystems). Varying combinations of environmental conditions, all based upon conditions likely to occur in real world waste treatment plants, are being assessed for each biological strategy.

The program has seven sub-goals:

(1) Examination of existing concepts and procedures in wastewater treatment, aquaculture, and hydroponic culture. Determination of mutually beneficial interrelationships between them. Selection of most promising methods.

(2) Development of systems providing for detection, control, and inactivation of bacteria, viruses and other potential health hazards in the culture media and the products produced.

(3) Evaluation of species with potential for culture.

(4) Evaluation of alternative culture systems.

(5) Development of the technology that can link the various components of the system into an operative entity.

(6) Development of essential knowledge relating the biology of culture species to the integrated systems.

(7) Evaluation of the economics of alternative approaches. Development of workable models directly applicable to the overall objective.

SPECIFIC TASKS

I. Evaluation of potential culture species

The evaluation of species is a complex task involving five major subject matter areas:

(1) Plant Physiology

(2) Algalogy

(3) Invertebrate Physiology

(4) Fish Physiology

(5) Pathology

Although the methodology of evaluation for each one of these areas is very different, the objectives of the evaluation are identical.

(1) Attainment of optimum productivity.

(2) Identification of production functions, all those factors that relate to allocation of resources within an operative system.

(3) Identification of interactions with other species and the treatment plant (e.g. demineralization of wastewater).

(4) Selection of those species that can be intensively cultured within the constraints of the species physiology and the design and water quality of the system.

CRITERIA FOR SELECTION OF SPECIES FOR CULTURE

The characteristics of each candidate species provide the criteria for investigations in connection with the culture systems under study. It is a combination of the environmental needs of the animal and the nature of the system under study which leads to the selection of species. The following is a list of traits used in preliminary screening of species for culture.

(1) Reproduce naturally in captivity or are capable of being stimulated to reproduce by modification of the environment, or use of hormones to stimulate reproduction in captivity.

(2) Have short larval period, and requirements for high survival thru larval period are known.

(3) Species can be grown in high density.

(4) Species feeds low on the food chain or demonstrates efficient conversion of foods.

(5) Short reproductive cycle and growth to maturity, favoring genetic selection and multiple crops per year.

(6) Hearty and disease resistant. Able to withstand fluctuations in the environment.

The choice of species for study and ultimately the potential for food production are reflected in spatial requirements and location in the water column. The design of systems for the species chosen includes consideration of combinations of different species having different dietary needs, feeding habits, and tolerances for environmental conditions. Disease susceptibilities and possible interactions (e.g. one species serving as carrier for disease of another) are also considered.

The species being tested in the program are mostly those for which a substantial body of practical cultural information already exists. This information is used to the fullest possible extent. In virtually all of these cases, however, the available information is not complete enough, or of high enough analytical quality, to permit the immediate development of optimal culture strategies and procedures. Thus considerable basic biological study is required for each form, no matter how widely used in other contexts.

II. Design of Culture Systems

The physical implementation of the project concepts is first being carried out in bench scale culture systems serving dual purposes:

(1) The holding and maintenance of experimental stock.

(2) Preliminary, small scale trials of apparatus that may be incorporated in later pilot scale tests.

There is often a gap between the biological understanding of a species for culture and the efficacy of large scale culture. It is the objective of this task that engineering expertise be merged with an intimate understanding of the organisms in culture.

Culture systems are being developed by review of the literature, combined with the experience of project team members, and consultation with commercial culturists. These inputs have provided initial designs for construction and testing.

III. Systems Development and Analysis

Decision making is a process central to the success of the project. From the initial literature survey to the final evaluations there must be an objective means of incorporating all information obtained into a conceptual model.

Each of the tasks can be looked upon as an investigation in a subsystem of a comprehensive system model. As the overall conceptual model is developed, appropriate sub-routines for these individual subsystems are investigated and incorporated in the model. As the model develops conceptually, specific inputs are demanded from the subsystems. This input demand provides a test of relevance for the output and design of specific research projects. The extents to which certain types of subsystems, such as algal cultures, can be used as indices for trends in the larger system are being investigated. In many areas available information is inadequate for the demands of modeling efforts. This points the way toward the design of further subsystem investigations. Thus the modeling effort is an important management and organizing mechanism for the development of the project and the development of management techniques for hydroponic and aquaculture system studies in general.

In the final analysis, the model generated will be more than evaluation of a pilot project. It will be a sophisticated means of projecting the concept implemented by the project to larger and more widespread applications.

Internal economics of the system are closely monitored at all stages.

IV. Water Quality

The inclusion of intensive hydroponic and aquaculture facilities in a wastewater treatment plant generates unique problems in water management. The dense culture of organisms in a highly perturbed environment requires that precise monitoring and control of water within the narrow constraints of optimum culture parameters be established. The interactions with and the benefaction of the final effluent are a central concern of the program.

The water quality aspect of the program is a series of activities that determines many of the design requirements in the final systems. Monitoring of numerous physical, chemical, and biological properties of water in process, both at points within the treatment process and in culture facilities, provides the data for necessary modifications. Activities within this task include:

(1) Identification of Water Quality Criteria: California Regional Water Quality Control Board requirements for wastewater are evaluated

in conjunction with reviews of culture organism requirements.

(2) Testing: One of the most important parts of the total program is determining the efficacy of systems, both in culture and effluent quality senses. Evaluation and subsequent modification of systems designs are based on the results of these tests. All relevant parameters are measured, both physico-chemical and microbiological.

V. Product Quality

Since the ultimate output of several of the proposed systems is food for animal and human consumption, the products produced must adhere to the most stringent quality control criteria. The most comprehensive testing of cultured organisms must document their acceptability for human consumption.

The concept of growing human foods in effluent waters is not widely practiced or accepted in the United States. Therefore, the broadest possible demonstration of product quality standards is essential, both with respect to public health and market acceptability. The major thrust of this task is to screen products for any chemical, viral or bacterial contamination. The following activities provide a base of information needed to document product quality.

(1) Identify Quality Criteria: A comprehensive survey of standards and regulations has been made. This includes all State and Federal regulations (e.g. Dept. of Public Health, FDA, Dept. of Agriculture).

(2) Preliminary Evaluation of Product Quality: Physico-chemical, virological and bacterial sampling of animals grown in bench scale models determines whether modifications in system designs are needed, and identifies specific problems.

(3) Development of Routine Monitoring Techniques: Animals reared under actual mass culture situations in pilot demonstration units will be screened for all pathogenic influences. The isolation and identification of all such organisms will quantitatively and qualitatively describe the extent of any contaminant in the product. Chemical analyses will be performed to detect the presence of any deleterious substances in the product.

(4) Evaluation: Product quality is evaluated against quality criteria identified.

TRAINING ASPECTS

One of the major benefits derived from having a program such as this carried out by a university is, of course, the fact that many of the people involved are students and others who are interested in making careers in the general subject area. The program provides opportunities for:

(1) Practical experience and financial support of graduate students working in a variety of related areas. These include students in UCLA programs in biology, public health, engineering, and environmental science and engineering.

(2) Masters thesis and doctoral dissertation subjects and facilities for graduate students in the disciplines mentioned.

(3) Postdoctoral level research apprenticeship experience for professionally qualified people in the disciplines involved, better preparing them for higher level jobs in these fields.

(4) Training of both professional and technical people from other countries who are interested in the project area.

STAFFING

The senior scientific staff involved in this program are all members of the regular University of California faculty. Their names, titles, and research fields are:

Co-Principal Investigators:

Malcolm S. Gordon, Ph.D., Professor of Biology and Director, IEEB, UCLA. Fish physiology.

John D. O'Connor, Ph.D., Associate Professor of Zoology, UCLA. Invertebrate physiology and endocrinology.

Co-Investigators and their research fields:

David J. Chapman, Ph.D., Associate Professor of Biology, UCLA. Algal physiology and biology.

Edwin L. Cooper, Ph.D., Professor of Anatomy, UCLA. Invertebrate physiology and pathology.

John Dracup, Ph.D., Associate Professor of Engineering Systems, UCLA. Systems analysis and organization.

O. R. Lunt, Ph.D., Professor of Botany and Director, (AEC) Laboratory of Nuclear Medicine and Radiation Biology, UCLA. Plant physiology, hydroponics.

Robert Mah, Ph.D., Professor of Public Health, UCLA. Microbial ecology.

Arthur Wallace, Ph.D., Professor of Plant Nutrition, UC Riverside. Plant physiology, hydroponics.

The research staff working on the program, under the general direction and supervision of the listed faculty members, is currently (April 1975) composed of three postdoctoral research associates, five research assistants, and five laboratory helpers.

CURRENT STATUS OF RESEARCH

The project is now (April 1975) approaching the end of its first year. As the project has developed, Tasks I and II described above have partially merged and then subdivided again, the new subdivision being based upon the functionally different needs of the hydroponic studies as compared with the artificial food chain, aquacultural studies. Presently available support levels for the work have also necessitated a partial functional fusion of Tasks IV and V.

Hydroponics: Substantial series of bench scale tests have been carried out using chrysanthemum plants, to determine the technical feasibility of growth of commercially valuable flowers using fully treated secondary stage effluent from the Tapia Park treatment plant as culture medium. These tests have been uniformly successful, but have demonstrated that the effluent is somewhat deficient is available iron for plant growth. Simple and cheap leaf spraying of dilute ferrous sulfate solutions reverses this deficiency.

There are preliminary indications that concentrations of some heavy metals in the leaves of the plants may reach levels sufficient to cause concern if efforts were made to grow leafy vegetable crops. It is improbable, on economic grounds, that leafy vegetables will become desirable culture species. The heavy metals involved probably derive from such sources as metal piping in homes, galvanized water fixtures, etc.

The bench tests have mostly been done at UCLA, using standing volumes of effluent, frequently changed on various schedules. A small experimental greenhouse has recently been completed on-site at the Tapia plant. This greenhouse permits studies under more realistic conditions of controllable rates and patterns of effluent flow through standard hydroponic growth tables. It also permits study of the effects on plant growth rates and yields of slightly elevated atmospheric carbon dioxide concentrations. Carbon dioxide produced in the primary treatment of wastewater is piped to the greenhouse to produce desired CO_2 levels.

Artificial food chains: The desired food chains for testing are short, initially involving no more than three stages. These are: (1) algae; (2) a herbivorous animal; (3) a primary carnivore animal.

Tests have been carried out on four genera of green and blue-green algae to determine the most suitable forms for mass continuous culture using secondary treated effluent as complete culture medium. Present evidence is that algae of the genus Scenedesmus show the best overall combination of growth and density characteristics. Extensive continuous culture (chemostat) studies are in progress to determine optimal light, temperature, etc. conditions, also maximum sustainable growth rates and culture yields.

The herbivorous animals selected for initial studies were cladoceran crustacea of the genus Daphnia, and various close relations. Extensive tests have been carried out with Daphnia pulex, demonstrating the feasibility of growing this form on a diet composed exclusively of the algae grown on effluent. This work is continuing, including now several other species of cladocerans. Studies are in progress of reproductive rates, growth rates, feeding rates, maximum culture densities and yields, etc. for continuous cultures of the various cladoceran species fed on several of the algal species found to grow best on the effluent.

Studies of culture of a carnivorous animal (a bony fish, probably the Channel Catfish, will be first choice) have been deferred until culture conditions and methods for the first two stages can be reasonably well defined.

Systems analysis: A fairly detailed computer model of the main features of a continuously productive three-stage artificial food chain is being constructed. A comparable modelling effort for hydroponic culture of higher plants is also under way. The reciprocal iterative process of basing experiments on requirements of the models, and of modifying the models on the basis of experimental results, has been begun.

Water quality and product quality: A detailed study has been completed of all regulatory agency requirements relevant to the project and its products. Physico-chemical and microbiological monitoring programs for both water and products have been established and are in operation. Beginning steps have been taken to investigate possible problems of virological contamination.

THE FUTURE

At the date of writing this description a decision was pending in the California State Water Resources Control Board and the Federal Environmental Protection Agency concerning a proposal for funding a pilot scale test of the technical and economic feasibility of a tertiary treatment system for the Tapia Park wastewater treatment plant based on the principles and findings of this program. If this decision is favorable, a two year program of substantially larger scale culturing of a wider range of higher plants and a variety of artificial food chains will be undertaken. These efforts will be supported by continuing systems engineering, economic analysis, and water and product quality monitoring programs. They will also be supported by a continuing research effort directed toward selection of good candidate species for culture, development of methods to forestall possible problems deriving from diseases of culture organisms, determination of the reality of concerns involving viral contamination, etc.

THE CONTAMINATION OF SOIL AND FOOD CROPS BY TOXIC ELEMENTS NORMALLY FOUND IN MUNICIPAL WASTEWATERS AND THEIR CONSEQUENCES FOR HUMAN HEALTH

By

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INTRODUCTION

It is now generally recognized, in scientific circles at least, that mankind faces an ecological crisis as a result of overpopulation and its attendant problems. While the most pressing of these problems is the shortage of food in parts of the world dependent on large-scale importation of food supplies, we also face a variety of problems arising from various kinds of environmental pollution and the prospect of exhaustion of mineral resources and fossil fuels. The seriousness of this situation has been underlined in recent years by the publication in The Ecologist of "A Blueprint for Survival," by Reports to the Club of Rome and by numerous "doomsday" publications.

While this paper is not concerned with the reasons behind our current predicament or with the philosophy inherent in the economic system which has produced it, the problems associated with the disposal of sewage sludge, and municipal wastewaters in general, are one important aspect of environmental pollution and should be considered in the context of the wider environmental problem.

The author's own interest[2] is in the dispersion of trace elements, originally mined in localized deposits, throughout the environment, so this paper will be confined to a consideration of the extent to which municipal wastewaters currently contribute to this process. The more ephemeral problems associated with the local creation of biological oxidation demand (BOD) will, no doubt, be adequately dealt with by other contributors.

Most of the relevant work in this field relates to sewage sludge or untreated sewage and there is, unfortunately, a dearth of published information on the possibility of metal pollution arising from effluents produced by sewage disposal plants after sedimentation.

EFFECTS OF APPLICATIONS OF SEWAGE SLUDGE ON SOIL COMPOSITION

If we take a birdseye view of what is happening to the trace-element composition of the soil, we can see that there are two broad trends. In rural areas there is a trend towards reduced availability of essential trace elements as a result of cultivation. Most of the operations involved in cultivating the soil tend to reduce the availability of most trace elements.[3] Liming has this effect, ploughing and harrowing aerate the soil, reduce availability by producing oxidizing conditions, and drainage and cropping accelerate depletion.

In urban areas we have a trend in the opposite direction and the availability of a wide range of trace elements in the soil is enhanced as a result of environmental pollution from such sources as domestic chimneys, industrial chimneys and motor vehicle exhausts. There is also trace-element contamination from materials which are deliberately added to the soil such as cinders, fly-ash, municipal compost and domestic rubbish. We also have to consider incidental contamination from bonfires, discarded car batteries, corroding galvanized iron and corroding objects made of copper, bronze and brass.

Table 1 illustrates the magnitude of the differences which already exist between the available levels of trace elements found in urban and rural soils. The rural figures are based on the analysis of 100 representative field soil samples taken in South-East Scotland and the urban figures on samples taken from domestic gardens and public parks[4] in five urban centres in the same area. The general level of contamination encountered is similar whether samples are taken from gardens or public parks[4] and there seems no reason to doubt that the levels in urban areas will be similar all over Western Europe and North America.

Table 1. Mean levels of "available" trace elements in urban and rural soils (ppm).

Extractant	Water B	EDTA Cu	Acetic Acid Pb	Acetic Acid Zn	Acetic Acid Cd	Total Hg
Rural arable	0.70	2.3	0.7	2.9	0.13	0.11
Urban	1.81	15.3	11.2	52.4	0.31	0.64

We now appear to be in a situation where the trace-element composition of urban soils is a reflection of the extent to which the soils have been contaminated, rather than a reflection of the composition of the parent material, and results supporting this conclusion have been published elsewhere.[5,6,7,8]

The extent of contamination of soils in the urban environment is now so great that it is now possible to identify most soils as urban or rural on the basis of their content of a few elements known to be general urban contaminants (for example, Zn, Pb and Hg). This is to say that the effect of contamination already over-rides any differences due to the composition of soil parent materials.

Since most rural soils are still relatively uncontaminated with trace elements, we have to consider the composition of the materials which are commonly added to agricultural soils with a view to increasing their productivity[9,10] and the effects of adding the metals such products contain, on the soil. The trace element composition of municipal wastewaters used for irrigation purposes and of sewage sludges are therefore of considerable interest at this time.

In densely populated countries, there is now considerable pressure to dispose of the waste products of society such as sewage sludge, or even domestic refuse, on agricultural land, and as the rural areas dwindle at the expense of urban expansion, this pressure will intensify. Since sewage sludge is largely organic matter and contains the major nutrients, N, P and K in useful quantities, there is no doubt that the idea that this product should be applied to the soil to allow the nutrients it contains to be recycled, has considerable appeal. The same considerations apply to irrigation with municipal wastewater. The older generation of conservationists tends to favor the idea of disposing of sewage sludge on agricultural land on the grounds that it contains organic matter (loosely called humus), which is regarded as being good for the soil, which is vaguely felt to have been damaged by repeated applications of synthetic artificial fertilizers.

Then there are marine biologists and those concerned with river pollution prevention, who would like to see sewage material disposed of on agricultural land because they want to keep this material away from water in rivers and estuaries. Then there are many people who still see pollution as simply a problem associated with the generation of BOD, so the dispersion of sewage material in soil, with its tremendous oxidative capacity, is seen as an adequate solution to the problem of its disposal. Unfortunately, the problem of disposal of this kind of waste has been complicated by the contribution made to it from industrial sources producing a variety of metallic wastes and we have to consider the effects of adding the metals these products contain on the soil.

In general, the trace-element composition of sewage sludge is similar to that of municipal composts since the bulk of the elements present derive from contamination of raw sewage from industrial sources and the metals in common use in our society find their way into both these waste materials. LeRiche[11] and Berrow and Webber[12] have published figures for the analysis of sewage sludges with respect to a number of trace elements and a feature of these results is their highly variable character and the presence of high and variable amounts of Zn. Berrow and Webber pointed out that the Zn present is in a highly soluble form and gave a median level for soluble Zn based on the analysis of 42 sludges over 500 times the level found in uncontaminated soil.

The author has analyzed ten samples of sewage sludge from towns in the UK and a comparison between the levels found and those present in 100 uncontaminated representative rural soils from South-East Scotland are given in Table 2. The levels reported in Table 2 are of the same order of magnitude as those reported by Berrow and Webber[12] but sludge, as at present constituted, is such a variable product that a mean value for any element commonly present as a contaminant is not of great significance. The presence of factories producing metal-rich effluents within the catchment area of a particular sewage disposal plant will

Table 2. Trace-element composition of sewage sludges and uncontaminated rural soils (ppm).

Extractant		Water	EDTA		0.5 N Acetic Acid			Total
		B	Cu	Ni	Pb	Zn	Cd	Hg
Sludges	Mean	13.6	81	8.1	65	552	3.3	6.7
	Range	3-29	11-181	2.0-17.5	1-557	112-1600	0.2-10.6	1.8-22.6
Soils	Mean	0.6	4.0	1.1	1.2	3.4	0.13	0.11
	Range	0.2-1.9	0.6-21	0.3-3.3	0.2-4.8	0.4-22	0.03-0.43	0.03-0.31

obviously make a great deal of difference to the final metal content of the sewage sludge (Mackenzie and Purves[13]). Indeed, the presence or absence of elements like Cd and Cr in sewage actually depends on whether there is an industrial source, such as a Cd-plating plant or tannery, in the area concerned. Other elements can be traced to local industry, for example, Co, Cu and Ni to foundry processes, alloy production and electroplating, and Ag to photographic processes.

In Scotland, most towns have an annual "trades" holiday when most factories close and only maintenance work is done. One would therefore expect a reduction in the content of some metals in the sludge produced during the holiday period, and figures quoted in Table 3 for sludge produced in the Scottish industrial town of Kircaldy, bear this out.

These results appear to indicate that in this town, the Cu, Ni and Pb present is largely of industrial origin while the B has a domestic

Table 3. Trace-element concentrations (ppm) in dried sewage sludges.

Extractant	Water	EPTA		0.5 N Acetic Acid	
	B	Cu	Ni	Pb	Zn
Sludge					
Holiday	18.3	11.2	5	5	1350
Working	6.7	27.2	14	590	1600

source. This may well be the case since washing powders and detergents frequently contain around 1 per cent soluble B and borax and boric acid are also used in pharmaceutical preparations.

Berrow and Webber[12] have reported that the level of Ni in one sludge fell markedly after an improvement in the recovery of this metal at an alloy factory and that five sewage sludges, which did not include industrial wastes, contained 30 per cent less Cu, Zn and B than 30 sludges based on industrial effluents.

If there is no industrial source of elemental contamination of sewage in the catchment area in which the sludge is produced, we would expect it to have a generally low metal content. The author has encountered a sludge which contained only 1.8, 2.8 and 0.16 ppm acetic acid-extractable Ni, Pb and Cd respectively; this is to say that its composition was not unlike uncontaminated soil. However, even this relatively uncontaminated sample contained 149 ppm acetic acid-extractable Zn, compared with a normal soil level of around 3 ppm, and the levels of Zn generally present in sludges appear to be high whether industrial effluents make a contribution to the composition or not. The origin of the high levels of Zn generally found in sludges is obscure, although it is clear that galvanized cisterns containing water used for flushing toilet bowls, cosmetics and pharmaceutical preparations are possible domestic sources.

The levels of trace elements found in the majority of sludges are so high that their use as fertilizer must inevitably lead to contamination of the soil. One cannot, therefore, generalize about the suitability of sludge as a fertilizer since each product must be judged on the basis of its trace-element composition and this may vary widely even from a single disposal plant.

There is adequate evidence that applications of sewage sludge to agricultural land does lead to substantial enhancement of the available levels of a number of potentially toxic elements. Table 4 gives the mean contents of available B, Cu, Pb and Zn in a number of sludge-treated soils sampled from fields in various parts of England. These samples were provided by the Henry Doubleday Research Association, Braintree, England. The corresponding mean levels found in soil samples taken from untreated neighboring fields and reference levels based on the analysis of 100 uncontaminated rural field soils sampled in South-East Scotland are given for comparison.

Table 4. Mean levels of "available" trace-elements in soils treated and untreated with sewage sludge (ppm).

Extractant	No.	Water B	EDTA Cu	Acetic Acid Zn
Sludge-treated	14	1.45	34.7	77.6
Untreated	12	0.95	16.2	35.2
Rural Arable	100	0.68	4.3	2.9

Although the differences between the mean level for the sludge-treated and untreated groups indicate contamination from sludge, the difference is statistically significant only in the case of Cu. However, a comparison with the levels for uncontaminated rural arable soils makes it clear that the failure to find statistically significant enhancement in the case of B, Pb and Zn was due to the fact that the general level of contamination in the industrial environment in which the control samples were taken was high and variable.

There can be no doubt that repeated applications of sewage sludge can have a drastic effect on soil levels of trace-elements and an example has been given by Patterson[14] of a market garden in Somerset, England which contained 1000 ppm Zn and 1500 ppm Pb extractable by acetic acid, after receiving an annual dressing of 8 tonnes per ha of sewage sludge for 30 years. This level of Zn is well above the author's estimate of the toxicity level for Zn in soil and it is not surprising that vegetable crops grown on this soil were severely damaged. Cases of Ni toxicity, affecting oats and potatoes, were also cited by Patterson[14] and there is the possibility of rendering land permanently sterile by making repeated heavy applications. Contamination of soil with respect to Cu, Pb and Zn appears to be virtually irreversible, for the levels of these elements were not substantially reduced on leaching columns of two heavily contaminated sludge-treated soils over a period of three months with a volume of distilled water equivalent to 40 m rainfall.[2]

Since the results given in Table 4 indicate that it is difficult to establish a contaminating effect of sewage sludge when the soils involved are already heavily contaminated with trace-elements from urban and industrial sources, a long-term study of the effects of addition of sewage sludge was commenced in 1971. Some of the results of this study have already been reported elsewhere (Mackenzie and Purves[13]). A dressing of sludge equivalent to 150 tonnes per ha was made to an area 25 m^2 and an adjacent area left as a control. Representative soil samples have been taken at 6 monthly intervals from these plots and the results of trace-element analysis are given in Table 5.

Table 5. Concentrations of trace-elements (ppm) in sewage sludge dry matter and in treated (T) and untreated (C) soils.

Extractant		Water B	EDTA Cu	Pb	Acetic Acid Ni	Zn	Cd	Total Hg
Sludge		11.0	18.1	14.5	18.5	570	3.3	23
Dec 71	C	0.83	3.1	0.8	0.9	3.4	0.14	0.15
	T	1.00	31	1.8	2.6	42	0.41	0.61
June 72	C	0.90	2.8	1.0	0.8	2.8	0.14	0.12
	T	0.74	9.0	1.4	1.4	17	0.26	2.1
Dec 72	C	0.92	3.7	0.8	0.8	3.7	0.12	0.09
	T	0.80	30	2.7	2.8	48	0.48	1.38
June 73	C	0.90	7.7	1.0	0.9	5.2	0.14	0.14
	T	0.96	15	1.2	1.4	20	0.24	0.56
Jan 74	C	0.73	3.8	0.9	0.9	3.4	0.13	0.10
	T	0.69	36	4.4	2.9	46	0.49	1.6
June 74	C	0.57	3.0	0.8	0.8	2.8	0.12	0.12
	T	0.64	50	3.9	2.9	49	0.54	2.1

The results indicate substantial contamination of the treated soil with respect to every element determined with the exception of B. This is understandable in view of the fact that this particular sludge did not contain a high level of B and any contamination with B would, in any case, tend to be removed by leaching with rainwater. Evidently, none of any of the other elements is leached to any great extent and there is no indication of any reduction in the level of contamination over the three-year period involved. There is apparently a progressive build-up of metals in the soil as the organic matter in sludge decomposes and the metals are adsorbed by soil colloids. The implications of this build-up have been discussed by Andersson and Nilsson.[15]

It is a matter of public concern that agricultural soils should be permanently contaminated to a marked extent with a wide range of metals derived from sewage sludge and we have to consider the possible consequences for plants, grazing animals and humans of the enhanced levels involved. Since man has evolved against a background of a food supply based on virgin soils which normally contain minute amounts of toxic elements like Pb, Ni, Cd and Hg, in a form available to plants, we run the risk of creating serious biochemical difficulties for ourselves if we allow the soil to be contaminated in the long-term with toxic substances which can pass freely into plants.

CONSEQUENCES OF TRACE-ELEMENT CONTAMINATION

The extent to which any element present as a contaminant in the soil is taken up by plant roots varies widely depending on the element involved. Some elements like Cu and Pb, are not readily taken up by most plants when present in excess and large increases in soil levels are not represented by corresponding increases in plant concentration. Other elements, such as B and Zn, are readily taken up and concentrations of around 10 times the values normally found in plant material growing on uncontaminated soils, are possible. Indeed, the phytotoxicity of B appears to be a consequence of the ease with which water-soluble B enters plants.

Since Zn is readily absorbed by plant roots and is also normally present in municipal wastewaters in high concentrations, the effect of additions of sewage sludge on herbage Zn levels is of interest. The levels of B, Cu and Zn in herbage cut from the treated control plots already described, at intervals up to a period of three years from the time of application, are given in Table 6.

Table 6. Concentrations of trace-elements (ppm) in over-dry herbage from sewage sludge-treated and untreated plots.

	Sludge-treated			Untreated		
	B	Cu	Zn	B	Cu	Zn
June 72	7.6	5.8	82	6.2	2.7	18
Aug 72	7.8	5.0	87	10.4	2.8	18
Dec 72	12.6	4.9	81	12.2	3.3	19
June 73	11.5	8.1	101	7.5	2.6	22
Oct 73	11.7	8.5	70	10.4	5.2	18
June 74		9.1	78		3.9	21

Although the composition of the herbage given in Table 6 does not suggest a hazard for grazing stock, any substantial increase in the levels of toxic non-essential elements in plants grown for human or animal consumption is cause for concern. It is therefore of interest that information should be available on the relationship between the levels of elemental contamination in the soil which can be produced by treatment with municipal wastewaters and the corresponding uptake by various crops. The establishment of phytotoxic threshhold levels for each element likely to be present as a contaminant in sewage is also of great agricultural importance.

The author has carried out a series of pot experiments over a number of years involving the additions of ranges of concentrations of the elements, B, Cu, Pb, Zn and Cd to light and heavy soils with a view to determining the phytotoxic levels for these elements for oats, clover, radishes and lettuces and although this work is not yet complete, the results already indicate that for each element, the relationship between the level in plants and the "available" level in the soil determined by using conventional extractants, is unique. The results of these experiments indicate that harmful effects on plants are possible when levels in the soil are encountered greater than 3 ppm water-extractable B, 30 ppm EDTA-extractable Cu, 200 ppm acetic acid-extractable Zn and 20 ppm acetic acid-extractable Cd. The element Pb appears to be reluctantly taken up by plants even from quite heavily contaminated soils and no phytotoxic effects were observed with Pb up to a level of 200 ppm soluble Pb added to the soil. Since it is not difficult to find examples of soils in urban areas, or soils contaminated as a result of heavy and repeated applications of sewage sludge, where these threshhold levels are exceeded, we can say that there will be some harmful effects on plants resulting from contamination with these elements. In practice, phytotoxic problems involving Zn, Cu and Ni toxicity are not uncommon on land repeatedly dressed with sludge and this product has become unpopular with some farmers in Scotland on this account.

It is also possible to say that where a plant is unable to grow normally as a result of an abnormally high uptake of a potentially toxic element, its suitability as food is suspect. We have, however, been protected to a considerable extent from the effects of metal contamination of soils by the reluctance of plants to take up some elements, notably Pb, from contaminated soil. In industrial areas, much of the increase in the concentration of metals found in plants is undoubtedly de to foliar contamination from airborne pollution, rather than from increased uptake via the root system (Beavington[18]), and many of the high Pb levels reported in plants in urban areas are probably due to surface contamination (Purves[4]). Certainly, Purves and Mackenzie[19] were unable to detect any enhancement in Pb content in the clean hearts of cabbages grown in urban areas.

Until a few years ago, the question of whether food grown on metal-contaminated land is suitable for human consumption was not given any consideration. As long as crop yields were not affected, nobody seemed to be worried about what the plants contained. However, there has been a change in the attitude of the general public to pollution and in 1971 there were scares in English newspapers about health hazards on building sites in Leicester and Croydon, which had previously been used as sewage farms. The soil on these sites evidently contained unusually high levels of Pb, Zn and Cu. Lead levels were reported between 300 and 6000 ppm at Croydon and between 120 and 1297 ppm at Leicester, and there was some concern over whether it would be safe to grow vegetables for human consumption in the areas concerned.

More recently, there has been an outcry in the Slough area, near London, over a scheme for distributing liquid sewage sludge by tanker to farmers who want it. The sludge in this area was found to contain 7500 ppm Zn, 1000 ppm Pb and 100 ppm Cd, and the news of its composition caused such an uproar that the Crown Estates Commission banned the application of sludge on its three tenant farms in the Slough area and more generally.

Among the metals likely to be present in sewage sludge, Cd, which is usually associated with high levels of Zn, probably presents the greatest health hazard, since it is both highly toxic and readily taken up by plants. Reference has already been made to the possibility of phytotoxicity due to Cd at levels of acetic acid-extractable Cd in soil greater than 20 ppm and papers have been published by John[20,21] and by Webber[22] illustrating that this applies to a number of crops and is associated with high contents of Cd in the plants. The normal level of Cd in plants grown in uncontaminated soil is less than 2 ppm in the dry matter but John[21] quotes a level of 668 ppm Cd in the dry matter of lettuce leaves after the addition to the soil of 200 ppm Cd as cadmium chloride. Unfortunately, quite high levels of Cd (up to 60 ppm dry matter) may be present in lettuce leaves before either visual phytotoxic symptoms or any significant reduction in yield are encountered, so that an apparently normal crop may be unsafe for human or animal consumption.

We do not yet know what level of Cd in the soil is dangerous but wide publicity was given a few years ago to the dreadful effects of Cd toxicity on humans in Northern Japan, where the irrigation of padoy fields with water contaminated from a Zn-smelting plant gave rise to an osteomalacia known as Itai-Itai disease in villagers who were dependent on the rice-crop. Lesser intakes of this metal have since been associated with a number of other illnesses. In the circumstances, any enhancement of soil Cd levels must be regarded as highly undesirable.

Nevertheless, sewage sludge is frequently applied to the land without reference to its possible content of Cd and in England, the Agricultural Development and Advisory Service (ADAS) actually published a paper in 1971 (ADAS Advisory Paper No. 10[23]) proposing rates and frequency of application for sewage sludges on the basis of their "Zn equivalent" an arbitrary figure based on the total content of Zn, Cu and Ni in the sludge. This paper appears to be based on the assumption that as long as phytotoxic symptoms and reduction in yield in crops are avoided, it does not matter how much metal-contaminated sludge is applied to agricultural soils. Thus, on the basis of very dubious assumptions about the connection between "Zn equivalent" and phytotoxicity, carte blanche has been given in England for the application of sludges, irrespective of their content of potentially toxic metals other than Zn, Cu and Ni. Since the maximum proposed application is 50 tons dry matter per acre per annum and this would be equivalent to adding a weight of sludge equal to the whole weight of top-soil in a period of 20 years, these recommendations could have serious consequences for stock and human health when applied to sludges containing an appreciable content of Cd. Berrow and Webber[12] have reported total Cd levels up to 1500 ppm dry matter in sludge and although, in sludges produced by sewage disposal plants in areas where there is no industrial source of Cd, the Cd content may well be negligible, it is clearly important that the level of Cd should be known before heavy and/or repeated applications are made to the land.

DISCUSSION

Sewage sludges and municipal wastewaters, as at present constituted, are highly variable products which contain unpredictable levels of a wide range of elements potentially toxic to both crops and stock and the consequences of their widespread application to agricultural land is incalculable unless their composition is monitored by trace-element analysis. However, if steps were taken to prevent metal-containing industrial waste products from contaminating human excrement before it enters sewage disposal plants, the level of elemental contamination could be much reduced and the sludge and effluent produced might well be suitable for use as a fertilizer. The application of sludge or sewage effluents to the land would then become a profitable stage in nutrient cycles involving N, P, K, Ca, Mg and S, instead of a source of pollution of soils with metals. The value of sewage materials as fertilizer has been discussed by Bunting[24] and possible benefits from the content of essential trace-elements in such materials emphasized by Rehling and Truog.[25]

It would, of course, be an extremely costly operation to separate industrial effluents and ordinary sewage and even if this could be achieved in a society which still equates industrial expansion with progress, the final products would still be contaminated to some extent with metals, particularly with Zn. However, most of the content of Cu, Pb, Ni, Cr, Hg and Cd would be eliminated and the metal content would be much less variable. Toxicity problems resulting from the use of these products in agriculture would therefore be much less likely. It is certainly true that the present practices of polluting estuaries with sewage or of producing huge quantities of sludge which has been rendered unmarketable because of high contents of metals, are nutritionally wasteful, in the long-term dangerous and cannot continue indefinitely.

The contamination of soils and crops with toxic metals derived from municipal wastewaters is simply one aspect of the general problem of dispersion of metals throughout the biosphere. The solution to this general problem lies in the design of industrial processes in which the metals used in industry are effectively recycled. It would be a pity if we were to run out of metals because we had used them to contaminate the environment irreversibly.

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Sampling and Isolation Methods For the Detection of Viruses in Municipal Waste-Waters

by Ebba Lund

In the recent years a number of review papers (8, 11, 23) have appeared on the methods in use for the detection of viruses in water and sludges. Although it would be most desirable, it has to be admitted that no single method exists, which might be applicable for all kinds of samples, in fact it seems that the state of the art is more close to the opposite: i.e. that there are some methods suited for small volumes of highly polluted waters and others for large volumes of relatively clean waters. The in-between-stages are much more difficult.

As also pointed out by Sobsey (23) "the lack of reliable, standardized techniques for detecting and quantitating enteric viruses in water has been a major handicap in our efforts to determine the public health significance of these agents". This is unfortunately still true, although significant advances in virus detection methods have been made. No technique is finalized in such a way, that it could reasonably well be described as a reference method. All methods are still on an experimental stage. They may have been found suitable for samples artificially contaminated with one or more different strains of viruses, but large amounts of data are not yet available. It is not known, if the methods may be equally well suited for all types of viruses, that may be present in polluted waters, or how the methods are working in a real world situation. As the progress is rapid any one who plans to start virus detection work should not do so without consulting directly laboratories directly involved in this kind of work, because important improvements may have come around so fast, that the results are not yet published.

Sometimes people actively engaged in the detective work feel almost embarrassed, that ready and foolproof answers are not available concerning the viruses in water. I think, we are inclined to forget, that the theoretical and applied virology is in an exponential growth phase, which started little more than twenty years ago after a lag phase of around 70-80 years. It is a very steep curve indeed, but the field of applied virology must by necessity come afterthe theoretical field,and quite a few persons find more merite - and probably rightly so - in studying molecular biology rather than dirty waters.

In the present period there are on both sides of the Atlantic well defined wishes from people concerned about methods to be used for analysis of waters, that the virologists stick out their heads and expose their necks and hand out some methods. The virologists only very reluctantly do so, but the coming edition of Standard Methods for the Examination of Water and Wastewater will contain a chapter on the detection of enteric viruses in water and wastewater, and so will the Manual in preparation by the European Regional Office on Analysis for Water Pollution Control. It is felt, that this is an important step forward, but also that this is done rather tentatively and with the view in mind, that these chapters will be subject to changes in the immediate future.

One of the questions of practical importance is: Who is going to do the work. In my opinion, and I share this opinion with a number of others, it is not advisable to let people not familiar with virological work do animal inoculations or tissue culture work. That restricts the number of potential laboratories for virus detective work quite considerably in all countries of the world. This seems very awkward, if you want to promote, from a public health interest, virological examinations, but I think it is unavoidable. Although it is quite possible to buy good-looking cell cultures from a commercial source as well as the proper media, I know so many examples of not-qualified use of such cultures as to be convinced, that inexperienced work in this field is worse than no work, because inreliable positive or negative results are worse than no results. Thus the actual virus isolation work must be done in a specialized laboratory by experienced people. For virologist, who wants to do water samples, there are also a number of new aspects to consider, if they previously worked e.g. with diagnostic samples, but in such cases it must be only a question of getting used to the specific problems and pitfalls.

Whereas the actual identification work must be done in a specialized laboratory, one could easily accept that the preliminary steps, i.e. the sampling and concentration procedures could be taken by a local laboratory well acquainted with ordinary water bacteriology and with a trained bacteriologist responsible for the work. There would be a number of important advantages connected with such divided efforts: Avoidance of transportation of bulky materials, saving in personnel with specialized skills, etc., and there might consequently be a cut in expenses for this type of work, which unfortunately is quite costly anyway. It must, however, be stressed, that even the preliminary work in a local laboratory should not be undertaken except in close cooperation with the specialized laboratory in such a way, that the personnel know, what they are doing. Because of the special risks involved in procedures, which may concentrate a number of pathogens, not only of viral nature, the personnel should be properly instructed and have necessary facilities to avoid the danger of becoming infected from the materials.

With such divided activities in mind the virus detective work from water - and also from sludge - may consist of in principle 6 steps:

1) Sampling
2) Transportation
3) Concentration
4) Decontamination
5) Transportation to the specialized lab.
6) Virus isolation and identification.

1. Sampling

Grab or dip samples are simply obtained by dipping a suitable container into the water to be examined. The technique is essentially the same as that used for bacteriological sampling, except that it in many cases (except for drinking water sampling) may be satisfactory to use a well cleaned plastic container. In some cases it may be advisable to take composite samples over 24 hours by a suitable sampling device, depending on how varied the composition of the water is on the particular sampling spot. By nature a dip sample would represent a relatively small volume. If the sample is going to be handled without too much difficulty, the volume may be in the range o.2-200 l range. In most cases the sample must be concentrated in same way to make the virus detectable. In some of the flow-through filter adsorption elution methods for concentrating viruses from large volumes of water a continuous sampling procedure is by necessity employed. The large volumes processsed could not readily be handled in any other way.

Swab samples

Melnick et al. (17) were the first ones to point out, that swab samples from wastewaters gave a higher yield in terms of virus positive samples than dip samples. Several authors have confirmed this and found quite considerable differences in the number of positive samples between the two types of sampling methods. On the other hand, as it is impossible to translate the findings obtained using swabs in quantitative terms, the swabs should only be used when the emphasis is on obtaining the highest possible number of positive samples rather than a good approximation to true quantity.

Sampling by means of swab samples is especially useful for wastewaters (untreated as well as treated). The opinions on the usefulness of swab samples for sampling from surface waters and sea waters differ somewhat, whereas there is a general agreement, that they would not be suitable for drinking water sampling. It seems that the swabs are important in all such cases, where the pollution is slight and an important part of the virus contents of the water is bound to particles that may be trapped in the swab. The swab sampling could thus probably be regarded as a primitive concentration method.

Swab samples may be obtained in the following way: Cotton wrapped in several layers of gauze (17), sanitary napkins or sponges of previously soaked polyethylene may be hung at the sample collection point. The pads should be clean, but need not be sterilized. After 24-48 days' exposure (1 day being sufficient) the swabs are placed in a plastic bag and transported to the laboratory in a suitable sealed container. In the laboratory the fluid in the swab is squeezed out through a hole cut in the plastic bag. Some workers adjust the swab fluid to pH 8.0 by means of NaCH and/or addition of 3 per cent beef extract or 2 per cent calf serum prior to recovering the fluid.

Sample size

The size of the sample will depend upon the extent of virus concentration of the water. A number of studies in which viruses have been detected in sewage treatment plant effluent have utilized samples as small as 200 ml. To obtain more representative samples it may be advantageous to collect samples several times bigger than the volume to be examined and then remove an aliquot. It is possible to demonstrate small amounts of viruses in hundreds of liters of water, but the costs involved in transportation, concentration, equipment etc. may be prohibitive for routine use even in big public health laboratories.

Virological examinations of water are especially useful in situations such as estuaries with e.g. oysterbeds, studies of new sewage treatment plants of important size, surveillance of wastewater reclamation systems, surface water to be used as drinking water supplies and special epidemiological studies.

2. Transportation of samples to the lab.

If the samples reach the laboratory within 24 hours, and the temperature does not exceed around 25^{o}C, there is no absolute need for refrigerated containers or the like, as the viruses that might be detected are fairly stable. For longer transportations or higher temperature refrigeration is necessary. In all cases the fastest possible transportation should be recommended. In the lab. the samples should be kept in the cold and processed without delay. Only if the samples are kept for more than 2 days prior to concentration, they should be frozen, as a loss in titer may result from freezing and thawing.

Chloroform could be used to inhibit growth of microorganisms during transportation. If the sample still contains residuals of chlorine (free or combined) the chlorine should be reduced by sodium thiosulphate as inactivation of the virus otherwise will proceed during transportation.

3. Concentration methods

Raw domestic wastewater from urban areas may contain up to around 10^5 $TCID_{50}$ per l. If at least 10^4 $TCID_{50}$ or 10^4 PFU are present per liter one may demonstrate virus by direct inoculation without any further concentration, but with most samples a concentration procedure would be necessary. Furthermore a purification may be necessary, if evaluations by PFU are wanted, because toxic components of wastewater may otherwise make the determinations impossible by destroying the cell sheet.

A number of different methods are employed for the concentration of the virus contents of wastewaters. In spite of the fact that some methods give good quantitative recovery with known amounts of viruses, it is most difficult to evaluate, whether they are quantitative when employed on field samples.

In addition to efficiency a method that deserves further study should be simple and require as little expensive special equipment as possible. In the following different methods are tentatively suggested and evaluated with an effort to indicate at least alternative methods where expensive equipment is not utilized.

The methods employed depend on the sample size found necessary because of the amount of virus expected to be present. The turbidity of the sample and other characteristics may, however, also influence the selection of a proper method.

Demonstration and attempt to quantitatively estimate viruses in relatively clean or finished waters may require 200-400 l samples or even more to get a reasonable sensitivity. If suspended particulates make filtering procedure difficult, other methods may be used, which could only be employed on smaller volumes, but then may be the concentration of viruses also is higher in this kind of sample, so that o.5-4 liter samples may be adequate. These samples could be dip samples or may be swab samples, which are really preconcentrated samples for qualitative use.

The methods which have been considered as tentatively the better suited for a number of sample types are: Simple and flow-through filter adsorption-elution, ultrafiltration, 2-phase polymer separation, and chemical adsorption-precipitation.

3.1. Methods useful for sample volumes of 200 ml-5 l

(in cases where the virus load is probably more than 1 infectious unit per 1)

Simple filter adsorption-elution systems

Certain filters of cellulose derivatives or fibreglass are commonly used to concentrate viruses. The membrane adsorption technique depends on the adsorption of virus to the membrane following by elution from the membrane. The concentrations obtained using artificially contaminated samples are reported to be very satisfactory, but the real world samples are more difficult to handle, and wastewater or treated wastewater will usually require prefiltering. Only if the filter pad from the prefiltering is collected and treated by elution the same way as the membrane filter itself, it seems possible to obtain a reasonably quantitative recovery.

Addition of salts such as $MgCl_2$ or $AlCl_3$ and lowering of pH to 3.0-3.5 improves the adsorption to the filter membrane, but care should be exerted, and the time factor becomes all important. Some viruses might be quite different from the ones tested, when working out the method.

The advantages of the membrane technique are their simplicity and speed. The problems ancountered is the possible removal of virus together with the gross particles, substances in the sample interfering with adherence of virus and may be less than optimal elution of virus from the membrane.

Adsorption to polyvalent cation salts or polyelectrolytes or precipitation by means of polyvalent cation salts

Viruses may be adsorbed on alum, aluminum hydroxide floc, ferric chloride, lime and on a number of polyelectrolytes. Especially the polyelectrolyte PE 60 (Monsanto Comp.) has been employed. Despite the demonstrated ability of PE 60 to concentrate viruses from waters, several difficulties have been repeatedly encountered by those attempting to use this adsorbent in developing standard virus concentration methodologies. Different production lots vary in their chemical characteristcs and the efficiency of PE 60 is not identical for different enteric viruses. The production of the PE 60 has been stopped by the manufacturer.

A method (14) employing alum precipitation and elution with trisbuffer of pH 9.o seems efficient, but is not sufficiently evaluated for different viruses. The size of the sample is in general limited by the bulk of precipitate, which readily clogs ordinary filters and collection of floc by centrifugation is not practical for large samples. A very promising method is that of England (5, 6). With the employment of both $Al(OH)_3$ and protaminesulfate precipitation it seems possible to get a good recovery of all the known enteric viruses, which may be present in polluted waters (i.e. enteroviruses, adenoviruses and reoviruses).

The aqueous polymer two-phase separation technique

The application of Albertsson's two-phase separation system for purification and concentration of viruses was described by philipson et al. (20), and it was later shown (15, 22) to work in wastewater samples, but the efficiency is reported to vary from 5-100 per cent. The method is simple and requires little technical equipments. It consists of adding polyethylene glycol and dextransulfate at a proper pH and ionic strength in order to obtain a two-phase separation with 1 per cent of the volume in the dextransulfate-phase containing the virus. It seems best fitted for 0.2-2.0 l samples. By adding another two-phase-separation step the degree of concentration may be increased an additional ten-fold step. It is useful for moderately or grossly polluted water, but may work erratically when used on sludge samples. It may be added as a final step to concentrate eluates from precipitates or filters.

Although the method is technically simple, it requires care in adjusted pH and ionic strength. It has been reported (3), that strains of Coxsackievirus type B 2 and echovirus 6 are inhibited by dextransulphate and that phase separation is not always obtained.

An important problem is that dextransulphate does not seem available any more in a number of countries. Dextran T 500 is being investigated, but does not appear to give as good a phase separation as that obtained with dextransulphate.

3.2. Methods for 1 l samples or more

The following methods seem applicable for 1-50 l samples in some cases even larger depending on the method. If swab samples have been applied, the methods of 3.1. are referred to.

The type of water considered would probably be surface waters, highly treated effluents and saline waters, all of low turbidity and with a load of virus around 1 infectious unit or less per l. Some samples will because of too high contents of particulate matter have to be treated as the samples mentioned under 3.1. This will perhaps mean, that the sensitivity will decrease, so that viruses present in low concentrations may not be detected, but filter materials may too easily be clogged to allow the use of some of the methods to be mentioned here.

Membrane adsorption-elution methods as described e.g. by Metcalf et al. could be employed. Nupen and Stander have employed flat membranes,and in a recent paper by Belfort et al. (1) hollow fiber membranes are employed. As is mentioned in their paper a method should be reliable, simple to operate, with lightweight, compact and inexpensive equipment. Although the results seem very promising, the method is still at an experimental stage and should be tested with different viruses, types of water, real worl samples etc., before it can be properly evaluated.

Limited experiences in my own laboratory indicate, that an adoption of the alum-precipitation, trisbuffer elution method employed by Lal and Lund (14) with advantage could be employed for the detection of virus in 10-20 l samples of e.g. coastal or surface waters. In stead of centrifuging the alum-precipitate a coarse filterpaper in a large funnel was employed. The filter paper is placed in a beaker and eluted there. On artifically contaminated samples the recovery is very good, and the concentration factor also, but the experience is very limited so far.

Soluble alginate filters

As described by Gärtner (9) and employed by several workers (e.g. 7) alginate filter may be made on a filterpaper. Following filtration sodium citrate is used to dissolve the alginate filter. A sample of one litre may be passed through a filter of 47 nm diam. with a good virus retention and nothing can adhere to the membrane, as it is dissolved without being toxic for cell cultures. However, the need for prefiltering seems even higher than for the ordinary membrane filters, because they clog more easily.

The filters are not suitable for samples larger than 1 l (to slow filtration rate). The sample should not contain more than a minimum of colloid material, and the filter is very sensible for a number of ions, e.g. as Na^+, and for EDTA, citrate and trisbuffer.

Neither small volumes of unclarified raw waters nor large volumes of highly treated waters have been conveniently processed by soluble alginate filter.

Tangential fluid flow ultrafiltration systems

A number of techniques have been described which may be employed, if samples of 20 l are sufficient. It could also be used as a secondary concentration method. It seems simpler to use than filtration by pressure or osmosis, but the filtration rate is slow.

Ultrafiltration systems appear to be promising for concentrating viruses from relatively large volumes of highly-treated effluents and finished waters. Surface waters would have to be pretreated, their utility for concentration from large volumes of natural waters has not as yet been adequately demonstrated.

3.3. Examination of samples of 100 l or more

Methods for concentrating small amounts of virus, i.e. less than one infectious unit in 1 l of water from large volumes of water are possible, although the recovery rate may be rather low and perhaps also the reproducibility.

Examinations of large samples of water (100 l or more) are possible only in a few special laboratories. The examinations are in general requiring special techniques although some of the techniques of 3.2. could be used also for larger samples, if the turbidity and organic load are low.

Flow-through filter adsorption elution systems

The Wallis-Melnick portable virus concentrator has been used with a succession of techniques, which may give very satisfactory results. A recent modification is described by Wallis et al. (24). The disadvantage is mainly the special equipment and the overall problem of filter clogging when used for slightly to moderately turbid waters. In the chapter on viral examination by Clarke to be included in the Standard Method this is the only type of method described.

Membrane cartridge filters

Hill et al. have evaluated the use of o.45μ Millipore Millitube, type HA artridge filters for recovering small amounts of poliovirus from 100 gallon (378 l) volumes of preclarified tap and estuarine waters. Adsorbed virus was eluted and a secondary two-phase separation step was added. At low levels recovering average 43%. Further studies on natural waters are needed.

3.3. Methods for sludges

From the point of view of cell toxicity sludge samples present extreme problems. On the other hand viruses may be detected from primary sediments and flocs from chemical treatment without any concentration step. The method of precipitating with polyvalent cation salts seems useful, but passing to new cultures from the original ones is very often necessary because of the cell toxicity. Two-phase separation is not useful for sludge samples.

Conclusions regarding concentration methods and their use

No universal method for concentrating enteric virus has been developed, but efficient and reliable concentration methods for detecting small quantities of viruses in large volumes of turbid natural waters such as river, lake and coastal waters are not yet available. Despite the fact that a number of comparative studies on virus detection methods have been made, additional, quantitative studies are needed to systematically evaluate the more promising methods that have been reported for various types of water.

4. Decontamination of samples

Except for the cases where the concentrated sample to be examined has been rendered sterile through the concentration procedure, the samples must be decontaminated prior to inoculation. Some workers do this by centrifugation at e.g. around 13,000 g or filtering through 0.5 μm pore size membranes. This is not to be recommended,as viruses adsorbed to particles may be removed in this way. As is common for use in diagnostic work on faecal specimens treatment with high doses of antibiotics (10 times the amount employed in the culture media) may be successfully employed if allowed to act for some hours prior to inoculation. (This applies to cases where inocula are about one tenth of the total volume of fluid in a cell culture). Higher concentrations may be toxic to the cells.

With some types of waters (21) 100 units/ml of penicillin, 100 μg/ml of streptomycin, 70 units of neomycin/ml, Fungizone (2-5 μ/ml) or Nystalin 70 units/ml and Polymyxin B (for Pseudomonas aeruginosa) has been successfully employed over many years.

The simplest treatment seems to be treatment with ether or chloroform. Some workers prefer ether, which from a layer on top of the water, others prefer chloroform, which separates below the water phases. In both cases care must be taken to remove traces, that may be harmful to cell cultures. Due to great risk of working with more than very small amounts of ether in an ordinary laboratory ether cannot be recommended for general use.

The following technique may be employed: A volume of chloroform of around 30 per cent the sample volume is added, and the sample is shaken accasionally during a one hour period. The water phase is separated from the chloroform phase by pipetting, and the water sample is treated by bubbling air through for around 10 min. Before inoculation the sample is left open to the atmospherr to remove remaining traces. The decontamination should be carried out in the local water laboratory before shipment to the specialized laboratory in order to avoid excessive growth of the contaminants.

5. Transportation to the specialized lab.

As the transportation of the concentrated materials does not envolve excessive costs and technical difficulties, these samples should be transported not only in the fastest possible way, but also refrigerated. For transportation over periods more than 24 hours shipment with dry ice could even be recommended. Processing in the specialized lab. should take place without delay.

6. Virus isolation and identification

Virus isolation from the concentrates are not in principle any different from other virus isolations except that there is in most situations an emphasis on the quantitative recovery of the virus and also may be a multiplcity of different virus types present in the samples.

Viruses only multiply inside a cell. The virus-host cell relation may be very specific. Viruses contain one nucleic acid (either RNA or DNA in single- or double stranded form), and their size is between 20-300 nm. Some virus particles contain a protein layer (the capsid) as the outer layer and other types are surrounded by a lipid-containing envelope. Each animal species, including man, may be host - usually transiently - for a high number of different viruses.

The extent to which the selection of isolation method can influence the detection of specific virus types differs considerably from the extent to which methodology can influenze the detection of specific types of bacteria. "Selective substrates" do not exist in virology to the same extent, that they do in bacteriology. This does not imply, that all known human viruses can be found by using one type of cell culture. In fact to cover the full potential range different celltypes and variations in methods for isolation must be employed and also inoculations in baby mice.

The viruses commonly encountered in polluted waters are mainly acid-stable, non-enveloped (and therefore relatively stable). They are enteric viruses, because they are transient inhabitants of the intestinal tract and are shed with the faeces in large quantities by infected individuals.

The viruses commonly found in water are: Enteroviruses (a group of picornaviruses, i.e. the small RNA-viruses), reoviruses (also named diplornaviruses because of their double-stranded RNA) and the adenoviruses, which are medium sized DNA-viruses.

In addition the hepatitis A virus (the virus of infectious hepatitis) should be considered. A number of candidate viruses have been suggested: The virus is may be a small DNA-virus (of parvovirus type)or perhaps an enterovirus. Although an immunoelectron microscopic method has made possible the demonstration of aggregates of virus-like particles in suspected faecal extracts a method for cultivation of this virus is not available. Evidence for waterborne spread of the hepatitis A virus is therefore epidemiological.

The number of subclinical enteric virus infections is large (perhaps a hundred fold or more) compared to the number of cases, where an infection causes overt disease. Therefore epidemiological evidence based on occurrence of disease may be an inadequate basis for evaluating the spread of enteric viruses. In addition one type of virus may give rise to diseases of quite different character and unlike viruses may cause the same clinical symptoms. For these reasons it is only by demonstration and identification of the virus itself, that the actual cause of disease can be determined.

In nearly all work carried out the concern has been centered around the viruses pathogenic to man. For different reasons this is somewhat unfortunate. The pollution of waters with viruses pathogenic to all other animal species should be given much more attention. Technically this only means employing cell cultures containing cells from the relevant animals species instead just cultures of cells of human or simian origin.

The demonstration of a certain type of virus by means of e.g. suckling mice or cell cultures does not furnish any information on the virulence of the particular strain. Such information may in principle only be obtained by experiments using the natural host or by knowledge about certain characteristics of the virulent or non-virulent strains, which may be used as genetic markers. Such markers are not available for more than a few of the enteric viruses. The virulence of any virus isolated must be assumed, unless there are special circumstances requiring a reference laboratory to undertake the investigation of genetic markers as in the case of polioviruses.

<u>The types of virus that may be demonstrated using a certain method of isolation</u>

In a mixture of different viruses, as may be expected in samples of urban sewage from larger cities, the ones that grow fastest and give the most pronounced cell destruction or appear in the highest concentration will be the ones most easily demonstrated. If an intensive study of a sewage sample is desired, one should in fact start from the beginning with aliguots of the sewage sample each time a specific virus was demonstrated and to this aliguot add a sufficient amount of antiserum to neutralize the already identified virus. Such a procedure could hardly be used in general for a number of practical reasons, and thus it must be accepted, that the presence of some virus types will be masked by others more fast growing or present in relatively higher concentrations.

To cover the full range of known enteric virus inoculations both in newborn mice and in cell cultures must be carried out. Only the Coxsackie-viruses among the enteric viruses are pathogenic for newborn mice, but some Coxsackievirus type B may more easily be detected in cell cultures. Inoculation of mice is often omitted in examinations of polluted waters and thus Coxsackievirus group A generally cannot be demonstrated. (A number of strains of Coxsackievirus A has been isolated either in primary or continuousline monkey kidney cultures or in human fetal diploid kidney primary embryonic kidney or human cell line cultures (HeLa cells).

Detection of viruses by inoculation in cell cultures

With the for Coxsackieviruses group A mentioned exception the known enteric viruses are cytopathogenic (i.e. they cause cell destructions, which are observable directly by low power microscopy). Cells of human and/or simian origin may be employed.

Inoculations in cell cultures of human cells (either primary cultures of embryonic cells or human amnioun cells, human diploid cells (e.g. WI-38) or continuous cell lines (e.g. amnion cells, HeLa cells, Detroit-6 cells etc.). Using selected human cells polioviruses, Coxsackieviruses group B, adenoviruses and most echoviruses may be detected. Adenoviruses will rarely be detected, unless blindpassage to new cultures is carried out. For reoviruses cell cultures of human origin may be employed, but prolonged incubation times (i.e. 4 weeks) may be necessary. Production of haemagglinitins may be demonstrated even in the absence of cytopathic changes.

Inoculations in cell cultures of primate origin

Monkey cell cultures may be used as primary cell cultures of kidneys from e.g. rhesus, cynomolgus or African green monkeys or cell lines of monkey cells like Vero cells or BGM cells may be employed. Using monkey cells polioviruses, Coxsackie B virus, reoviruses and a broader spectrum of echoviruses may be demonstrated, but very rarely adenoviruses. If it is possible to have a regular supply of primary (secondary or tertiary) human embryonic lidney cells, one single cell culture type could be expected to cover the whole range of viruses. If this is not possible, it must be recommended to inoculate the samples in two kinds of cells (e.g. HeLa cells and primary monkey cells or at least some type of simian cells. This in order to pick up as many as possible of the echo- and adenoviruses. For specific purposes a single type of cells may be sufficient, but the limitation in the spectrum of detectable virus must be realized.

Blind passages are often useful or even necessary, e.g. for detecting viruses in cell-toxic samples, for detecting adenovirus, or if it is desired to demonstrate hither to unknown viruses. The number of viruspositive samples can be expected to increase slightly even for polio-, Coxsackie- and echoviruses. For the demonstration of reovirus prolonged incubation of the primarily inoculated cultures seems more helpful.

Quantification of viruses

In principle two methods are available for titration of viruses, which are cytopathogenic (i.e. cause microscopically observable cell destruction in cultured cells).

6.1. End point titrations expressed in units of $TCID_{5o}$ (tissue culture infective doses on 50% base) or as MPNU.

Serial ten fold dilutions of the virus containing material is set up, and each dilution is inoculated in a number of tube cultures (3-5 tubes or more) using inocula of e.g. o.1 ml. The dilution giving microscopically observable changes (e.g. after one week at $37^{o}C$) in half the inoculated cultures contains per o.1 ml 1.o $TCID_{5o}$. This value may be estimated by means of an evaluation according to Reed and Muench or to Kärber.

The titration error using 5 tubes per dilution is around $10^{\pm 0.3}$. This end point titration could also be applied to titrations in laboratory animals e.g. mice. The titration unit would in this case be a correspondingly obtained infective dose (ID_{50}) or lethal dose (LD_{50}). For mice titrations employing 6 mice per tenfold dilution step the titration error is usually somewhat bigger, probably at least $10^{\pm 0.5}$. End point titrations expressed in Most Probable Number of Cytopathogenic Units (MPNCU). As suggested by Chang (2): If the sample is probably containing only a small number of virus particles and with the hypothesis that 1) these virus particles are not giving plaques 2) or do not occur in sufficient number for an end point titration 3) and that the whole sample must be used primarily, the Most Probable Number of Cytopathogenic Units (MPNCU of Chang) could be suggested. In fact such a method then seems the only way out.

6. 2. The viruses found in wastewater (except most of the Coxsackievirus group A strains) may be titrated in terms of plaque-forming units (PFU).

The plaque technique (Dulbecco) is essentially equivalent to the bacterial colony count in a solid substrate. The principle of the technique is, that a monolayer of cells is exposed to a virus-containing fluid allowing sufficient time to let the virus adhere to the cells. The fluid is then usually removed,and a nutrient agar overlayer is placed over the cell monolayer. The virus produced in the primary infected cells is thus kept from spreading except to the neighbouring cells, and gradually the focus of infected and destroyed cells is big enough to be observed by the naked eye, if the remaining cell layer is stained with a vital stain. The number of plaques produced thus corresponds to the number of infectious virus particles of the suspension. In the appendix the technique is described briefly.

For work with a suspension of free, i.e. not-par-

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THE CURRENT STATUS OF BACTERIA AND OTHER PATHOGENIC ORGANISMS IN MUNICIPAL WASTE WATER AND THEIR POTENTIAL HEALTH HAZARDS WITH REGARD TO AGRICULTURAL IRRIGATION.

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1.0. GENERAL INTRODUCTION:

The problems treated under the heading of this paper represents one of the most difficult problems of the main theme: "Renovation and Reuse of Waste Water through Aquatic and Terrestrial Systems".

In aquatic systems the factors causing dispersion and decay are studied to a much higher degree (cf. Bonde 1968), and valid knowledge is much easier to aquire because of the mixing by turbulence of the streams and lakes facilitating representative sampling.

The distribution of microorganisms in the aqueous environment is quite often (cf. Bonde 1963) well approximated by the Poisson distributions opening a lot of possibilities for computation and statistical tests. This is not the case to the same degree in sediments, sludges, or soils, where no turbulence and mixing are at hand. Microorganisms in water as in soil are always part of some structure which is only imperfectly broken by sampling, mixing, and homogenization, even in water samples. In soils and sediments compound Poisson distributions are much more generally found, as is also the case with other solid materials such as corn-borers in silos. In some experiments with multiplication of Cl.perfringens in soil samples (Bonde 1963) a bigger variation was found between subsamples taken a few cm's apart than any variation caused by changes in the experimental conditions.

The above considerations should indicate that quantitative studies on microbiological processes in soils are much more difficult and for that reason also more rarely performed than in waters. The following presentation will, therefore, be less functional and dynamic and more of a reeling off of knowledge concerning individual organisms, with some reference to their behaviour in water.

The processes active in agricultural irrigation are related to these known from recharging partially treated sewage to underground acquifers, and to the processes of slow sand filtration (Robinson 1969, Ives 1969, Huisman & Wood 1974).

What is known, theoretically, about mechanisms of filtration derives from slow sand filtration processes with flow rates of about 0.2 m/h. Filtration rates exceeding this will have no biological effects at all, or only an incidental and slight nitrification.

The physical mechanisms in operation are: 1) straining, 2) interception, 3) diffusion, 4) inertia, 5) sedimentation, 6) attachment, and 7) detachment (of particles). For these mechanisms mathematical models may be set up, but these are not equally applicable and predictive for the biological processes as those made for waters as already mentioned.

Domestic waste water, waste water from abattoirs and dairies, as well as slurry from stables contain infectious agents from the animal-human reservoir. Sick animals and human beings as well as symptomless carriers excrete pathogens which may later occur in waste water, sludge, and slurry. Their species and numbers are determined by the following factors:

1) The epidemiological situation in the region.
2) The treatment to which the waste water is subjected.
3) The rate of tourism and the extent of trade with live animals, food, and feedstuffs across the borders.

Wastes of the animal-human populations may either be deposited in waters or on/in soil. We are now becoming increasingly aware that the aquatic biotopes are sensitive ecosystems. And overloading with waste matter will cause adverse ecologic changes and accumulation of infectious and toxic substances in the part of the food chain of the water that is used for human consumption. Disposal of wastes in aquatic biotopes will result in uncontrollable spreading of pathogens; soil, on the other hand, is able to retain contamination within a limited area owing to its absorption capacity and its biomass. There is no uncontrollable spreading and no profound ecologic changes. Through generations, farmers have deposited domestic animal waste, which in many countries by far exceeds the amount of human waste, on the ground. In Denmark the amount of feces and urine originating from animal husbandry is about 50 million tons per annum and the farm land about 3,000 000 hectares.

The physico-chemical conditions of aquatic and terrestrial ecosystems differ in principle, e.g. with respect to the contents of organic/inorganic substances, temperature, humidity, oxygen content, carbon dioxide content etc. In the top layers of the soil there is a possibility of dehydration, action of sunlight, fluctuations in temperature, so that in certain geographical areas those layers are practically sterile.

The consequences of contamination of soil with pathogenic bacteria, parasites, virus, fungi, and rickettsia depend on the following factors:

1) The survivability of pathogens in/on soil.
2) The type of crops, their use and treatment before consumption.
3) The degree and time of contamination in relation to the treatment of the crops and their consumption.
4) The type of soil.
5) The treatment of the soil.
6) The climate.

Water containing abattoir waste presents a special problem when used for irrigation. Table 1 shows guidelines for the use of waste water and sludge on agricultural and horticultural fields, woods etc.

TABLE 1

RECOMMENDED DISPOSAL (+) OF WASTE WATER AND SLUDGE

	Agriculture	Horticulture, market gardens, orchards	Woods, plantations, road borders
Purified sludge+)	+	+	+
Non-purified sludge, without abattoir waste	only root crops for animals	non-edible products only	+
Non-purified sludge with abattoir waste	cereals, seeds, industrial crops	-	-

+) Purified sludge is sludge which has been subjected to decontaminating treatment (pasteurization at 70°C for 25 minutes, composting or drying and storing for 6 months) (Å.Jepsen, unpublished).

2.0. CONSEQUENCES OF CONTAMINATION OF SOIL.

Theoretically, contamination of soil may result in secondary spreading as follows:
1) Seepage to groundwater, 2) Surface run-off, 3) Spreading to human beings and animals via consumption of crops, 4) Spreading by rodents, birds, and insects.

2.1. Seepage to Groundwater.

Normally, the absorption capacity of the soil and its capacity for self-purification prevent contamination of groundwater when pathogenic organisms are deposited on the ground. Investigations have shown that the risk of bacteria passing through the soil systems is negligible, and contamination of groundwater is a theoretical problem, provided the soil conditions are normal and the waste is not deposited in excavations where the distance to the groundwater is very short.

2.2. Run-off with Surface Water.

Pathogens deposited on soil may by spread more or less uncontrollably through drain pipes, ditches, and streams, and may be carried to surface waters used for bathing or drinking by man and animals. Leaching and run-off transmit pathogens from primary receiving bodies of soil to water. But apparently this mode of spreading involves no great risk.

2.3. Spreading to Man and Animals through Consumption of crops.

This form of spreading definitely represents the greatest health hazards. Contaminated crops in themselves represent a danger, especially with respect to parasites. And vegetables, fruits etc. used in the household may contaminate other foodstuffs which may provide good conditions for propagation so that infective doses are produced. The consequences of contamination of crops depend on the survivability of the infectious agent. Table 2 shows the survival of pathogens as indicated by various research workers.

TABLE 2

PATHOGEN AND TOTAL COLIFORM SURVIVAL. SURVIVAL ON CONTAMINATED FARM PRODUCE AND FODDER.

Organism	Produce or fodder	Survival time
Salmonella	Fodder	12- >42 days
	Root Crops	10- 53 "
	Leaf vegetables	1- 40 "
	Berries	6 hrs.- 5 days
	Orchard crops	18 hrs.- > 2 days
Shigella	Fodder	< 2 days
	Leaf vegetables	2 - 7 days
	Orchard crops	6 days
Enterovirus	Root crops	15-60 days
	Leaf vegetables	15-60 "
Ascaris eggs	Leaf vegetables	27-35 days
		< 2-3 "
Endamoeba histolytica	Leaf vegetables	
Total coliforms	Fodder	12-34 days
	Leaf vegetables	35 days

The survival time will, of course, be influenced by temperature, sunlight, moisture etc. to a varying degree, and should be considered with caution.

2.4. Spreading of Pathogens by Rodents, Birds, and Insects.

Pathogens deposited on the soil will inevitably be introduced into the wild fauna, where they may persist for varying lengths of time, possibly with consequences for the fauna itself, but there may also be uncontrollable spreading to man and domestic animals. For example, eggs of Taenia are still infective after passage through the intestinal canal of seagulls.

3.0. SPREAD OF HUMAN PATHOGENS VIA WASTE WATER, SLUDGE, AND STABLE SLURRY.

3.1. Salmonella typhi and Salmonella paratyphi.

Müller (1968) showed that certain Salmonella species could survive for more than 9 months in sludge. Mair (1904 and 1905) showed that up to 16% of Salmonella typhi could survive for 2 weeks at 20°C, and that the bacterium was still demonstrable in soil after 70-80 days. Apparently, growth of members of the Salmonella group in sterilized soil under natural conditions has not been proved for certain.

In contrast to Salmonella typhi, Salmonella paratyphi B are often present in waste water and sludge. Finnish, Swedish, German, and Danish investigations (Ojala 1966, Johannsen 1969, Hess 1974, Grunnet & Nielsen 1969, Kristensen 1969, 1970) show that Salmonella paratyphi B is often the predominant Salmonella serotype in waste water, for which reason use of the water in the agricultural sector involves certain risks. The survival of these serotypes, specifically pathogenic to man, appears to be significantly better in wet soil than in aquatic biotopes, where their time of survival ranges from 3 days to 3 weeks (Aubert 1972).

3.2. Vibrio cholerae and Vibrio cholerae NAG.

In some countries V.cholerae and V.cholerae NAG are constantly or intermittently present in waste water and sludge. The increased tourist traffic has contributed to a wider spread of cholera. In 1960 cholera was recorded in 6 countries; during the period 1965-70 in 39 countries. The survivability of those bacteria in soil seems not to have been conclusively investigated. In aquatic biotopes they survive from 2 to 42 days (WHO, 1970).

Recent examinations (Ko 1973, and Kristensen 1974/75, unpublished) show that Vibrio cholerae NAG is often isolated from untreated town sewage. German examinations showed that the bacterium could be isolated from all the samples of Köln sewage examined, and Danish examinations have corroborated those surprising results.

The epidemiological situation of cholera and recent observations concerning V.cholerae NAG should invite greater caution when considering the use of waste water for irrigation in market gardens, orchards etc., where food is produced for human consumption, and often eaten without previous decontaminating treatment.

3.3. Enteropathogenic E.coli

In sanitary bacteriology the role of E.coli as an indicator of fecal pollution has been dominant for many years. In recent time, however, it has been more and more evident that E.coli may play a much more active and unpleasant part to man. Strains causing infection of young children, and in the urinary tract were first known, but later on also the production of an enterotoxin of choleralike action has been demonstrated. The disappearance rate of E.coli in receiving waters is quite rapid. In brackish sea water Bonde (1972) found a 90% die-away in about 60 min. and a total disappearance in 4 hours. In more salty waters or in areas with higher temperatures and more sunlight the die-away is more rapid, in fresh water it can be considerably longer depending upon temperature, sunshine, predatory organisms, etc.

In soils E.coli is known to persist for considerably longer times. Cuthbert et al. (1955) reported a persisting of 14-20 weeks. However, other coliforms will survive E.coli in both water and soil.

The distribution of the enteropathogenic serotypes seems to be quite varying in different parts of the globe, and it must also be envisaged that considerably more types than the ten types generally recognised may cause diarrhoea (Bonde 1963; Cook 1974) and urinary infections.

Irrigation of soil with sewage irrespective of the degree of treatment is a serious hazard to man, in particular to young babies and to some kinds of young animals (piglets, calves, chicken, lamb) and may be transferred by vegetables and lettuce. Furthermore, coli strains of sewage are quite often resistant to antibiotics and may transfer R factors to the pathogenic Gram negative rods present. Soil soaked with sewage in warm climates may possibly be ideal places for such catastrophic events. Pathogenic E.coli must be considered a real danger associated with irrigation of agricultural lands. Although up to 6000 cases of gastro-enteritis in young children caused by E.coli is reported each year even in devellopped countries (England, Cooke 1974), the danger from irrigation is presumably even more serious to young animals, and large doses of the same strains can also cause disease in adults. Serratia marcescens and non-pigmented Serratias are quite common in polluted water and must also be brought to mind in this context.

3.4. Cl.perfringens

is exceptional among anaerobic bacteria by its ready growth in simple media and its distinctive characters, facts which make a quantitative estimation probable also in soils. Because of the spores it can remain for a long time at a given locality. However, ubiquity and growth in nature have never been proved. Its presence in soil has had catastrophical and well-known consequences in times of war (gas-gangrene) and in the causation of epidemics in grazing lamb, sheep, and calves. Among its toxins, one (α-toxin) can also produce gastro-enteritis with man. Estimation of the importance of Cl.perfringens in a given situation (causation of epidemics etc.) will always depend on counts of the organisms, as a few spores may always be present as a background pollution. In virgin soils numbers of 1-10,000 per g of soil have been claimed, mostly as spores. In soils and sediments polluted by feces or sewage the numbers may amount to 100,000 or more per gramme very often mainly as vegetative cells (Bonde 1963).

In raw sewage the amount of Cl.perfringens per ml is generally about 1,000 per ml. In conventional treatment plants the reduction is modest, sedimentation reducing the numbers to 36-38% biological treatment to 16-17%. Treated sewage will thus very often contain 150-200 cells + spores per 1 ml. Chemical treatment and conventional chlorination has no effect on spores.

Cl.perfringens might thus cause serious problems in hygienic respect. However, wound lesions of a kind that might entail gas-gangrene are seldom on agricultural soils, and this infection is much more effectively treated than 50 years ago. Vegetables may be heavily polluted by Cl.perfringens but will not generally be applied in dishes which can give growth and production to the α-toxin. Epidemics among young animals grazing on irrigated soils might be a possibility (in case types B, C, or D are present).

Cl.perfringens must be remembered with watchfulness. Further reference can be given to the monograph of Bonde (1963) which gives further details.

3.5. Bacillus species.

Within the genus Bacillus only one species, Bacillus anthracis, is generally accepted as a pathogen to warm-blooded organisms. However, virulent and toxigenic strains are frequent within the closely related species B.cereus, and strains of other species have also from time to time been associated with various kinds of disease. The insect pathogenic species B.thuringiensis is taxonomically closely related to the anthrax-cereus group of organisms. Other insect pathogens, B.larvae, popilliae, and B.lentimorbus belong in another group with bulging spores, and formation of toxins by some organisms of this group, as well as insect pathogenicity, has recently been described.

Among these are B.sphaericus, and strains of the species circulans and alvei. As will be mentioned later on, strains of these groups have also occasionally been associated with human disease.

Production of exotoxins have thus been demonstrated in more species than B.anthracis and cereus.

Actually, some Bacillus strains are Gram negative and a formation of endotoxins might be envisaged from some species (B.circulans, brevis, and sphaericus). Non-antigenic enzymes such as DNA-se is formed by species to which pathogenicity has been ascribed.

There is thus evidence that virulent strains are found in more groups than anthrax-cereus, supporting the reports on clinical cases.

Bacillus species are common in soils and dust and also do occur in sewage, however, in relatively small numbers. Mud and soils of great estuaries in tropical and sub-tropical areas are known to be important permanent reservoirs of B.anthracis, and the risk from Bacillus spores in soils after irrigation should not be minimized. The possible transfer of B.anthracis is of course obvious, but the toxin producing B.cereus are also mostly spread by cornproducts and may well be conveyed after irrigation. Likewise, the species producing disease in silk-worms can have this origin.

B.licheniformis is typically an organism of water and sewage and is by its ability to reduce nitrate to nitrogen gas very important in sewage treatment; also B.polymyxa is found in sewage treatment plants. In filter sands on water works Bacillus species are frequent (often B.brevis and B.subtilis). The cereus and sphaericus groups seem to be more frequent in soils and sediments.

However, the quantitative occurrence of Bacillus spp. in different kinds of sewage is not too well studied, and the occurrence or the predominance of any one species will also be a question of the whole ecological situation more than of the fecal pollution. Treatment plants and dust storms may for instance be more important sources of Bacillus species in an agricultural area than fecal matter. For further information reference is given to Bonde (1975).

3.6. Pseudomonas - Aeromonas.

Several organisms of these two genera of Gram negative, oxidase+ sporeless rods are pathogenic to plants, reptiles, and fish; only one species, Ps.aeruginosa, however, is important as a pathogen to man and warm-blooded animals (cf. Bonde 1963, Hansen & Bonde 1973).

Most species of Pseudomonas (i.e. the fluorescens-putida, alcaligenes, pseudoalcaligenes, and stutzeri groups) can multiply in nature, and the number of pseudomonads can rise considerably in slow sand filters and during irrigation of sand-dunes.

The effect of this growth must be considered mainly beneficial to purification and shall not be mentioned further.

Thus Pseudomonas aeruginosa alone shall be considered; this species is always demonstrated in sewage and often in polluted receiving waters, however, generally in quite small numbers and only where more recent pollution has taken place. In dried sludges they can survive for months but not multiply and probably also so in soils (Bonde 1963).

Ps.aeruginosa are present in much smaller numbers than are E.coli, besides the occurrence of Ps.aeruginosa is probably depending upon the climatic conditions (Bonde 1963, versus Israeli results).

The demonstration of Ps.aeruginosa may be facilitated by its ability to multiply in Tetrathionate broth at 42°C together with Salmonella sp. (Grunnet, Gundstrup & Bonde 1974).

In this context, only the gastro-enteritis caused by Ps.aeruginosa is of importance, however, probably of smaller importance than that of E.coli.

3.7. Cocci.

Although Staphylococcus aureus and Str. pyogenes are of importance in swimming and wading pools, the only coccus of possible interest for irrigation of soil is the fecal straptococcus, which is very resistant to adverse conditions and are present in sewage in large numbers (1,000-100,000 per ml).
As far as is known these have mostly veterinary interest.

4.0. SPREADING OF BACTERIA PATHOGENIC TO BOTH MAN AND ANIMALS BY WASTE WATER, SLUDGE, AND SLURRY (ZOONOSES).

Zoonoses are infectious diseases which are naturally transmitted from vertebrate animals to man and vice versa. Agents causing zoonoses are frequently present in human-animal waste.

4.1. Salmonella of the Gastro-enteritis Group.

Most species of Salmonella attack both man and animals. Salmonellosis, being one of the most widespread and important diseases, demands special attention in relation to the use of waste water, sludge, and slurry as agricultural fertilizers. Quantitative examinations carried out in the U.S.A., the Netherlands, Germany, and Denmark, show that Salmonella are constantly present, often in large numbers, in waste water and sludge.

Table 3 shows the result of Danish examinations of waste water and sludge from biological treatment plants.

TABLE 3.

Date	Crude waste water	Mecanic-ally treated waste water	Biologic-ally treated waste water	After second settling	Primary sludge	Secondary sludge	Digested sludge
1970							
25/08	>240	46	0.30	0.14	>2400	23	16.5
26/08	1.70	12	0.34	0.09	>2400	35	120
27/10	80	0.50	0.49	0.49	175	1	175
28/10	3.95	80	0.95	0.70	120	11.5	120
1971							
12/01	1.7	12	0.31	0.22	>600	0	6.75
13/01	0.39	3.95	5.40	0.13	>600	19.75	>600
23/02	0	0.08	0.11	0.02	1.38	0.50	1.00
24/02	0	0	0.02	0.02	1.88	1.13	1.95
27/04	27	80	0.17	0.13	400	60	12.25
28/04	12	17.5	0.14	0.14	60	60	60
06/07	>120	46	0.17	0.33	135	5.75	60
07/07	>120	>120	0.95	3.3	230	19.75	88

(Fenger, Krogh, Krongaard & Lund, 1973).

Similar German examinations (Hess, 1974) showed that the concentrations of Salmonella in fresh sludge, aerobically stabilized sludge, and digested sludge were 10^4, 10^2 and 10^2/100 ml respectively. According to Danish examinations (Kristensen 1970) biological treatment of waste water reduces the Salmonella content by approximately 99% (see table 4). With chemical treatment the reduction was even greater (Kristensen, unpublished).

TABLE 4.

QUANTITATIVE EXAMINATIONS OF THE CONTENTS OF SALMONELLA IN TREATED AND UNTREATED SEWAGE WATER (BIOLOGICAL PURIFICATION PLANT REPRESENTING 46,000 INHABITANTS). EXAMINATIONS ON THE DECIMATION OF ESCH.COLI I AND SALMONELLA IN A BIOLOGICAL PURIFICATION PLANT.

Date	Number in influx per 100 cc	Number in outlet per 100 cc	Number from outlet as % of number from influx
03.06.69	0.20	0.07	35.00%
17.06.69	1.40	0.08	5.71%
01.07.69	22	0.42	1.91%
15.07.69	28	0.62	2.22%
29.07.69	24	0.98	4.10%
12.08.69	>240	0.46	0.19%
26.08.69	160	0.52	0.33%
09.09.69	160	0.28	0.18%
23.09.69	>240	0.22	0.09%
07.10.69	160	2.80	1.75%
21.10.69	0.78	0.08	10.26%
04.11.69	0.20	0.26	130.00%
18.11.69	0.40	0.18	45.00%
09.12.69	0.20	0.09	45.00%
16.12.69	0.20	0.08	40.00%
12.01.70	12	0.49	4.08%
26.01.70	3.95	1.70	43.04%
10.02.70	3.95	0.06	1.54%
24.02.70	12	0.23	1.92%
10.03.70	2	0.31	15.50%
02.04.70	Salmonella not detected	0.09	
22.04.70	8.50	0.79	9.29%
05.05.70	17.50	0.43	2.46%
19.05.70	4.90	0.46	9.39%

According to German investigations (Hess, 1974) the survival of *Salmonella* in sludge, slurry, and wet soil was 100-200 days, > 200 days, and 70-500 days, respectively. Generally, the time of survival is longer in wet soil than in aquatic biotopes.

Salmonella infections in relation to irrigation with waste water or waterlogging of pastures by sewage-contaminated surface water have often been described in literature (Jack & Hepper 1969; Bicknell 1972).

Danish examinations show that *Salmonella* are demonstrable in 3% of fecal samples taken from pigs (Skovgaard & Nielsen 1972), and abattoir waste water may be considered a causative factor of disease (salmonellosis).

4.2. Yersinia.

During the last 5-10 years *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* have claimed increasing attention in the fields of both human and veterinary medicine. In Sweden, yersiniosis is considered to be just as frequent as salmonellosis. In Denmark, Finland, Poland, the U.S.A., Canada, and Japan the disease is regarded as an essential problem in human medicine. The cycle of transmission is still unknown, but perhaps domestic animals, especially pigs, act as excretors. Waste water and slurry cannot be precluded as determinant factors in the spreading of those bacteria. But demonstration of the bacteria in soil, slurry, and waste water is difficult owing to the lack of sufficiently selective methods for propagation and isolation. The few findings made so far do not give a reasonably good picture of the extent of Yersinia in nature. It has been demonstrated in surface water, but apparently not in soil. Whether, or to which extent, waste water, slurry, rodents and domestic animals play a part in the spreading ecology and as normally used infection chains has not yet been clarified.

4.3. Listeria.

Listeriosis is a zoonosis of less importance than those mentioned above. But the infectious agent is definitely related to waste water. Dutch investigations (Kampelmacher, 1969) showed that up to 29% of the groups of persons exposed to infection excreted Listeria monocytogenes with feces, while up to 13.3% of cattle excreted the organism. Examinations in the U.S.A. (Welshimer, 1971) showed that Listeria could be isolated from plants or soil samples from 11 out of 12 farms in the spring season, whereas in the autumn the results were negative. Today, many researchers regard Listeria as a ubiquitous bacterium which may be able to propagate even at low temperatures in the soil-plant environment, especially on moist, decaying parts of plants. Living plants do not seem to provide good conditions for growth or survival.

4.4. Mycobacterium.

Tubercle bacteria may be present in waste water from sanatoria and abattoirs, sometimes also in dairy waste water and town sewage. An examination in the U.S.A. (Greenberg, 1957) showed the following contents of mycobacteria in waste water and sludge from a tuberculosis hospital:

Crude waste water	1500,000/litre
Biologically treated waste water	10,000/litre
Crude sludge	100,000,000/litre
Anaerobic sludge	10,000,000/litre

Generally, mycobacteria are extremely resistant against physico-chemical action. They survive for months in soil and sludge. Therefore, waste containing tubercle bacteria should not be deposited on soil. In this connection it should be pointed out that Mycobacterium bovis is just as pathogenic to man as Mycobacterium tuberculosis. Danish examinations have shown that mycobacteria may be isolated from abattoir waste water if there is tuberculosis among the domestic animals. Danish infection experiments on cattle grazing pastures irrigated with Mycobacterium-containing waste water showed that the exposed cattle did not become tuberculin-positive even after several months experiments (Jepsen & Roth, 1950; Greenberg, 1957).

4.5. Brucella.

Brucellosis is a zoonosis of great importance in those countries where it is still prevalent among cattle, sheep, and pigs. Brucellosis is often localized in the udder, consequently milk and dairy waste water are among the spreading factors. About 0.1% of the milk weighed-in at the dairy is spilt on the floor, hence avoiding pasteurization, and some of the pathogens will end up in the waste water. Dairy waste water, in particular, is often sprayed on agricultural fields and may thus contribute to the spreading of pathogens commonly localized in the udder (Brucella, Mycobacterium, Coxiella burnetii, and Foot and Mouth virus). Apart from Brucella, these pathogens appear to be relatively resistant to drying. Abattoir waste water should also be given serious consideration in connection with brucellosis. Detailed knowledge about the survivability of Brucella in soil is not available; but in liquid manure the time of survival is said to be > 10 weeks (Rankin, 1969).

4.6. Pathogenic Leptospira.

Leptospirosis is an important zoonosis in many countries. Pathogenic leptospires are often present in material containing urine, hence also in waste water and slurry. Infected cattle may excrete leptospires for months in numbers up to 100,000,000/ml. The time of survival for the pathogenic, strictly aerobic leptospires is in domestic waste water 12-14 hours, in aerated slurry up to 60 days, and in soil 3-15 days (Smith, 1955). Pathogenic leptospires seem to be very sensitive to dehydration and ultraviolet rays, so in the dry top layer of the soil it only survives 30 minutes. Under favourable conditions in water-saturated soil it has been observed to survive for 180 days.

5.0. BACTERIA WHICH ONLY SEEM TO BE PATHOGENIC TO DOMESTIC ANIMALS.

5.1. Mycobacterium paratuberculosis.

Paratuberculosis is of considerable interest to the animal husbandry sector, affecting cattle and sheep. The bacteria are excreted with feces and are carried with waste water, sludge, and slurry. In soil it seems to be able to survive even longer than mycobacteria (Larsen et al. 1956).
Danish Examinations have revealed an infection percentage of 1.8 in the cattle herds (Jørgensen, 1972). Abattoir waste water, together with slurry, may form an integral part of the spreading ecology of the bacterium. In slurry, the time of survival is only 30 days owing to the urine content. Paratuberculosis is a serious disease, causing considerable economic losses to cattle farmers.

6.0. PARASITES PATHOGENIC TO MAN.

The problem of parasites in waste water, sludge and slurry deposited on/in soil is very serious because parasite eggs are highly resistant in soil and sludge, and because the infective dosis is very small. Some parasite eggs (taenia) are infective immediately on excretion, whereas others (*Ascaris* and *Trichuris*) have to undergo an extraenteral maturing process. Parasites like *Entamoeba histolytica* and *Diphyllobothrium latum* seem to be considerably reduced in numbers in digesting tanks. Large and heavy eggs (*Trematodes*, *Ascaris*) are usually found in the sludge phase, whereas e.g. taenia eggs more often follow the water phase.

6.1. Protozoa.

6.1.1. Entamoeba histolytica.

In tropical-subtropical areas, especially, *Entamoeba histolytica* is an important intestinal parasite which will be carried to receiving bodies of soil or water with waste water and sludge. As shown in Table 4, its time of survival is short on plants, but in soil, protected against light and dehydration, it may survive for weels or months. This parasite is often transmitted by contaminated vegetables and fruits.

6.1.2. Giardia lamblia.

Like *Entamoeba histolytica*, *Giardia lamblia* is a unicellular intestinal parasite. In recent years its extent and importance have become more widely recognized. Its time of survival in soil and sludge is not known in detail, but waste water and sludge must be considered determinant factors in the persistance and extent of this parasite.

6.1.3. Other unicellular parasites.

Protozoa, like Entamoeba coli, Endolimax nana, Balantidium coli and Dientamoeba fragilis, and perhaps Toxoplasma gondii (Isospora gondii), might be spread with waste water and sludge. However, there is doubt about the pathogenicity of some of these protozoa.

6.2. Nematodes.

6.2.1. Ascaris lumbricoides.

Normally, Ascaris lumbricoides is considered specifically pathogenic to man, and Ascaris suum specifically pathogenic to pigs; but the question is still under discussion. Ascariasis is a common intestinal disease, where the patient may excrete up to 45000 very resistant eggs per gram of feces. In some countries, e.g. Italy, infection percentages of more than 40% have been observed. In the same investigation, the infection percentages for Trichuris, Enterobius and Taenia were 14%, 0.25% and 1.25%, respectively (Boventer, 1947).

German experiments on the use of untreated waste water in market gardens showed that the combination of untreated waste water and vegetables is a gamble. 80-90% of the exposed population got roundworms. In this connection it should be mentioned that Ascaris eggs, in particular, are very resistant; they can survive for months or years in wet soil. Danish examinations (Jepsen, 1974, unpublished) showed that eggs of roundworms were found in soil knocked off potatoes, which had grown in sludge-treated soil, in quantities of 2 eggs per 50 g soil. The presence of Ascaris in sludge, slurry and waste water is one of the most serious hygienic problems connected with waste water.

6.2.2. Trichuris trichiura.

Owing to its wide distribution and the considerable resistance of its eggs, Trichuris is an essential problem in relation to the disposal of waste water.

6.2.3. Enterobius vermicularis.

This nematode vauses disease, especially in children, but the infection percentage is much lower than in case of Ascaris and Trichuris.

6.2.4. Other nematodes.

In tropical/subtropical regions waste water contains eggs of Anchylostomum, which may develop into infective larvae in warm and humid areas.

7.0. PARASITES HAVING MAN AS FINAL HOST AND ANIMALS AS INTERMEDIATE HOST.

7.1. Cestodes.

7.1.1. Taenia saginata.

Taenia saginata is a tapeworm of great importance in many countries. Its persistance is due to an interplay between man and cattle, and disposal of waste water on agricultural fields is therefore instrumental in spreading the organisms. Taenia eggs are considered less resistant than e.g. Ascaris eggs. Danish experiments (Jepsen & Roth, 1950) with irrigation of pastures with sedimented waste water showed that all 20 calves used in the experiment developed cysticerci in the musculature after 4-5 months. This proves that cattle tapeworm can be disseminated via waste water, so in areas with a high incidence of taeniasis it is not advisable to use waste water for irrigation.

7.1.2. Taenia solium.

Taenia solium use the pig as intermediate host. It is not so common as Taenia saginata, but it is considered more dangerous.

7.1.3. Other cestodes.

Other cestodes like e.g. Diphyllobothrium latum require the presence of man, fish, and small aquatic animals to complete their life cycle. So disposal on/in soil does not usually contribute to the spreading of this parasite.

8.0. PARASITES SPECIFIC TO DOMESTIC ANIMALS.

Disposal of waste water, sludge and slurry on agricultural fields may also cause problems relating to the livestock only.

Parasitic diseases in cattle usually spread during the pasturing period. The pastures are contaminated by grazing cattle or by waste water, sludge and slurry. Modern animal husbandry seems to provide favourable conditions for parsitism, for example through automatic dung removal requiring less bedding, and the collection of liquid manure in tanks which have to be emptied frequently on the fields because of their relatively small size. An important factor is no doubt the high content of water and the small amounts of bedding in the slurry, since the bedding provides the slurry with the proper structure for the aerobic heat-producing composting processes that will kill bacteria, virus and parasites. As a result of these new tendencies in animal husbandry an increasing number of pathogenic agents in slurry becomes available to grazing animals. Table 5 and 6 show that fecal samples and slurry may contain many parasites of economic importance.

Swedish examinations show that gastrointestinal nematodes may become a problem in connection with slurry and abattoir waste water. Danish and Swedish researchers have found that Ostertagia and Trichostrongylus are important parasites, but also Cooperia, Haemonchus, and Nematodirus deserve attention.

The fact that infective parasite stage survive in slurry and abattoir waste which products are often applied directly onto grazing fields may increase the economic losses of

TABLE 5

FREQUENCY OF PARASITE EGGS, LARVAE, AND OOCYSTS IN 1972 FAECAL SAMPLES FROM CATTLE, 559 TAKEN FROM COWS, 1116 FROM YEARLINGS, AND 307 FROM CALVES.

Species	Cows		Yearlings		Calves		All cattle	
	No.	%	No.	%	No.	%	No.	%
Trichostrongylids	201	36.0	590	52.9	24	7.8	815	41.1
Eimeria spp.	32	5.7	325	29.1	98	31.9	455	23.0
Moniezia benedini (Bendel)	7	1.3	25	2.2	2	0.7	34	1.7
Trichuris spp.	0	0.0	13	1.2	2	0.7	15	0.8
Nematodirus helvetianus	0	0.0	9	0.8	5	1.6	14	0.7
Capillaria spp.	0	0.0	5	0.5	1	0.3	6	0.3
Strongyloides papillosus	0	0.0	0	0.0	1	0.3	1	0.1
Dictyocaulus viviparus	0	0.0	4	0.4	-	-	4	0.2

TABLE 6

FREQUENCY OF PARASITE EGGS AND LARVAE AND COCCIDIO OOCYSTS IN SOLID AND LIQUID MANURE. n=number of examined samples.

Species	Solid manure n=118, Surface n=63		Depth of 0.5 m n=55		Liquid manure n=86	
	No.of positives	%	No.of positives	%	No.of positives	%
Trichostrongylids						
Viable eggs	20	31.8	15	27,3	55	64.0
Infective larvae (L3)	8	12.7	7	12.7	5	5.8
Viable eggs or infective L3	26	41.3	19	34.3	58	67.4
Eimeria spp.	0	0.0	0	0.0	5	5.8
Moniezia benedini	4	6.3	1	1.8	15	17.4
Trichuris spp.	5	7.9	1	1.8	7	8.1
Nematodirus helvetianus	1	1.6	2	3.6	16	18.6

internal parasites to animal husbandry. Such losses were demonstrated in a Danish field experiment where gastro-intestinal parasitism in young calves resulted in a loss of bodyweight of 50 kilograms per animal compared with control calves grazing pastures where the built-up in the pasture of the herbage contamination by parasites was broken by moving in July to other pastures (Henriksen et al., in press).

Among other parasites being spread by slurry and abattoir waste are Eimeria, Dictyocaulus, Strongylus, Ascaris and Fasciola. It should be noted that parasite eggs deposited on pastures in the autumn will usually be infective next spring. Ploughing of waste water-irrigated fields does not solve the problem, since both bacteria and parasites survive best in the soil, where they are protected against light and dehydration. It has been observed that certain parasite eggs (gastro-intestinal nematodes) have hatched in the soil, after which the larvae migrated to the surface (Persson, 1974).

9.0. VIRUS.

Virus problems in connection with disposal of sludge, waste water, and slurry on soil will not be discussed here, as they will be dealt with separately in this symposium. But it should be mentioned that intestinal virus (polio virus, E.C.H.O.virus, Coxsackie virus, Adenovirus and Reovirus, and probably also infectious hepatitis virus) represent an integral and still fairly unknown problem in relation to wastes from the animal-human population. Other important viruses like Foot & Mouth virus are excreted with milk and feces. Dairy and abattoir waste water contains such viruses before clinical symptoms have incited special measures to be taken.

10.0 RICKETTSIA.

A rickettsia species of importance, Coxiella burnetii causing Q-fever, is found in dairy and abattoir waste water. Unlike other rickettsia, it is highly resistant to physico-chemical actions and, for example, survive low pasteurization. It is highly resistant to drying; hence the use of spraying methods should be avoided in areas where Q-fever is prevalent. Q-fever is a zoonose affecting especially dairy workers and farmers. Cattle, sheep and goats are natural reservoirs, often without clinical symptoms. In England, slightly less than 7% of the milk samples examined were contaminated. In the U.S.A. about 50% of 1634 cows examined had specific agglutinating antibodies in their whey, and not less than 23% of 840 cows shed Coxiella burnettii in their milk (Biberstein et al. 1974). It must be underlined that Q-fever is recognized to be an increasing problem in U.S.A. Observations reveal a sevenfold invrease in serum reactors among dairy cows compared with the situation 25 years ago, and it must be emphasized that infected cattle excrete Coxiella for up to 200 days.

CONCLUSIONS AND RECOMMENDATIONS.

It must be stressed that it is generally possible by any kind of indirect or direct reuse to obtain waters that will meet bacteriological standards, but problems of colour, taste, odour, and froth are insurmountable. Salinity is another difficult problem.

Technically, surface clogging may be an obstacle to recharging water through soil. In many instances, however, partially treated sewage is used for recharge or irrigation, some communities even by sewage from neighbouring areas for this purpose (i.a. City of Panama, Orange County plan, Long Island, Tel Aviv).

It will furthermore be evident that application of untreated or less thoroughly treated sewage is unjustifiable. Likewise a thorough control is needed with the process, the crops harvested and the personel.

The processes taking place and the consequences of agricultural irrigation are different, but not automatically greater than in water.

Reuse of water after irrigation and infiltration is a necessity and much research is needed to get the full benefit of this possibility.

THE EFFECTS OF LONG TERM ACCUMULATION OF HEAVY METALS AND SELECTED ORGANIC COMPOUNDS IN MUNICIPAL WASTEWATER ON SOIL

dr.ir. F.A.M. de Haan[1)]

1. INTRODUCTION

Following water and air, soil is nowadays generally considered as the third main environmental component. This finds expression in, amongst other things, the development and establishment of statutory regulations directed towards the protection of the quality of these environmental components. Such legislation aims at the maintenance of sound water, air and soil conditions within the limitations as imposed by natural circumstances and by processes brought about by nature.

This sequence of interest and consequently the sequency in the development of laws and bills involved goes without saying for several reasons. In the first place the pure state of the compound can, although not always unambiguously, much more easily be defined in the case of water and air than in case of soil. Soils do have an extremely wide variation in composition, depending e.g. on type of parent material, human influence and time of subjection to other soil developing influences like climate related conditions such as temperature, humidity and precipitation, and vegetation. This variation not only impedes the definition of "pure soil", but also results in completely different soil properties and consequently

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different processes and reactions following the addition of the same compound or material to different soil types. This constitutes an extremely complicating factor in the development and establishment of legislative measures.

Secondly, an undesirable composition of soil is, in contrast to the situation with water and air, not directly perceptible. This is so because soil acts as an intermediate in the composition of water flowing through the soil system and in the composition of plants grown on a soil under consideration. Due to the mutual interactions between soil, ground-water and vegetation, the soil composition is usually reflected to a certain degree in the composition of water and vegetation involved. In agriculture this mutual interaction is made use of by fertilization of soils in order to increase the yield or improve the quality of crops. In many cases soils possess a fairly extended buffering capacity, which allows the presence of compounds in soil at a certain level before plants and water are influenced to an undesirable degree.

In fact, the interactions mentioned above are purposively applied in the case of land disposal of waste water, using the soil with its intrinsic biological and chemical properties as a treatment system provided by nature. Recent literature generates increasing evidence (Ewert et al., 1973; De Haan, 1972; De Haan et al., 1973; Thomas, 1973) that the results of waste water renovation by land disposal are at least competitive with and usually even preferable to other ways of treatment, both with respect to purification and with respect to treatment costs. Thus percentages of removal may amount to as much as 98% for BOD_5, 80-85% for nitrogen and 96% for phosphates in case of land disposal of raw domestic sewage water. On top of these excellent treatment results the benefit of recycling plant nutrient elements must be considered when the disposal system is kept in or taken into agricultural use for crop production. In specific situations the complete plant fertilization may be obtained from waste water applications, thus making the combination of waste water treatment and crop breeding on the same place to a very profitable one. It will be clear that an economic evaluation of such systems is strongly influenced by the costs of artificial fertilizers on the one hand and costs of waste water treatment in treatment plants on the other. Recent problems with energy supply evidently favor land disposal systems for both reasons. Especially if waste water of relatively innocent nature is involved, as is the case when considering e.g. potato starch processing waste water, destruction of vaste amounts of plant nutrient elements seems an unwarranted waste.

However, due to the restricted storage capacity of soils and as a result of the reflection of soil composition in the quality of plant and water, land disposal may find its limitations. Only in case of complete and permissible recirculation, i.e. when all compounds added are taken up by plants without exceeding undesirable levels within the plant, the soil will not be subject to alterations as a result of waste water renovation by land disposal. In all other circumstances a gradual build-up of the excessive compounds will result, eventually leading to a possible situation of soil pollution. Since most waste waters do contain a variety of compounds which are not required or even unfavourable for plant production, land disposal of wastes essentially may induce undesirable accumulation of compounds in soil.

And so one arrives at the decisive point: to add waste products to the soil and thus use the soil system as a means for environmental quality improvement? or to protect soils against all composition changing influences except those directed towards crop production? The last approach not only would prohibit renovation of most types of waste water by land disposal, but also the application to soil of the concentrated remainder of waste water treatment in sewage plants: the sewage sludge. A definite decision in favor of one of these approaches is hard to present. The wide variation in properties of different soils (and to a lesser degree also of different waste products) makes it impossible that such a decision be of general validity.

One of the most impeding hesitations in this respect is brought about by the uncertainties as regards future developments in soil following prolonged additions of waste materials which contain less desirable compounds. This makes it evident that predictive methods to estimate long-term effects take a key position in the above decision making procedure. The major themes of such predictions are related to transport and accumulation of hazardous elements or compounds in soil, their availability to plants and consequently their long-term effects on food chain and water quality.

This contribution is confined to the description of the accumulation in soil of several specific heavy metals and of a couple of organic chemicals. The soils under consideration have been used during a number of decades for land disposal of wastes and waste water renovation. The compounds are more or less arbitrarily chosen and their fate in soil will be treated as an example. Prior to the description of field data, a general discussion is presented on factors which govern accumulation of compounds in soil. A good understanding of these factors forms a prerequisite in the explanation of the results.

2. INTERACTIONS GOVERNING TRANSPORT AND ACCUMULATION OF COMPOUNDS IN SOILS

For the description of the liquid phase transport of compounds in porous media like soils, use may be made of the theory of chromatography. Then the conservation equation is usually taken as a point of departure, stating that the rate of change of the content of a certain compound per volume element of porous system due to transport processes equals the net rate of flow through the boundary planes of the volume element under consideration. Introducing the prime factors which govern the concentration of the compounds in the liquid phase and hence its mobility in the soil system, the conservation equation in its most basic form may be represented as:

$$\frac{\delta(q_i + \varepsilon.C_i)}{\delta t} = - \nabla.F_i + P_c + P_b \qquad (1)$$

The left hand side of equation (1) represents the rate of change of the amount of compound i which is adsorbed on the adsorption complex, q_i, and of the amount in solution which is found as the product of the moisture content, ε, and the solution concentration, C_i.

$\nabla.F_i$ stands for the difference in flux of compound i through the boundary planes of the volume element during the time period under consideration. This term thus comprises the transport processes involved, i.e. convective transport and diffusion in the liquid phase. Also the phenomenon of dispersion, which refers to the spreading of a polluting zone as a result of differences of flow velocities at different places in the porous system, may be accounted for by $\nabla . F_i$.

The P symbols in equation (1) represent production factors, i.e. concentration governing interactions other than adsorptive bonding on the adsorption complex of the soil solid phase. Such interactions may be of pure chemical nature, P_c, in case of dissolution and precipitation of solid salts, or of biological nature, P_b, when referring to e.g. the microbiological decomposition of organic compounds. Depending on the absolute contribution of the production process to the concentration in the liquid phase these production terms may have a positive or negative value.

Thus the three main categories of interactions which affect the mobility of compounds in soil may be listed as:

adsorption on the exchange complex of the soil solid phase

precipitation and dissolution reactions

biological decomposition and turn-over reactions

A quantitative elaboration of equation (1) requires, in addition to the introduction of the mathematical formulations depicting the transport processes leading to the flux term, the mathematical relationships which describe the concentration regulation by the above groups of processes. Such a quantitative treatment has been presented by Reiniger (1970) and Reiniger and Bolt (1972) for the cationic exchange Na-Ca in soil columns, in which case the production terms of equation (1) may be obliterated.

For the pollutants under consideration here, viz. heavy metals and organic chemicals, usually more than one of the above listed groups of interactions (and actually sometimes even all three) are involved, rendering the mathematical description of the transport and accumulation phenomena fairly complicated. For most compounds of interest such a quantification is even still prohibited by lack of information about the actual relationship describing the concentration governing processes.

2.1. ADSORPTIVE BONDING ON THE EXCHANGE COMPLEX

The three main soil constituents which exhibit adsorption of compounds at their solid-liquid interfase to a considerable extent are clay minerals, soil organic matter and oxides and hydroxides of e.g. iron, aluminum and manganese. The adsorption mechanisms may be of various nature, viz. London - van der Waals attraction, Coulombic attraction as a result of opposite electric charge of adsorber and adsorbate, H-bridge formation, metal-ion bonding and salt bridge formation.

The predominant negative electric charge on most soil colloids at relevant pH values renders positive electrostatic adsorption of cations and exclusion of anions one of the most striking phenomena in soils. In case of bonding of organic chemicals on soil constituents usually also one or more of the other mechanisms listed above may be involved.

Considering the electrostatic adsorption of heavy metals when present in cationic form, it must be mentioned that the valency of the ion is of prime importance with respect to the adsorption strength. For the more common cations in soil like Na^+ and Ca^{2+} this valency effect has been accounted for by introducing in the ion exchange equations the so-called reduced ion concentration ratio. The ratio of the amounts of both exchanging ions adsorbed is then taken proportional to the ratio of the concentrations in solution in which each concentration is raised to a power equal to the reciprocal value of the ion valency. The proportionality factor is commonly indicated as the exchange constant. Its value indicates the relative affinity of the adsorber for both cations for reasons other than the ion valency (like e.g. the radius of the hydrated ion). Also in case of homovalent cation exchange the relative affinity of the adsorber is reflected in the proportionality factor between the ratios of the amounts adsorbed and of the concentrations of both ions in solution.

Under normal conditions the adsorption complex of most soils is mainly constituted by common cations like Na^+, K^+, NH_4^+, Ca^{2+} and Mg^{2+}. From a practical point of view it is thus evident that attention in the past has mainly been limited to exchange reactions of these cations. Thus soil science literature provides exchange constants and selectivity coefficients for the abundantly occurring cations and the major soil colloids, whereas only very scarcely information is presented on the exchange behavior of specific cations like heavy metals. Consequently these values usually remain to be determined for the predominant adsorber of each soil system of interest.

A compilation of literature data on selectivity constants for exchange reactions of a number of metal-ions and several different adsorbers is presented in table 1. The high affinity of many adsorbers for

table 1

Cs is well known. Also the fixation of K especially by illitic type clay minerals has received considerable attention, since this constitutes an important fertility problem on many soils. The high affinity of most illites for K is due to the fact that potassium ions may diffuse into the clay crystall lattice, thus prohibiting further exchange against other cations.

The exchange studies on heavy metals performed so far all pertain to relatively high degrees of saturation of the adsorber with the heavy metal ion (cf. table 1). It must be mentioned here that if the exchange range is indicated as 0.0 - 1.0 this actually means that the fractional composition turned out to be about 0.1 - 0.9. Since heavy metals ususally prevail at low concentration levels only, such exchange studies are of limited practical applicability. As was pointed out by De Haan (1975) increased values for selectivity coefficients of heavy metals at low degrees of adsorber saturation are highly probable due to the occurrence of adsorption sites with high specific affinity on most adsorbers.

A second important problem related to exchange studies of heavy metals is constituted by the formation of many types of complexes in natural systems. While the more common ions like Na and Ca are unambiguously present as either mono- and divalent cations, respectively, the ion valency of heavy metals may be considerably influenced by complex formation. This is not only the case with respect to chelates but also with respect to relatively simple inorganic complexes. Hahne and Kroontje (1973) described this phenomenon for the complexes of the heavy metal cations Cd^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} with the anions OH^- and Cl^-. Depending on the pH of the system and the chloride concentration these divalent heavy metal cations may be transformed to monovalent cationic, to uncharged and even to negatively charged complexes. It is evident that the adsorptive behavior of the heavy metal is completely dependent on its ionic form.

The adsorption of organic chemicals, e.g. pesticides, is even more governed by characteristic properties of the organic molecules on the one side and of the adsorber on the other. The variety and diversity of these compounds prohibits the establishment of general rules for these interactions. Accumulation of such compounds in soil reflects the combined result of their mobility as influenced by bonding on the soil matrix and of their persistence as influenced by the biological or photochemical degradability. Voerman and Besemer (1970) studied the accumulation of the organochlorine insecticides dieldrin, lindane and DDT, and of the organo phosporus compound parathion, as a result of a repeated application on a light sandy soil throughout a period of 15 years. The chemicals were all applied at two different rates. The experimental results are presented in

figure 1 figure 1, indicating the low mobility of all compounds involved. Only in case of dieldrin and DDT, trace amounts were found below a depth of 20 cm.

The persistence of the chemicals is reflected in the percentages

table 2 recovery, which are presented in table 2. On this specific sandy soil the persistency of all three organo chlorine chemicals studied was considerably higher than of parathion.

In sharp contrast to the restricted mobility of the above four chemicals is the behavior of the herbicide bromacil, as described by

Leistra et al. (1975). They applied bromacil annually at two different rates to a sandy loam and a sandy clay loam during a time period of seven years. The final distribution in the soil profile as found one year after

figure 2 the last application is graphically presented in figure 2, indicating a much more gradual distribution pattern and a recovery of the compound at much greater depth.

2.2. CHEMICAL PRECIPITATION AND DISSOLUTION REACTIONS

Many compounds, either harmless or hazardous, may occur in the solid phase of soils in a form different from the adsorbed state. With respect to e.g. heavy metals it may be visualized that a certain metal occurs in the soil solution and consequently at the adsorption complex, if present. As was discussed before the fractional saturation of the adsorber with the ion will depend on the specific affinity and on the concentration in solution. In addition to these states of appearance the heavy metal may also prevail in the form of solid salts. This then means that the solution concentration is not only governed by the ion exchange characteristics of the system but also by the solubility of these salts.

Different, both stationnary and dynamic, aspects are involved in this phenomenon. The stationnary aspects pertain to the equilibrium situation, i.e. to the concentrations in solution that must be expected when the system is at equilibrium conditions. The dynamic aspects pertain to the rates of the processes of precipitation and dissolution. Until recently the attention in this field of interest has predominantly been focused on the first mentioned aspects.

The solubility of solids is governed by a number of system parameters, their sequency of importance depending on the type of salt under consideration. As most outstanding representatives of these parameters may be mentioned: pH, oxygen content and CO_2 pressure.

Considering as an example of the heavy metal manganese the solid phases MnO_2, MnOOH and Mn_2O_4, one may arrive as follows at a stability diagram in which the activity of manganese ions is presented as a function of pH and the partial oxygen pressure. Combination of the chemical reaction:

$$MnO_2(s) + 4H^+ + 2e^- \rightleftarrows Mn^{2+} + 2H_2O \quad \text{with } \log K^o = 41.6 \tag{2}$$

with

$$H_2O \rightleftarrows 2e^- + 2H^+ + \tfrac{1}{2}O_2\ (g) \quad \text{with } \log K^o = -41.54 \tag{3}$$

leads to the resultant equation

$$MnO_2(s) + 2H^+ \rightleftarrows Mn^{2+} + H_2O + \tfrac{1}{2}O_2\ (g) \text{ with } \log K^o = 0.06 \tag{4}$$

Since the activity of the solid phase and of water equal unity it follows from equation (4) that

$$(Mn^{2+}) = \frac{10^{0.06}.(H^+)^2}{(O_2)^{\frac{1}{2}}(g)}$$

$$\text{or: } -\log\ (Mn^{2+}) = -0.06 + 2pH + \tfrac{1}{2}\log(O_2)(g) \tag{5}$$

For a partial oxygen pressure of 0.2 and 10^{-6} atmosphere equation (5) results in, respectively:

for MnO_2

$$-\log\ (Mn^{2+}) = 2pH - 0.41 \tag{6}$$

and

$$-\log\ (Mn^{2+}) = 2pH - 3.06 \tag{7}$$

figure 3

In figure 3 these relationships are represented by the MnO_2 lines, as in the above calculation MnO_2 was assumed to be the solid phase which governs the manganese activity in solution.

In a comparable manner one may arrive at the following sets of relationships for the other two solid manganese phases:

for MnOOH:

$$-\log (Mn^{2+}) = 2pH - 4.61 \text{ for } P_{O_2} = 0.2 \quad (8)$$

$$-\log (Mn^{2+}) = 2pH - 6.93 \text{ for } P_{O_2} = 10^{-6} \quad (9)$$

for Mn_3O_4:

$$-\log (Mn^{2+}) = 2pH - 6.77 \text{ for } P_{O_2} = 0.2 \quad (10)$$

$$-\log (Mn^{2+}) = 2pH - 7.65 \text{ for } P_{O_2} = 10^{-6} \quad (11)$$

As follows from these equations and figure 3, the activity of manganese ions in solution increases hundredfold for each decrease of the pH with one unit. Of the three solid phases considered here MnO_2 constitutes the most stable form.

In addition to the ones mentioned above, more manganese minerals may occur in soil, all with their own solubility relationships. The huge influence of the pH on the solubility explains that the addition of certain materials to the soil, e.g. in the form of sewage sludges, exert a much larger influence on heavy metal concentrations and mobilities than should be expected on the basis of heavy metal content of that specific material. The decrease of pH as a result of nitrification following sewage sludge application may result in an increased Mn-concentration in solution which greatly exceeds the direct effect of the amount of Mn added with the sludge (King and Morris, 1972).

A comparable approach as the above may be applied to minerals of other heavy metals, e.g. Cu and Zn. Such solubility diagrams all have in common that they pertain to equilibrium situations and do not provide any information on the rate of the processes involved. However, quantitative information on the kinetics of precipitation and dissolution reactions is required in order to evaluate the chemical production term of equation (1). Such studies on reaction kinetics have recently been started with respect to the precipitation and adsorption of phosphate ions (Chen et al., 1973; van Riemsdijk, 1975). They are equally indispensable for a better understanding of heavy metal behavior in soils.

2.3. BIOLOGICAL DECOMPOSITION AND TURNOVER REACTIONS

As a result of the biological, and especially microbiological activity in soils, most compounds are subject to conversion and turnover reactions following their addition to the soil. This is not only the situation with respect to "fresh" organic matter originating form e.g. manure or foliage but also for more specific materials like organic chemicals, oil, natural gas or the organic compounds of sewage water and sewage sludges.

Conversion of such fresh organic matter into more stable compounds is strongly enhanced by the activity of aerobic heterotrofic micro-organisms. At sufficient oxygen supply these reactions terminate in the production of CO_2 and H_2O, whereas the increase of "stable" organic matter in the soil results from a.o. dying and decomposing microbial cell material.

In the case of pesticides these biological conversion reactions may constitute the main processes for disappearance of the compounds from the soil system. It is thus evident that especially in this field of interest attention has been given do decomposition reactions because they usually are the main factor governing the persistence of the chemical. Decomposition of a certain compound is reflected in equation (1) by a negative value for P_b.

Hamaker (1972) presented a comprehensive discussion of the mathematics of such biological decomposition processes. Equations describing the reactions may be grouped into two main categories, namely the so-called power rate equations and the hyperbolic rate equations. The first mentioned category may be represented by the general equation:

$$\frac{\delta C_i}{\delta t} = -kC_i^n \qquad (12)$$

in which k stands for the rate constant and n for the order of the reaction.

The hyperbolic rate equations are of the type:

$$\frac{\delta C_i}{\delta t} = -\frac{k_1 C_i}{k_2 + C_i} \qquad (13)$$

indicating that the rate of decomposition depends upon the concentration and upon the sum of concentration and other factors.

In many cases it is hard to decide from experimental data which of both rate models is best applicable. As described by Leistra (1973) calculations on transport and accumulation of pesticides in soil usually take the simplified power rate model as a point of departure, if at all a biological production factor has to be taken into account. This simplified model assumes the decomposition to be a first order reaction. In that case the rate simply relates to the concentration which is still present, according to:

$$\frac{\delta C_i}{\delta t} = -kC_i \tag{14}$$

Broadbent (1973) discussed the biological incorporation in the soil of the organics from sewage sludge and the factors influencing this process. In organic matter turnover reactions, first order kinetics are usually limited to a short time period following the addition of the fresh material.

One of the main problems in inserting the biological production factor into equation (1) is brought about by the limited applicability of laboratory determined decomposition rates to practical circumstances as occurring in the field. The actual variation of factors which influence the decomposition in practice is usually much larger under field conditions than in the laboratory studies. Moreover, decomposition experiments are commonly conducted at optimal conditions for microbial activity, which conditions are met very rarely only in practice.

3. ACCUMULATION IN SOIL PROFILES ON SEWAGE DISPOSAL FARMS

In the vicinity of Tilburg, a town in The Netherlands with at present about 155,000 inhabitants, two farms are situated which have been used during a relatively large number of years for the disposal of town sewage water and for the application of sewage sludge. These farms are both situated on sandy soil and will be indicated here as the "community farm" and the "monastery farm", respectively.

The community farm has a total surface area of about 100 hectares and has been used during the last 50 years for the disposal of raw town sewage water. This waste water is at present applied to the soil at a rate of roughly 250 mm once each month. The farm is in agricultural exploitation as permanent pasture, used almost exclusively for grazing. The waste water is applied by a relatively simple system of surface flooding. Syphons are used in order to connect parcels to be flooded with raised supply ditches. Results with respect to waste water treatment, and with respect to phosphate removal from the waste water and the resultant accumulation in the soil profile have been described elsewhere (de Haan, 1972; Beek and de Haan, 1973). It was found that the phosphate bonding in this case was constituted by at least two different bonding mechanisms, which were suggested to operate in an alternative way as adsorption and precipitation reactions.

Part of the monastery farm, which has a total surface area of about 70 hectares has been arranged for the disposal of waste water of a brewery connected with the monastery. The remaining surface area has been used during the last 15 years for application of sewage sludge of one of the towns waste water treatment plants. This sludge is diluted to a dry matter content of about 3% and applied by means of a sprinkling irrigation system. Most of the farm is in exploitation as pasture, although parts are also used for the production of a.o. silage corn and silage rye. The total yearly application rate amounted in 1973 to 550 metric tons of raw sewage sludge per hectare. This total amount is divided over a number of applications, following the grazing of a certain parcel.

It is a general rule on both farms that cattle are vaccinated against anthrax before they are put out to graze.

3.1. RESULTS FOR ALDRIN AND DIELDRIN

The town of Tilburg is known for its textile industry. These industrial activities are mainly situated in that part of the town of which the sewage water is treated in the plant which supplies the sludge for application on the monastery farm. In the autumn of 1974 it was observed for the first time that the dieldrin content of the milk of this farm

table 3

was far above the residue tolerance level. Table 3 presents values of residue tolerance levels as maintained in The Netherlands for a number of pesticides. The dieldrin content of the milk of this specific farm was found as 1.2 ppm, thus being almost 10 times higher than the acceptable level. The milk is still delivered for digestion in dairy industry. Careful dilution with uncontaminated milk ensures reduction of the dieldrin content below the residue tolerance level.

Analysis of the sewage sludge indicated this material as dieldrin source. Although not yet irrefutably proven it may be assumed that this dieldrin originates from wool laundry works which discharge their wastewater on the specific treatment plant. Analyses of 12 sludge samples, collected over the period 1973 up to 1975, showed a mean dieldrin content of 7.9 ppm on dry matter, with 20.0 and 1.4 as highest and lowest value, respectively.

It is evident that this pesticide contamination is also reflected in the results of soil and plant analysis. The pesticides mentioned in table 3 were all found at non-detectable level except for aldrin and

table 4

dieldrin. Table 4 provides some information on the contents of these compounds in soil, silage corn, silage grass and silage rye.

Although the above data are preliminary and too superficial to allow definite conclusions, it may probably be assumed that rye is the most efficient accumulator of the plant species studied. The dieldrin content of this rye far exceeds the residue tolerance level, thus rendering this material unsuitable for cattle fodder. Although at somewhat lesser degree this same is true for the silage corn and silage grass. Accumulation of dieldrin in the soil profile is mainly restricted to the top soil layer. This is in agreement with the information provided by figure 1, indicating a low mobility of dieldrin in soil.

3.2. RESULTS FOR SEVERAL HEAVY METALS

The soil on both the community farm and the monastery farm are gradually contaminated, and possibly polluted, with heavy metals present in the raw municipal sewage water and the sewage sludge, respectively. As is well known heavy metal contents of sludge of a certain waste water treatment plant may vary considerably with time. This is even more so for sewage water. Reliable data on heavy metal content of the wastewater under consideration here are not yet available.

The sewage sludge of the monastery farm was in 1973 regularly sampled and analyzed by the Institute for Soil Fertility. The results on total heavy metal content, as mean value of 6 samplings, are presented in table 5, together with a number of sludge analysis data as found in the literature. It is shown that the heavy metal content of this specific sewage sludge is relatively high, except for Zn and probably for Pb which prevail roughly at comparable level as found elsewhere.

Both farms are surrounded by uncultivated woodland on sandy soil from which they have been reclaimed. Soil profiles on this original forest land and on a parcel of the farm which received sewage sludge during the last 15 years were sampled to a depth of 100 centimeter. The soil samples were digested with a 1:1 mixture of concentrated nitric acid and perchloric acid. Contents of Zn, Cu, Pb and Cd were determined by atomic absorption. Results for the untreated profile and for the sewage

figure 4 sludge disposal profile are presented in figure 4. It is shown that the accumulation of these four heavy metals is entirely limited to the upper 30 - 40 centimeter with far the highest concentration in the toplayer of 5 cm. It must be mentioned here that the contents are expressed on weight basis. This imposes an apparent distortion on the distribution profile since the volume weight value of the top layer is considerably lower than of the deeper soil layers. This is even more so for the untreated profile where the organic matter content of the top 5 cm amounted to 47.8% as against 12.4% on the sludge profile.

The data of figure 4 in principle allow a balance sheet calculation in which the total amount added of a certain heavy metal is compared to the accumulation in the profile. Differences between these values may be caused by withdrawal of the metal with agricultural products and by leaching to deeper soil layers or to the groundwater. Such a balance sheet calculation is omitted here because of a number of uncertainties with respect to the constituting parts. First of all data on heavy metal content of the sludge in the past are not available. Secondly the total amount of sludge added over the entire period is insufficiently known, whereas also the withdrawal with plant material, milk and meat is unknown. Moreover, the heavy metal content of plants grown on this farm has undoubtedly not be the same during the entire application period.

table 6 Table 6 provides information on the heavy metal content of grass grown on both farms and on a comparable sandy soil which has not been contaminated with heavy metals (Van Driel, 1975). Although the sludge and the sewage water are usually applied directly after grazing of a certain parcel when the grass leaves are still very short it may be possible that the heavy metals found in the plant material originate in part from attachment on the leaves. Therefore determinations were performed prior to and after washing with T-pol. All elements mentioned in table 6 were measured by atomic absorption except for Hg, As and Sb which were determined by neutron activation analysis.

Washing of the samples results in a partial removal of the heavy metals. In accordance with expectations this is especially so for the samples from the disposal farms. The distinction between washed and unwashed samples is of significance in the evaluation of the uptake by plants from the soil and not with respect to the heavy metal uptake by the grazing animals, which consume the grass as it is.

Land disposal of the raw sewage water involved apparently results in an increased content of Cu and Zn (about 2-fold) but especially of Ni (about 20-fold). Repeated sewage sludge application even leads to much higher values, also for Cd and Pb. According to World Health Organization advices increase of the level of these both elements in human nutrition should be avoided.

Values for tolerances of heavy metal uptake by animals have not yet been sufficiently developed to evaluate the above data. It is, however, beyond doubt that the contents in the grass as well as in the soil are approaching or exceeding acceptable levels, especially on the monastery farm. There the high copper content of the soil already prohibits sheep breeding.

In the Netherlands a special Committee is working on the development of tolerance levels for heavy metals in soil in relation to land disposal of waste products. According to the preliminary standards maintained in this respect, the yearly increase of the heavy metal content on the monastery farm is exceeding the acceptable level, thus requiring an (at least temporary) stopping of the sludge application.

The above data do not allow conclusions on the precise bonding mechanisms of the different heavy metals in the soil. Continued studies are needed to provide such information, which is also required in order to predict further behavior of the metals following the termination of sludge application.

Earlier investigations indicated that the soil on the community farm could be used for at least another number of decades for an extremely effective removal of phosphate, and probably also of nitrogen and BOD_5, from the sewage water. The experiences on the monastery farm indicate that the limitations in the use of soil to this purpose are not set forth by these relatively innocent compounds but by the accumulation of heavy metals and specific organic compounds when present in the waste product to be disposed off.

4. SUMMARY

Increasing waste production and demand for high quality water generates a growing interest to use the soil in waste disposal systems and in waste water renovation practice.

Compounds present in the waste materials may partially or completely be recycled in this way. Soils usually have a large storage capacity for a wide variety of compounds. In relation to waste disposal and waste water renovation systems in which soils are involved it is of utmost importance to have means for the prediction of long term effects.

The different interaction mechanisms are discussed to which compounds are subjected when present in the soil liquid phase. These can be listed as adsorptive bonding, precipitation and dissolution reactions, and biological decomposition and turnover reactions. It is reasoned that adsorption studies on heavy metals in soil should preferably focus attention to the low concentration range at which these metals usually prevail. The solubility diagram of several manganese solid phases is treated as an example. It is discussed that especially the kinetics of chemical dissolution and precipitation reactions are of importance in transport and accumulation studies. Some attention is given to biological decomposition reactions, since these predominantly govern the persistence of most organic compounds in soil.

Accumulation results are described for dieldrin and aldrin and for several heavy metals in soils of two different farms, indicated here as community farm and monastery farm. These farms have been used during the last 50 years for municipal waste water renovation and during the last 15 years for sewage sludge application, respectively. Especially the application of sewage sludge results in unacceptable dieldrin levels in

milk and roughage, and in unacceptable accumulation of heavy metals in soil and grass. Accumulation of such hazardous compounds put limitations to the use of soil in waste water renovation.

5. ACKNOWLEDGEMENT

The author is very much indebted to:

W. van Driel of the Institute for Soil Fertility

M. Heuver of the Extension Service for Plant Protection

K. Harmsen and V.J.G. Houba of the Laboratory of Soils and Fertilizers

for providing additional information and data.

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TABLE 1

Selectivity coefficients for the exchange reaction: ion B versus ion A, for a number of metal ions and different adsorbers (Blom, 1975).

adsorber	reaction B/A	mean value of exp. selectivity Coefficient	fractional composition of adsorber with exchanging ion	equilibrium salt concentration, eq/l	literature reference
Chambers (montmorill.)	Cs/Rb	4.1	0.0 - 1.0	0.001	Gast (1972)
"	Cs/K	19.8	0.6 - 1.0	0.01 - 0.4	Faucher and Thomas (1954)
"	Cs/Na	40.3	0.2 - 1.0	0.04	Lewis and Thomas (1963)
"	Cs/Sr	18.3	0.2 - 1.0	0.05	Gaines and Thomas (1955)
"	Cs/Ba	16.2	0.0 - 1.0	0.02	Loven and Thomas (1965)
Kaolinite	Li/Na	0.67		0.001	Gast (1972)
"	K/Na	7.8		0.001	"
"	Cs/Na	18.2		0.001	"
Clayspur (montmorill.)	Al/Na	3.7	0.2 - 0.8	0.05	Bruggenwert (1972)
"	La/Na	3.7	0.4 - 0.8	0.05	"
"	La/Ca	1.8	0.0 - 0.4	0.05	"
"	Al/Ca	1.9	0.0 - 1.0	0.05	"
Camp Berteau (montmorillonite)	NH_4/Sr	2.08	0.3 - 0.9	0.01	Fripiat et al. (1965)
"	NH_4/Ca	2.86	0.1 - 0.8	0.01	Van Bladel (1966)
"	Co/Na	1.94	0.3 - 1.0	0.01	Maes (1973)
"	Zn/Na	1.88	0.3 - 1.0	0.01	"
"	Ni/Na	2.01	0.3 - 1.0	0.01	"
"	Cu/Na	2.01	0.3 - 1.0	0.01	"
Wyoming bentonite	Cu/Ca	0.94	0.0 - 1.0	± 0.02	El-Sayed et al. (1970)

TABLE 2

Percentages recovery of four different organic chemicals following repeated addition to a sandy soil during a time period of 15 years (after Voerman and Besemer, 1970).

Compound	Dieldrin	Lindane	DDT	Parathion
Low application rate	15	3	20	0.1
High application rate	19	8	34	0.1

TABLE 3

Residue tolerance levels of several organic chemicals (ppm)

	Milk dairy products	Meat	Mash roughage
HCB	0.3	0.5	0.03
HCH	0.3		0.01
Lindane	0.2	2.0	0.20
Heptachlor + epoxyde	0.125	0.2	0.03
dieldrin + aldrin	0.125	0.2	0.02
DDT + metabolites	1.25	1.25	0.15

TABLE 4

Aldrin and dieldrin content (ppm on dry matter) of plants and soil of the monastery farm (data by courtesy of M. Heuver).

	Aldrin	Dieldrin
soil	< 0.01	0.71
	< 0.01	0.41
	< 0.01	0.70
	< 0.01	1.51
	< 0.01	0.88
silage corn	< 0.01	0.05
	< 0.01	0.01
silage grass	0.08	0.09
	0.08	0.14
	0.05	0.08
	0.02	0.05
silage rye	-	0.22
	-	0.79

TABLE 5

Heavy metal content (ppm on dry matter) of different sewage sludges

Metal	Michigan[1)]	England[2)]	Sweden[3)]	Monastery farm[4)]
Cd	12	-	7	21
Cr	380	250	86	4200
Cu	700	800	560	1280
Ni	52	80	51	190
Pb	480	700	180	510
Zn	2200	3000	1567	2250
As				18
Hg				3.6
Sb				73
Ag				122

references: 1) Blakeslee, 1973

2) Berrow and Webber, 1972

3) Beggren and Oden, 1972

4) Van Driel, 1975

TABLE 6

Heavy metal content (ppm on dry matter) of grass grown on comparable soil at different degrees of contamination. - and + indicate non-washed and washed with T-pol, respectively (data according to Van Driel, 1975).

	Uncontaminated Sandy soil	Community Farm	Monastery Farm
Cd -	0.3	0.24	3.6
+	0.3	0.19	2.9
Cu -	11	21	68
+	9	14	38
Ni -	1	19.0	19
+	0.7	15.4	15
Pb -	7	7.2	24
+	4	4.4	10
Zn -	74	140	330
+	68	126	280
Hg -	0.04	0.06	0.17
+	0.03	0.03	0.05
As -	0.2	0.58	0.8
+	0.2	0.36	0.2
Sb -	0.20	0.22	1.6
+	0.10	0.15	0.9

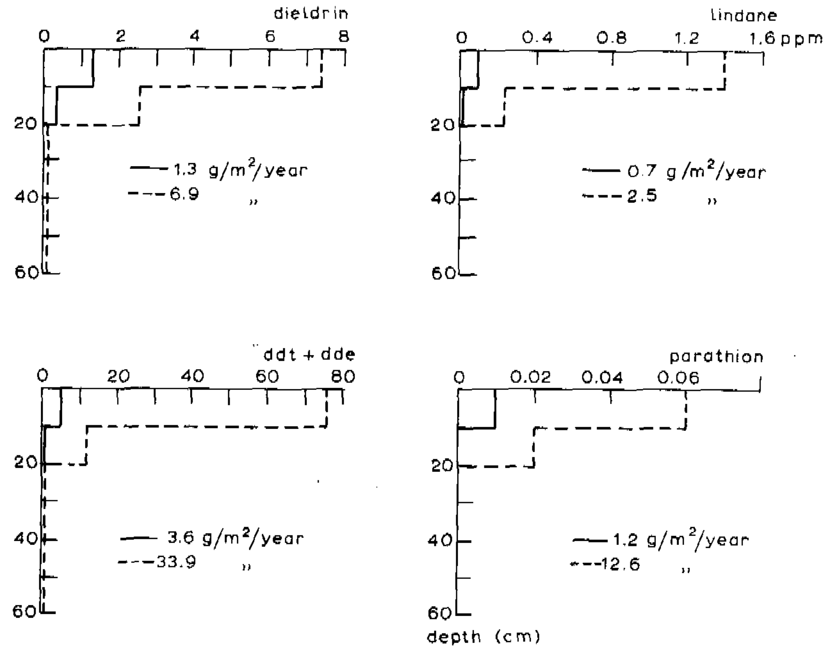

Fig. 1. Accumulation in soil of 4 organic chemicals repeatedly applied at two different rates during a period of 15 years (after Voerman and Besemer, 1970)

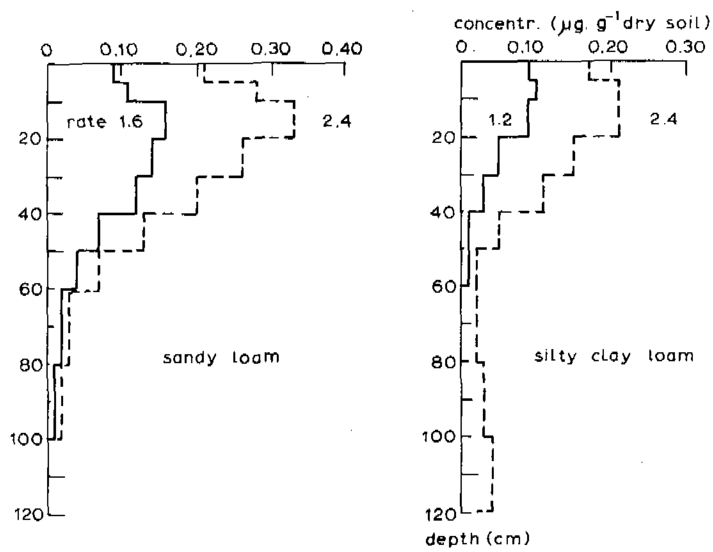

Fig. 2. Distribution pattern of bromacil in two different soils after 7 years of application at two rates (after Leistra et al., 1975).

Subscripts for figures:

Fig. 1. Accumulation in soil of 4 organic chemicals repeatedly applied at two different rates during a period of 15 years (after Voerman and Besemer, 1970).

Fig. 2. Distribution pattern of bromacil in two different soils after 7 years of application at two rates (after Leistra et al., 1975). Application rates are in kg/ha/year.

Fig. 3. Solubility diagram of three different manganese solid phases as a function of pH and partial oxygen pressure.

Fig. 4. Distribution patterns of 4 heavy metals in two profiles of comparable soil. One of the soils received large amounts of sewage sludge during a period of 15 years.

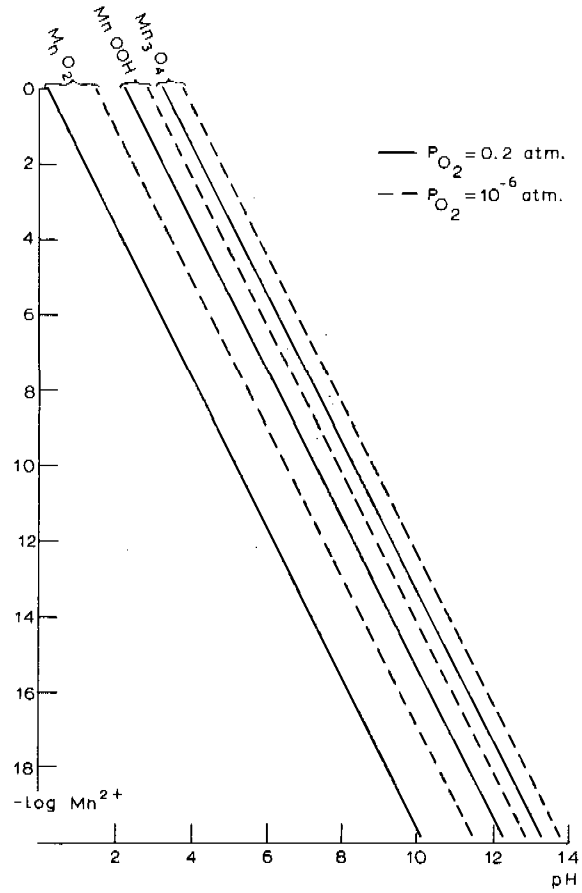

Fig. 3. Solubility diagram of three different manganese solid phases as a function of pH and partial oxygen pressure.

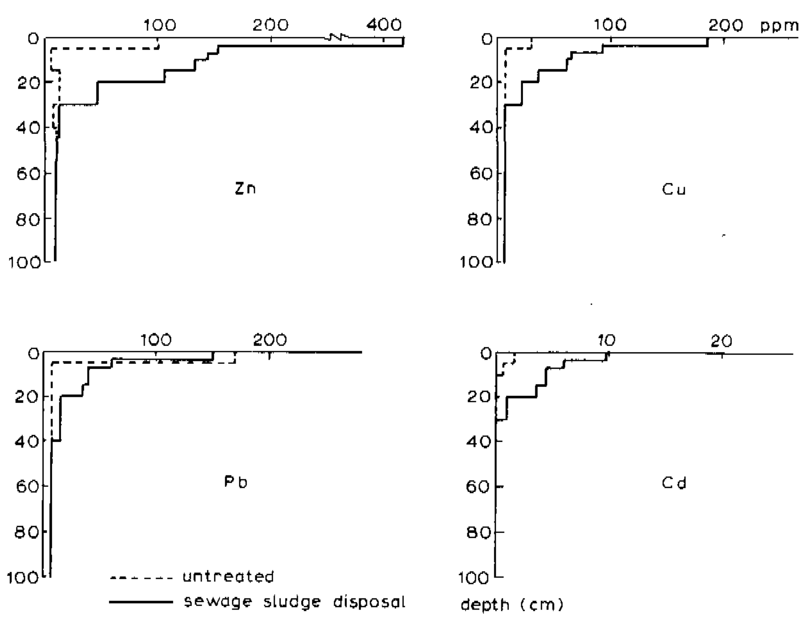

Fig. 4. Distribution patterns of 4 heavy metals in two profiles of comparable soil. One of the soils received large amounts of sewage

SURVIVAL OF VIRUSES IN SOIL UNDER NATURAL CONDITIONS

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This study was supported in part by grants from the City of St. Petersburg and the Rockefeller Foundation (RF-74064)

ABSTRACT

Studies of virus survival and movement in soils under natural conditions were undertaken in three geologically different areas, two have been in progress for over a year and the third, only recently initiated. The approach has been to establish the quantity of virus being discharged onto the soil in effluents or sludge and to determine virus movement in the soil by testing groundwater from monitoring wells.

Techniques designed to disrupt solids to free embedded virus showed 68.3 to 90% of virus present in influent and effluent samples to be solids-associated. Isolations were made from dried, caked sludge which had been exposed to the hot Florida sun for 13 days. Additionally, isolations were made from completely digested sludge after >60 days in the digester at 34°C.

Evidence is presented indicating virus percolation to depths of 10 and 20 feet, lateral movement of at least 7 meters and survival of virus in soil for at least 28 days.

SURVIVAL OF VIRUSES IN SOIL UNDER NATURAL CONDITIONS

INTRODUCTION

Theoretically, survival and movement of virus in soils under natural conditions are dependent upon many variables, some of which are presently little understood and perhaps some, not yet recognized. Most of the data currently available related to virus survival in soil(s) describe[1,2,3] experimental results when these variables have been examined in relative isolation in the laboratories, i.e., "free", adapted, laboratory strains of virus in various ionic solutions were inoculated onto soils which had been strained, sterilized and packed into uniform columns. When these data are evaluated on the basis of natural conditions, i.e., solids-associated, wild virus suspended in wastewaters which are characterized by their variability, deposited on bacteriologically contaminated, heterogenenous soils which permit slow to rapid, vertical and horizontal flows, it is evident that the evaluation must be done with perspicacity to avoid unwarranted conclusions.

Many of these laboratory studies have utilized bacterial viruses which have some of the properties of animal viruses but differ extensively in others. In the main, these studies have shown that bacteriophage were more readily trapped and/or adsorbed in the upper four inches of soils tested than were animal viruses. One of the major limitations of laboratory experimentation has been the emphasis placed on retention of virus,removal by concentration,in the upper few inches of the soil rather than removal by inactivation. Concentration of virus has long been accomplished by adsorption followed by desorption procedures and virus infectivity has been relatively unaffected by the process.[4,5]

Therefore, little has been accomplished if virus in effluents is merely being concentrated in soils. Is it possible that such a concentration may lead to undesirable results by sudden release of large multiplicities of virus?

Lefler and Kott[6] have recently reported studies which afford at least a partial answer to the question. Utilizing poliovirus 1 and f2 phage they conducted several adsorption, desorption and survival studies in sand. Various adsorption and elution media were employed. Briefly, they showed that sand to which poliovirus type 1 had been adsorbed, continued to yield virus through repeated washings. Most virus was eluted in the first wash but some could be demonstrated in the sand after 10 washings. They also showed poliovirus survival for 77 days in dry sand and 91 days in sand moistened with distilled water or oxidation pond effluents. The latter provides large quantities of proteinaceous materials which tend to protect virus. When the holding temperature was reduced to 4-8°C, 20% of the virus in oxidation pond effluents survived for 175 days. A natural die-off or inactivation of soil-adsorbed virus does occur but the parameters of this phenomenon are unclear. Their studies also demonstrated that virus suspended in distilled water was adsorbed poorly by sand columns. In the presence of multiple select ions, over 50% of the applied virus was found in the effluent. However, when 0.01 M $MgCl_2$ was added to distilled water, no virus was found in the column effluents, clearly demonstrating the ion concentration-dependency of virus adsorption.

In summary, laboratory data are not definitive. Depending on the experimental conditions, animal virus may or may not be adsorbed by soils regardless of column length. The major contributions of laboratory studies are (1) the overwhelming evidence that some portion of applied virus is adsorbed by soils, can be desorbed and emerge as infectious virus and, (2) distilled water, analogous to rainwater, can elute virus from soils.

The earliest evidence for virus survival under natural conditions resulted from epidemiological investigations of waterborne infectious hepatitis outbreaks.[7,8,9,10] They have indicated that passage through soils of virus laden cesspool and septic tank seepage have resulted in contamination of potable water sources. A recent American Water Works Association Committee Report[11] summarized waterborne disease outbreaks in the United States and Canada during 1971 and 1972. Data concerning hepatitis outbreaks in these years showed four occurring in areas served by municipal systems; two related to use of untreated groundwater, one to inadequate or interruption of chlorination, and one to contamination of the distribution system. In the preceding 25 years, such outbreaks were due to contamination of the distribution system rather than treatment deficiency.[12] In semipublic and industrial systems, untreated contaminated groundwater continues to be the important factor. Survival of virus in soils under natural conditions would appear to be the obvious conclusion from these data.

More direct evidence for virus survival and movement in soils was the isolation by Mack[13] of poliovirus type 2 from 50 gallons of water drawn from a 100 foot-deep well located more than 300 feet from the edge of a wastewater drain field. This isolation was made during the investigation of a gastroenteritis outbreak in patrons of the restaurant using the well water. Bacterial studies of food and water had failed to elicit a causative agent.

With these data as background, what might one anticipate as to percolation and survival of virus present in secondary wastewater used for spray irrigation and/or sludge used for soil building? Would virus be trapped in the upper few inches of the soil and in time be inactivated or would the adsorbed virus be released due to physiochemical alterations in the soil? Although techniques presently available for virus concentration from large volumes of water containing low multiplicities of virus are poor at best, the Epidemiology Research Center embarked on studies in an effort to answer these questions, at least in part. The general approach has been to determine the virus load entering and leaving the treatment plant in the influents, effluents and sludge. Once it was shown that significant quantities of virus were being released, groundwaters were tested to determine virus survival following percolation. To date we have conducted studies in three geologically different areas, supported in part by the City of St. Petersburg, the Rockefeller Foundation, and the St. Johns River Basin Planning Commission. Data accrued in the St. Petersburg and Rockefeller studies have been published in detail elsewhere[14,15] but will be discussed briefly herein.

MATERIALS AND METHODS

Study Sites

In St. Petersburg the test site was located across the street from the City's 5 MGD activated sludge Northwest Wastewater Treatment Plant (NWWTP) which serves a large residential and business area. Delivery of the effluent from the plant was via four inch polyvinylchloride (PVC) pipes connected to five lateral feedlines with five rainbird sprinkler heads attached equidistantly along the feeder lines, forming a 300 X 300 foot grid (See Figure 1). Application rates were varied between 2 inches and 11 inches week^{-1} depending on the study plan at a given time. Monitoring wells were strategically situated.

Geological evaluation of the site(16) indicated a 5 foot layer of Immokalee sand overlying a 3-5 foot-thick, semipermeable organic layer which effectively retarded vertical flow and resulted in two different water levels; the upper, 3-4 feet below land surface and the lower, 7-8 feet below land surface. An underlying 45 foot Immokalee sand layer with little or no silt or clay terminated in a 5 foot-thick sandy clay confining layer which protects the Floridan aquifer. This aquifer is not used as a potable water supply source in St. Petersburg due to salt intrusion.

In Gainesville, secondary effluents from a package treatment plant serving a mobile home park with a population of ca 500 people, are being discharged into a cypress dome which is nothing more than a bowl-like depression in the sandy soils of a clay-containing organic material which retards vertical flow. The name "cypress dome" is derived from the dome-like silhouette seen frequently in the Florida flatlands which results from decreasing ages and heights of trees from the center of the dome to the periphery.

In the experimental dome, the surface of the depression is covered with black organic material ca 2 feet in depth overlying ca 6 feet of natural surface sand. An organic semipermeable layer with clay interspersed throughout represents the first confining layer at 6 feet. Below this is a tan to gray 6 foot-thick layer of sand with silt and clay content increasing with depth until at ca 20 feet, solid blue clay is evidenced. Chlorinated effluents are delivered to the site through a 4 inch PVC pipe which surfaces in the center of the dome, allowing the effluents to flow into the dome with little aerosolization and/or turbulence. A series of ten monitoring wells were installed to depths of 10 feet (See Figure 2).

In Wildwood, a small municipality of ca 2,500 people, effluents from their primary treatment plant are discharged into a ditch which releases its flow across a spring fed swamp (See Figure 3).

Sample Collection and Handling

Small samples (250-500 ml) of primary, secondary, and chlorinated effluents, sludge and mud were collected in disposable plastic containers. Large samples (50-100 gal) of surface or groundwaters were processed on site and the virus concentrating membranes placed in disposable plastic bags in the presence of 20-50 ml of field eluting medium (5% beef extract buffered to pH 9.5 with Tris and NaOH). After collection, all samples were held on wet ice until delivered to the laboratory.

Virus Concentration Procedures

Virus in all small samples was concentrated by polyethylene glycol (PEG) hydroextraction.[17,18] The specimen was placed in a dialysis bag (25 Å average pore size) and exposed to PEG (Carbowax 20,000) overnight at 4°C. The following morning sufficient eluting medium (Phosphate buffered saline pH 7.4 containing 2,000 U penicillin and 2,000 μg streptomycin ml^{-1}) was added to the dialysis bag to make a final volume of 5 ml. The eluant was well mixed with the residual in the dialysis bag before the supension was removed. The sample was centrifuged at 20,000 X G for 30 minutes and supernatant stored at -70°C until assayed for virus on Buffalo green monkey kidney (BGM) cells.[19]

Virus in monitoring well samples of 50-100 gallons was concentrated by the membrane adsorption technique (MA)[20,21,22] incorporating diatomaceous earth.[23] Test waters were pretreated by lowering the pH and adding cations. In the laboratory, membranes (142 mm diameter cellulose nitrate membranes, 0.45 μm porosity, and an AP-20 prefilter processed separately) were homogenized with mortar and pestle in the presence of alundum and eluting medium. The homogenate was held on a rotary shaker for an hour before centrifugation at 1,200 X G for 15 minutes. Since August, 1974, when we obtained our sonicator (SoniFier, Model W-350, Ultrasonic, Inc.) this rotary shaker technique has been replaced by sonication at 100 W for 15 minutes in a rosette cooling cell. After centrifugation, supernatant was decanted and concentrated by PEG as previously described.

Solids-Associated Virus Concentration

Sludge and mud samples were processed as follows, as were pellets resulting from final PEG centrifugation of a few influent and effluent samples. Three approaches to free particulate-embedded virus were used.

Each sample was pretreated with the addition of sufficient beef extract to achieve a final concentration of 3%. NaOH was added to produce a pH of 9. Samples were then subjected to one of three techniques, i.e., sonication at 100 watts for 15 minutes in a rosette cooling cell, mechanical stirring at 4°C for 18 hours, or fluorocarbon (1,1,2 trichlorotrifluoroethane) extraction. In this latter, equal parts of fluorocarbon and sample were blended in an homogenizer. Following treatment, specimens were centrifuged at 1,200 X G for 20 minutes.

Supernatant was removed and concentrated by PEG as previously described.

Virus Assay

Details of this have been reported elsewhere.[14,15] Briefly, the total concentrates were inoculated in 0.5 ml amounts onto BGM cell cultures, adsorbed, washed and nutrient agarose added. On day three nutrient agarose containing a vital dye was added. Bottles were examined daily for 14 days for appearance of plaques which were picked and passed for plaque verification and identification by neutralization or hemagglutination-inhibition techniques.

RESULTS

Influent and Effluent Sample Isolations

Virus content of the influent and chlorinated effluent samples at the three test sites varied extensively from sample to sample within and among the treatment plants. Despite the small size, 250-500 ml, 85.4% (35/41) of the Gainesville influent samples yielded isolates and 88.7% (47/53) of the St. Petersburg samples. The quantity of virus present was consistently higher in Gainesville, ranging between 8 and 907 PFU/liter with 42.4% (14/33) of positive samples having >100 PFU liter. In St. Petersburg, 90.6% (48/53) of samples yielded virus with a range of 1-140 PFU/liter. Only 4.1% (2/48) of the samples had >100 PFU/liter. The study at Wildwood was just recently initiated. One sample which was concentrated by PEG and solids discarded showed 10 PFU/liter. A second sample treated by sonication showed >161 PFU/liter.

Chlorinated effluent samples showed a reverse relationship, In Gainesville only 4.4% (4/9) samples tested were positive with a range between 4 and 12 PFU/liter, whereas, in St. Petersburg, 82.6% (71/86) were positive with a range between 2 and 212 PFU/liter. Of these, 64.8% (46/71) had 1 to 12 PFU/liter, 29.6% (21/71) had 13 to 100 PFU/liter, and 5.6% (4/71), >100 PFU/liter. At Wildwood, a single chlorinated effluent sample yielded 14 PFU/liter.

Because it was felt that at least a portion of the virus present in wastewaters is an integral part of solids, pellets from the centrifugation of several PEG concentrated samples were recovered and sonicated to disrupt the solids. Table 1 shows the results. From 68.3% (278/407) to 90% (27/30) of virus in each of four samples tested was derived from the sonicated solids of influents and effluents from Gainesville.

Sludge Sample Isolations

A variety of sludge samples yielded virus following treatment by one or more of the techniques employed. Table 2 shows the findings when aliquots of sludge from common sources were treated by sonication, stirring and freon extraction. On 12-2-74, a gallon of sludge was obtained from the final digester faucet at the NWWTP in St. Petersburg. This was held at 4°C in a walk-in refrigerator until processed. On the day of processing, the material was well mixed and 500 ml aliquots removed. Storage times varied between 7 and 60 days as seen in Table 2. No one treatment process resulted in consistently positive results. Rather, each technique was positive 60% (3/5) of the time. It is of interest to note the relatively large number of isolates made from sludge obtained from the SWWTP. This material was obtained as the sludge disposal trucks were being loaded. It had undergone complete digestion, i.e., >60 days retention time at 34°C. No new sludge had been added to the digester over the preceding 7 days. Even after additional storage (7-8 days) at 4°C a total of 43 PFU were demonstrated in 3,000 ml of the sludge.

Additional sludge samples were treated by sonication as shown in Table 3. Two of the samples are of particular interest, i.e., field and sludge drying bed. The former, which yielded 1 PFU even after storage at 4°C for 45 days after collection, represents sludge which had been sprayed onto the field 72 hours before collection. This was retrieved from a small puddle on the field where it had been exposed to sunlight over the two day period. The latter sample, which yielded 24 PFU, was obtained from the sludge drying bed servicing the Wildwood primary treatment plant. The material was in the form of dry cakes after having been exposed to sunlight over a 13 day period. Operators were preparing to "scrape" the bed to permit deposition of fresh sludge.

Groundwater Isolations

One month after virus containing chlorinated effluents were sprayed on the test site in St. Petersburg, virus was demonstrated in weir waters (See Figure 1). Positive findings occurred whether the application rates were 2 or 11 inches week^{-1}. However, no virus was found in the 10 foot wells until September, 1973, *ca* 2 years after the study was initiated. No effluents had been applied in May, permitting a drying out of the field. Starting in mid-June, the application rate was 2 inches week^{-1}. Heavy summer rains, 10 inches in July, 10 in August and 8 in September, resulted in soil saturation including the 3-5 foot-thick organic layer located *ca* 5 feet below ground surface. As these waters began to recede rapidly on September 27, difficulty in filtering water from the 10 foot well was experienced and coincided with the first virus isolation made from this source. A second occurred on October 3, and on October 4 there was a burst of virus (78 PFU) demonstrated. On November 19, a sample was negative but on November 26, a Coxsackie B-4 was isolated. All isolations were made from 50 gallon samples. No further isolations have been made from the 10 foot well.

A 20 foot well sample in 1972 had been negative. Further testing was not done until positivity had been demonstrated in the 10 foot well. All samples from the 20 foot wells were 100 gallons. Those taken in the morning and afternoon of October 10 yielded 67 and 14 PFU, respectively. The following morning no virus could be demonstrated. All subsequent samples have been negative, as well. However, we have not experienced the same type of soil saturation which occurred at that time.

At the cypress dome in Gainesville, application of effluents was initiated in April, and on May 15 virus was isolated from a 50 gallon sample drawn from well A-16 located ca 7 meters outside the southwestern rim of the dome pond (See Figure 2). However, an observation tower had been erected in the center of the dome cutting through the clay confining layers. This probably resulted in some loss of integrity of these layers and may have permitted rapid movement of virus-bearing dome waters into deeper strata.

No effluent was applied to the dome from May 19 through July 2 due to heavy rainfall. On June 14, wells A-9 and A-11 both yielded virus isolates; 2 PFU both identified as poliovirus type 2 from the former and 1 PFU of polio type 1 from the latter.

DISCUSSION

Virus survival and movement in soils under natural conditions is, undoubtedly, due as much to the form in which the virus enters the soil, i.e., "free" or solids-associated, as it is to the various other parameters related to the physiochemical characteristics of the soil. For this reason it was imperative to establish whether or not the virus present in effluents and sludge were actually solids-associated.

Innumerable studies[24,25,26,27] have verified the presence of virus in the liquid portions of effluents and a few have shown virus in digested sludge.[28,29,30] The importance of solids-associated virus has been suggested by others[31,32] although, to date, no confirming data have been forthcoming. Lund has even suggested that quantitation of virus in wastewater and sludge is invalid if solids are removed before assay. She addressed the problem by direct inoculation of sludge into cell culture. A similar approach was used with influent samples by Buras in Israel.[33] In our studies, techniques designed to disrupt solids has strengthened Lund's statement since 68.3 to 90% of virus present in influent and effluent samples from the package treatment plant were solids-associated. The protective coating afforded by solids should encourage relatively long-term survival in soils. Although a sludge drying bed is not soil, the presence of virus in dried caked sludge after 13 days exposure to the hot Florida sun appears to demonstrate the potential for extended survival in soils of solids-associated virus.

As has been shown, it was relatively easy to establish that well protected viruses are applied to soils when secondary wastewaters and/or sludge is applied. Conversely, it is extremely difficult to ascertain virus survival and movement in soils. The indirect approach we have used was to determine the extent of groundwater contamination at the immediate site of application and at sites removed therefrom.

The major problems associated with this approach are the inefficiency of the virus concentration techniques and the "grab sample" monitoring which must be used. In reality, the demonstration of even a single PFU in 50 gallons of groundwater taken at a 10 foot depth is remarkable in view of the aforementioned problems. The demonstration of 78 PFU can only be attributed to happenstance, being in the right place at the right time. The sequential appearance of a comparable virus surge in the 20 foot deep well would appear to confirm the theory that once virus enters a high water-soil ratio zone when the pH is in the alkaline range, little if any adsorption would occur. Virus which reaches the water bearing zones, would move with the groundwater whether it arrived in this zone via normal percolation, rapid wash-through after desorption, or via fractures and/or channels in the soil. The isolation in Gainesville from the 10 foot well 7 meters outside the dome attests to this.

The length of time a virus can survive in soil under natural conditions remains an open question. However, the isolation of poliovirus types 1 and 2 from monitoring wells during the period when no virus laden effluents were applied, demonstrates at least a 28 day survival period. The long-term (91 days) survival of free adapted virus used in the Lefler-Kott experiments would appear to indicate that solids-associated wild virus should survive over an even longer period. Unfortunately, as would appear to be indicated by the St. Petersburg studies, the presence of virus in groundwater is dynamic and thus, presents real problems in determining survival time.

It is of interest to note that Cooper, et al.,(34) experienced this "virus surge" phenomenon in their lysimeter studies which are analagous to soil studies in that virus which appeared in their leachates traversed

5 or 10 feet of ground, municipal refuse. Even in control lysimeters which were not seeded with virus, their indigenous virus loads resulted in detectable "virus surges". The investigators attributed this to the irregularity of virus distribution in the urban wastes used in the lysimeters and their non-uniform flow of water increments. In our present study, wide variations in virus content of wastewaters were shown at both treatment plants tested. Additionally, since the surge of virus was demonstrated in St. Petersburg following heavy rains, alterations in the physiochemical status of the soils may have enhanced desorption of virus and/or precluded adsorption resulting in the observed virus surge.

Recognizing the inefficiency of test procedures, studies which our laboratory has undertaken, i.e. to determine the fate of virus applied to soils through wastewater and sludge application, may appear to some to be an exercise in futility in view of the large number of negative findings. Others may question the validity of a single PFU in a specimen. We acknowledge the ever present possibility of laboratory contamination but discount it since not a single control bottle in three years of study has been contaminated. Additionally, hundreds of negative specimens have been processed and in no instance has an isolate been made from wells during dry seasons. If laboratory contamination were occurring, it should have appeared more frequently and in specimens taken from control wells and/or wells in areas removed from the underground water flow.

There is no question that to the "pure" scientist such studies are fraught with so many variables that true experimentation is impossible. They are right, but if Snow(35) had been intimidated by variables he would never have removed the pump handle in London by which he succeeded in stopping the cholera epidemic at a time when the causative agent was unknown. We can at least isolate and identify virus contaminants even though our concentration techniques are almost as primitive as was Snow's removal of the pump handle. However, if wastewater spray irrigation and sludge disposal on land are to be continued and extended, I believe our data justify expanded studies of virus survival in various types of soil under natural conditions since the demonstration of a single PFU is adequate evidence that large quantities of virus are actually present. The alternative, of course, is to inactivate virus before these biological wastes are discharged into the environment.

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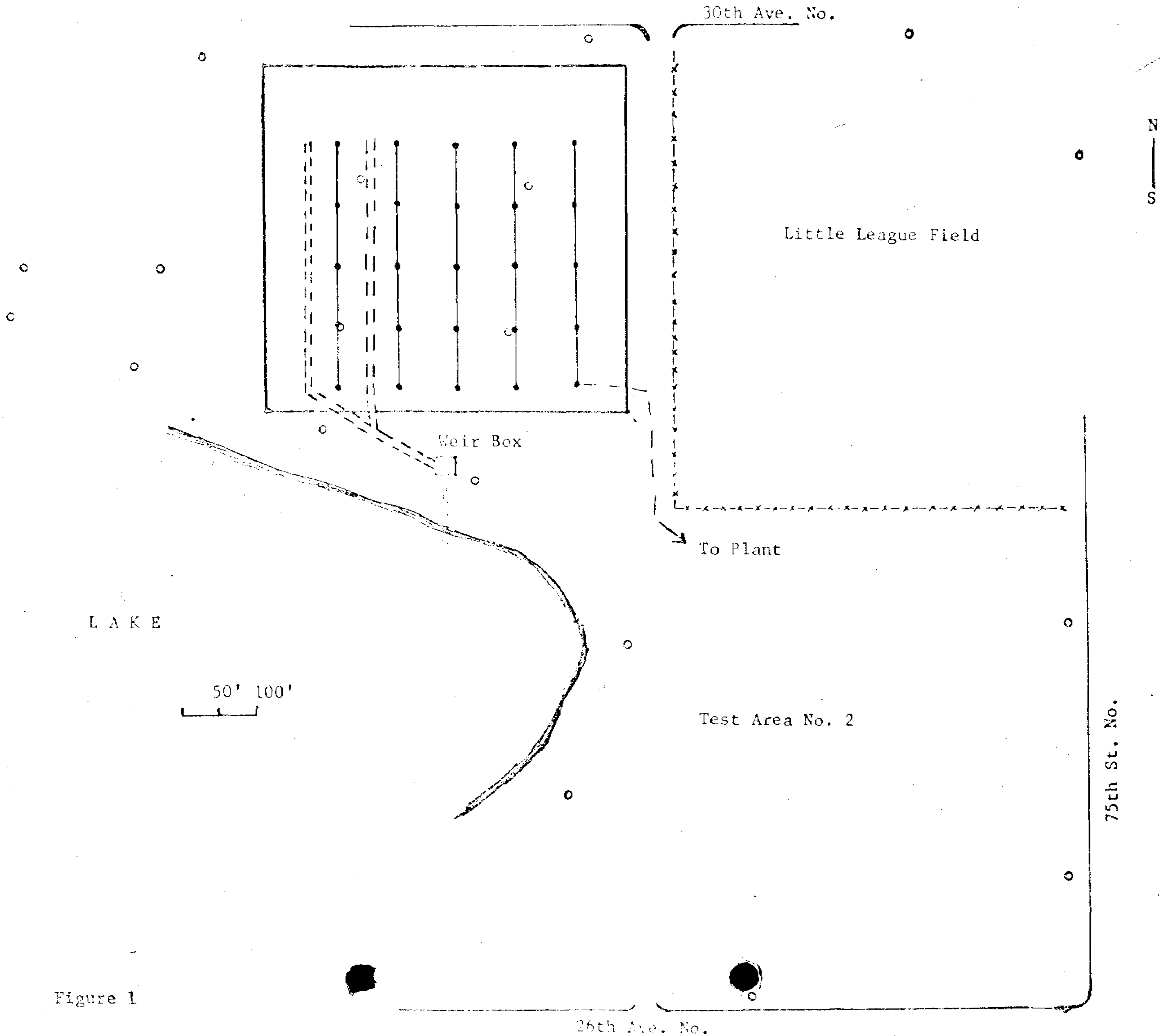
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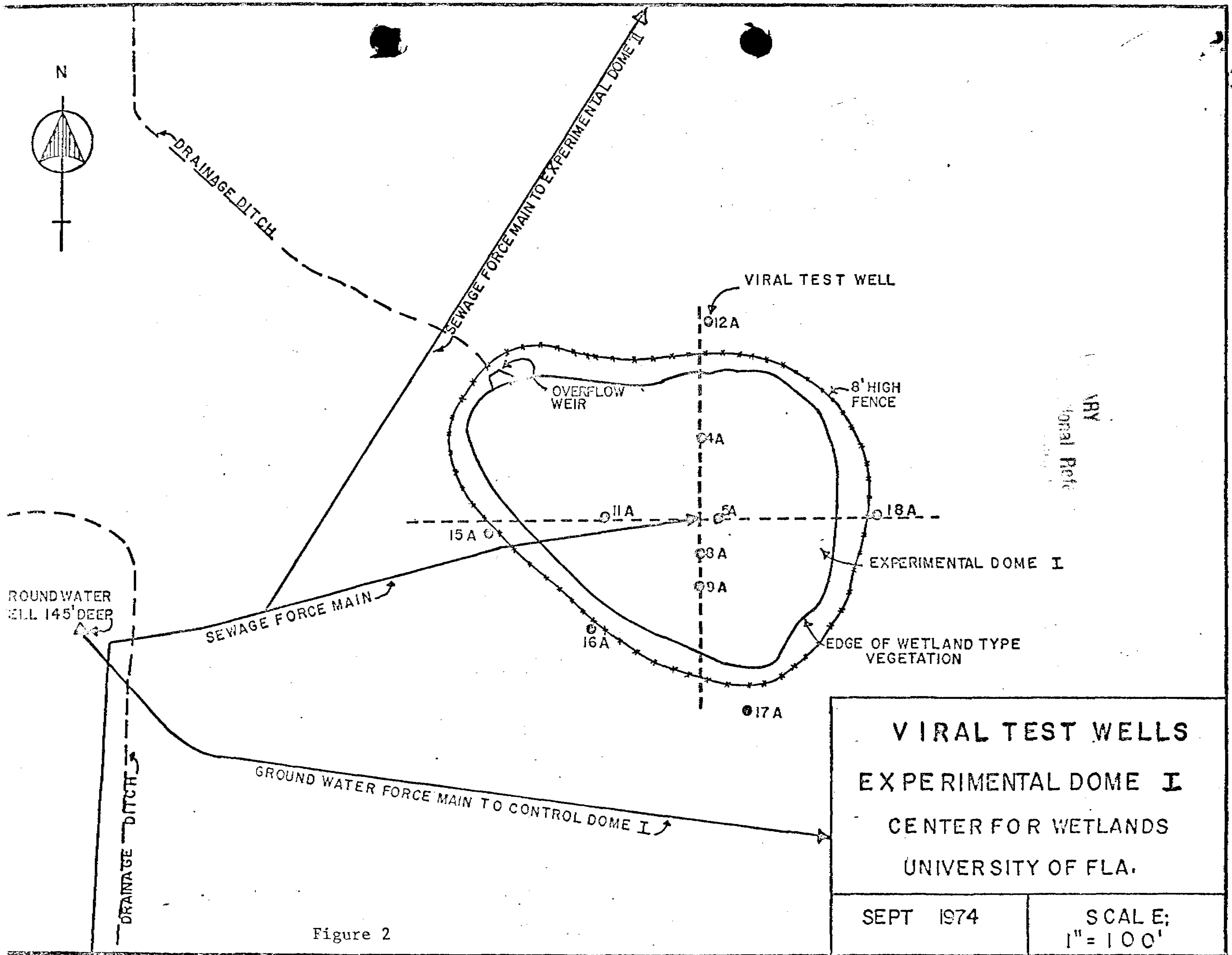
Figure 1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16A
EDGE OF WETLAND TYPE VEGETATION
17A
GROUND WATER FORCE MAIN TO CONTROL DOME I
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CENTER FOR WETLANDS
UNIVERSITY OF FLA.
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SCALE;
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Figure 2

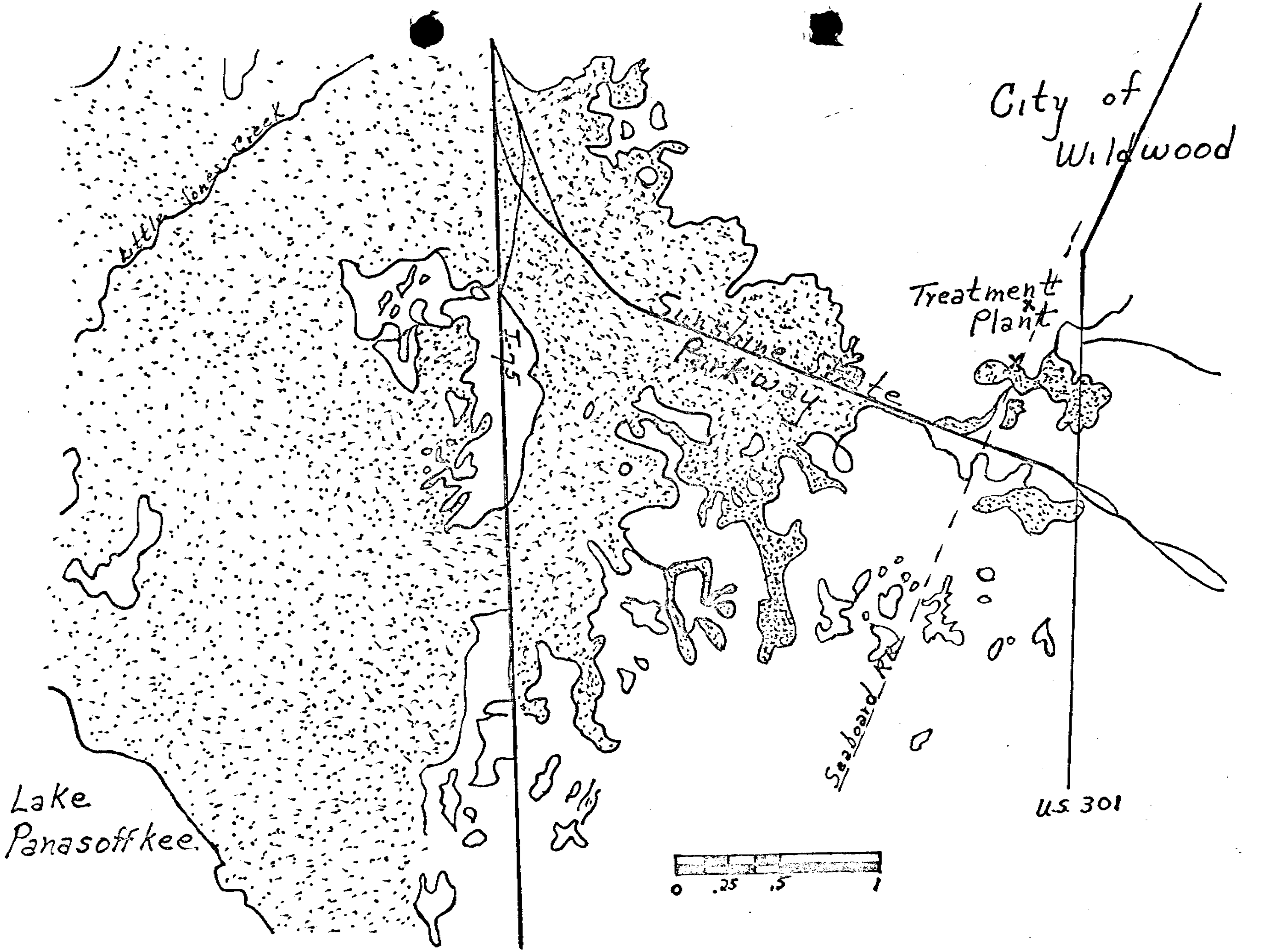
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Figure 3.

PUBLIC HEALTH IMPLICATIONS OF MUNICIPAL WASTEWATER REUSE

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I. INTRODUCTION

One of the primary considerations in the proper disposal of wastewater has traditionally been the protection of the health of the population directly served and of those that might be exposed to the wastes downstream. In earlier times, the main concern has been with the possible spread of communicable diseases by human body wastes, but as time went on, an awareness of the hazards to health associated with organic and inorganic chemicals from industrial and agricultural wastes has developed. This same basic concern with the protection of the public health must remain the sina qua non of any and all programs for water renovation and reuse.

Experience has shown that health requirements must be given very careful attention from the initial planning stages in order to ensure that adequate health criteria be established to meet various water objectives. Only when such health criteria have been set, is it possible to determine the design of treatment systems to meet such criteria on a sure and consistent basis.

II TYPES OF CONTAMINANTS

The degree of concern about the various types of potential contaminants is of course dependent on the type of water reuse being considered. Such concern from the health point of view might be quite minimal for most microbial and chemical contaminants in the case of surface irrigation of industrial crops and would become extremely acute in the case of any planned reuse for domestic consumption. What follows is a brief review of the main microbial and chemical contaminants that may appear in wastewater which bear some relationship to various reuse strategies.

A. Microbiological Contaminants

Since John Snow's classical investigations of cholera in London in 1854, it has been understood that water can serve as an efficient vector of human pathogenic microorganisms of sewage origin. We know today that the raw wastewater of a community usually carries the full spectrum of pathogenic bacteria, viruses, protozoans and helmenths associated with the enteric diseases endemic in the community which are excreted by clinical cases and carriers. During periods of epidemics of enteric diseases the concentration of pathogens can increase many-fold.

The agents of the following enteric diseases have often been detected in municipal wastewater: bacillary and amoebic dysenteries, cholera, typhoid and para typhoid fevers, Salmonella gastroenteritis, tape worm infections, shistosomiasis, ascariasis and a number of virus diseases including poliomyelitis.

It is worth noting that the pathogenic agents of diseases not known to exist in the community can at times be detected in the wastewater, since the organisms may be excreted in quite high concentrations on a regular basis by undetected carriers. In a study of the appearance of Salmonella organisms in 96 samples of wastewater and polluted water in Tel Aviv, we were able to isolate 229 Salmonella strains which included 34 serotypes. (Yoshpe-Purer and Shuval, 1970). The dominant type was S. paratyphi B. Many of the isolated organisms were rare in the community while a number had never been isolated from human cases in Tel Aviv. In another study carried out during the cholera outbreak in Jerusalem in 1970 we were able to detect Cholera vibrios in wastewater from neighborhoods where no clinical cases of the disease had as yet been reported.

Such findings provide further support to the premise that raw wastewater must be considered a most serious potential source of a wide variety of enteric pathogens, irregardless of whether serious enteric disease epidemics are present in the community or not.

Kehr and Butterfield (1943) pointed out that while the level of coliforms found in community wastewater is fairly constant, the ratio of typhoid organisms to coliforms is a function of the endemic disease rate in the community. Thus, for any given concentration of coliforms found in wastewater the risk of pathogens being present would be 10 or 100 fold greater in certain Mediterranean, South American or Asian countries, than in the United States, since some such countries have 10 or even 100 times greater enteric disease rates. The risks associated with wastewater reuse using equivalent treatment processes would be correspondingly greater in such countries.

Enteroviruses present a particularly difficult problem since studies indicate that viruses are more resistant to inactivation by natural factors in the water environment and to most water and wastewater treatment process than are coliforms.

This means that under many circumstances a low coliform count in an effluent destined for reuse may not provide a clear assurance that the effluent if free of potentially infectious enteric viruses that survived the treatment processes.

Our studies (Shuval, 1970) and work of others indicate that domestic sewage carries from 10 - 100 enteric viruses per ml. Enteroviruses found in wastewater may include more than sixty types, all of them considered pathogenic to man. These viruses include poliovirus, echo viruses and coxsackie viruses. Adenoviruses and reoviruses clinically considered respiratory have been found in wastewater. Most important of all is probably the virus of infectious hepatitus, which has been shown by epidemiological studies to have caused over 50 water-borne epidemics. Although techniques for detecting this virus in water are now being developed, it had not been possible to do so in the past.

Methods have now been developed for detecting low levels of viruses in large volumes of water (Shuval and Katzenelson, 1972). It is likely that in the future routine virus assays of wastewater for some high levels of reuse will be required since coliform tests appear inadequate, in certain cases.

B. Factors Affecting the Degree of Risk

The degree of risk of infection from sewage-borne pathogens in any reuse project depends on many factors, including the efficiency of wastewater treatment processes in removing or inactivating the pathogens, the survival of the pathogens in the wastewater effluent, in soil and on crops in the case of agricultural reuse, and the infectivity or minimal infectious dose required to cause infection in man.

1. Removal by Treatment Processes

While it is well accepted that conventional biological wastewater treatment processes provide only minimal removal of enteric bacteria, disinfection can often provide very high levels of bacterial inactivation. It is possible by optimal combinations of wastewater treatment and chemical disinfection to consistently achieve coliforms counts in treated effluent no higher than 100/100 ml. Enteric viruses however are usually many fold more resistant to chlorination than are coliforms (Shuval, 1975a). With advanced wastewater treatment technology including physico-chemical methods, it appears possible to effectively remove essentially all pathogens from an effluent stream. We have shown that ozone may prove to be particularly effective in such cases for the inactivation of viruses (Katzenelson _et al_, 1974). Ozone is a most effective virucidal agent.

Our studies indicate that under controlled conditions a 99% kill of poliovirus can be achieved with 0.1 mg/l of ozone residual in under 10 second while the same concentration of chlorine residual would require 10 minutes and iodine 100 minutes to achieve the same results. If effluent reuse for certain purposes calls for total effective removal of pathogens it now appears to be within the limits of developing technology to achieve this goal.

The reliability of these processes remain to be evaluated under actual field conditions. Considering the health risks that may be involved in the case of a mechanical or human failure, such treatment processes should be fail-safe and monitoring procedures established to ascertain the microbial quality of the effluent before its distribution for reuse. This is now becoming technically feasible with the development of new procedures for the rapid detection of bacteria and viruses in water.

The question of microorganisms' survival in soil and on crops will be discussed under the heading of agricultural reuse.

2. Minimal Infective Dose

With the possibility of obtaining significant reductions in the number of pathogens by active treatment processes or by die away in the soil or on crops, one must ascertain how many pathogens must be injested to cause infection or disease in man. It has been established that for certain salmonella bacteria a person must injest many millions of viable organisms in order to become infected. For this reason, such salmonella infections are most often associated with certain contaminated foods held at room temperature for periods of many hours thus enabling the massive multiplication of the initial inoculum of the pathogen. On the other hand, the ingestion of a few typhoid baccilli appears to be sufficient to cause infection in a certain percentage of susceptible humans who may have a low level of resistance. Very low levels of enteroviruses in water or on crops may present a potential health risk. It has been experimentally established that ingestion of as little as one tissue culture infectious dose of poliovirus (Plotkin and Katz, 1967), and other enteroviruses is sufficient to infect a percentage of susceptible persons. The minimal infective dose for infectious hepatitus has not been determined, but epidemiological evidence seems to indicate that the ingestion of but a few organisms might be sufficient cause of infection in some persons.

The ingestion of a relatively small number of cholera organisms may also lead to human infection. Infection with protozoan or helminthic pathogens may occur with a small number of ingested organisms as well.

With the above considerations in mind, it becomes apparent that very high removals of enteric pathogens are essential in any type of water reuse associated with human consumption of crops, body contact sports or consumption as drinking water. The same goes for any form of reuse where effluent is sprayed into the air and aerosolized microorganisms can be dispersed over relatively wide areas, particularly in the vicinity of residential zones. It has been demonstrated that inhaled enteric bacteria can cause human infections in doses many fold lower than when ingested (Sorber and Guter, 1975). Inhaled salmonellae, for example are 1000 times more infective.

Since we cannot determine in advance the exact type of communicable disease organisms that may at times be present in the wastewater stream destined for reuse, it is reasonable to assume that it is a distinct possibility that highly infectious disease agents will indeed be present and that the ingestion of a very few of such organisms may cause human infection. Health criteria for different forms of water reuse must be based on this conservative assumption.

C. Chemical Contaminants

The unbridled increase in the use of hundreds of new and often structurally complex synthetic compounds in industry and agriculture has resulted in the appearance of many of these potentially toxic materials in municipal and industrial wastewater streams. Many of these chemicals which appear in wastewater are known not only for their acute toxic effects but for their chronic effects which can be detected only after long periods of exposure. Materials having carcinogenic, mutagenic as well as teratogenic effects have been isolated in wastewater, polluted surface water and drinking water from surface sources. Trace metals which may at times reach toxic concentrations have also been found on many occasions in wastewater streams, particularly those carrying a high percentage of industrial wastes (Shuval, 1962).

1. Detection of Microchemicals in Wastewater

Numerous efforts have been made to gain a better understanding of the toxic hazards of modern synthetic chemicals that find their way into wastewater and ultimately into drinking water sources. However, there are many difficult problems in concentrating, extracting and identifying such compounds, many of which maybe, still unknown, breakdown products of more complex chemicals that have undergone partial biodegradation.

The refractory organic components that remain after biological wastewater treatment and natural biodegradation processes in rivers often have been assayed as carbon chloroform extract (C.C.E.) on the assumption that this test serves as a rough screening method for the presence of many of the potentially toxic synthetic organics. For example, the lightly polluted

Columbia River at Bonneville Dam was found to contain 24 p.p.b. of C.C.E. while the heavily polluted Detroit River near Wyandotte yielded 465 p.p.b. (Middleton, 1960). Recycled wastewater at Chanute, Kansas contained 992 p.p.b. of C.C.E. The effluent of the Denver Wastewater Treatment Plant slated for eventual reuse for domestic purposes contains 2478 p.p.b. of C.C.E. as compared to 59 p.p.b. found in the municipal water supply (Linstedt et al, 1971).

More recently a test for total organic carbon (T.O.C.) has been introduced as an assay for residual organics. Effluent from well operated biological sewage treatment plants may contain 40-60 p.p.m. of T.O.C. while the Ohio River at Cincinnati which may at times carry as much as 15-20% sewage effluent has been reported to show a T.O.C. of 20 p.p.m. during certain periods (Cleary et al, 1973).

The Rhine River in the Netherlands contains 10 p.p.m. of T.O.C. on the average while the treated water after dune infiltration and the use of some activated carbon prior to rapid sand filtration still contains 4 p.p.m. of T.O.C. In both the C.C.E. and T.O.C. test little can be said of the exact nature of the organics present particularly since it is known that part of the refractory organics may be humic acids or similar compounds of natural origin. However many efforts have been made to identify the specific organic chemicals present in water and wastewater effluent.

Middleton (1960), using the carbon chloroform extract method (C.C.E.) for concentrating the organics, identified from polluted riverwater, the presence of D.D.T., aldrin, orthochloronitrobenzene, tetralin, naphthalene, chloroethyl ether, acetophenone, diphenyl ether, pyridine, phenols, nitriles, acidic materials; miscellaneous hydrocarbons including substituted benzene compounds, kerosene, synthetic detergents, aldehydes, ketones and alcohols. Some of these substances are known to be toxic. Many other compounds undoubtedly present, remained unidentified. Bunch, et al (1970), employing the best methods available to them at that time, were able to recover and identify less than 40% of the soluble organics remaining in biologically treated sewage and these were described only in general terms such as ether extractable matter, protein, tannin, lignin and alkyl benzene sulfonate.

The introduction of newer methods for sample concentration and analysis has led to vast improvements in identification and measurement of organic micropollutants in wastewater and polluted surface water sources.

The development of concentration techniques for organics such as by means of macroretricular resins and reverse osmosis is rapidly replacing activated carbon adsorption used in the C.C.E. method which has been shown to be inadequate in many respects. High resolution gas chromatography in combination with mass-spectrometry (G.S.-M.S.) aided by computer analysis has provided much new information on the microorganics in water.

More than 1000 organic compounds have been identified in water by using such powerful techniques. In one such study drawing on data from numerous collaborating laboratories, 289 organic compounds detected in water are listed (W.H.O., 1975b). Many of these compounds have known toxic effects. A few are known as carcinogens.

D. Presence of Carcinogens in Wastewater

Evidence is gathering from numerous sources that cancer causing chemicals are being released regularly into the water environment from municipal and industrial wastewater sources.

Heuper and Conway (1964) outlined the main sources of carcinogens that can appear in waste water as follows:

1. Petroleum Products -- Petroleum refinery wastes containing polycyclic aromatic hydrocarbons, fuel oil, lubricating oils and cutting oils are being introduced into lakes and rivers from garages, service stations, petrochemical plants, metal-

working plants, and ships. Contamination of public water supplies may also result from the use of kerosene, methylated naphthalenes and similar petroleum products used as vehicles of insecticide sprays, or enter water from rain contaminated with air pollutants or from tarred or asphalted roads.

2. Coal Tar -- Effluents from gas plants, coke oven operations, tar distilleries, tar-paper plants, and wood pickling plants all contain carcinogens. Coal tar, pitch, creosote, and anthracene oil are known human carcinogens.

3. Aromatic Amino- and Nitro- Compounds -- Amino compounds such as beta-naphthylamine, benzidine, and 4-aminodiphenyl are known to be human carcinogens from results of occupational exposure of workers in dye and rubber industries. These compounds along with their nitro-analogues are released by dye and rubber manufacturing, pharmaceutical factories, textile dyeing plants, plastic production, and others.

4. Pesticide, Herbicide, and Soil Sterilants -- Compounds such as DDT, Dieldrin, Aramite, carbon tetrachloride, acetamide, thioacetamide, thiourea, thiouracil, aminotriazole, several urethane derivatives, isopropylchlorophenyl carbamate, and beta-propiolactone are capable of eliciting benign and/or malignant tumors in various organs of experimental animals.

A new source of concern is the formation of certain halogenated hydrocarbons possessing toxic qualities as a result of the chlorination of wastewater effluent or treated water drawn from rivers heavily contaminated with organics. Jolley (1973) identified over 50 chlorinated hydrocarbons in chlorinated domestic wastewater effluent in the U.S. While Rook (1974) observed that chlorination of polluted river water in the Netherlands produced compounds such as chloroform, carbon tetrachloride, dichlorobromomethane, chlorodibromomethane, tribromomethane, and traces of other halomethanes and haloethanes. Bellar et al (1974) have added further support to these findings and have shown that chloroform concentrations ranging from 37-152 p.p.b. were present in chlorinated drinking water in five communities receiving water from either the Ohio or Mississippi River. This is estimated to be 10 times higher than the concentrations found in chlorinated drinking water from ground water sources. Chloroform and carbon tetrachloride are definitely considered as carcinogens.

One must now ask whether the presence of cancer-causing chemicals in such low concentrations in water can actually cause cancer in man.

E. <u>The Risk Associated with Low Levels of Carcinogens in Water</u>

From Reports of the World Health Organization (1964) and the U.S. Public Health Service (1970), one must conclude that there is growing recognition by scientists that the majority of human cancers are due to chemical carcinogens in the environment. It has been estimated that somewhere between 60 to 90% of human cancers are environmental in origin and that the low levels of carcinogens to which the general public may be exposed could be the responsible causative agents (Epstein, 1974). For example dieldrin, a widely used chlorinated hydrocarbon insecticide, has been found to be carcinogenic in the lowest concentrations tested, 100 parts per billion (p.p.b.) while the chemical aflatoxin, one of the most potent carcinogens, has been shown to produce liver tumors in trout when present in feed at concentrations as low as 0.4 p.p.b.

Although there still is much to be learned about environmental carcinogens and the risk associated with exposure to very low concentrations, there is a strong case in support of the position that "no level of exposure to a chemical carcinogen should be considered toxicologically insignificant for man." (Epstein, 1974).

Most information on the carcinogenic properties of chemicals has resulted from chronic animal experiments and the question of extrapolating the data to humans remains a problem. However there appears to be increasingly strong evidence that indicates that materials found to produce cancer in animals will generally produce cancer in man. If anything, it is felt by science that animals may not be sensitive enough to certain materials which could produce cancer in man at very low exposure levels.

The possibility of long term exposure to low levels of carcinogens by population groups consuming renovated water either directly or indirectly may be a prime factor in evaluating the safety of direct water reuse programs. Of particular concern is the report of Harris (1974) on the implications of cancer-causing substances in Mississippi River water. The study presents presumptive epidemiological evidence which suggests a significant relationship between cancer mortality among white males and drinking water obtained from the Mississippi River in the New Orleans area. The report concludes that their analysis strongly suggests that drinking water from the Mississippi River is causally related to cancer mortality in the more than one million persons in Louisiana that depend upon that source for their drinking water supply.

A study by the U.S. Environmental Protection Agency (E.P.A., 1972) of water in the lower Mississippi River reported the presence of heavy metals such as mercury, arsenic, lead, copper, chromium, zinc and cadmium as well as numerous organic chemicals found in the finished water supplies (chloroform, hexachlorobenzene, zylene, ethyl benzene and dimethylsulfoxide) were listed as having induced histopathological changes during chronic toxicity studies on animals. Three compounds (chloroform, benzene and carbon tetrachloride) were listed as carcinogens. The report (E.P.A., 1972) recommended "that municipal water treatment plants install treatment facilities designed to obtain removal of organic contaminants and heavy metals. . ." They suggested that "continuous use of activated carbon would probably be required to remove the trace organics in the water supplies." The treatment plants in the area studied did not introduce activated carbon treatment as recommended.

The case of New Orleans can be considered as a prime example of <u>indirect</u> wastewater reuse with few of the precautions that are now generally recognized as essential to remove deleterious chemicals that appear in the contaminated raw river water.

Although the results of the New Orleans study cannot as yet be considered as conclusive evidence that cancer is in fact caused by consuming such contaminated water, the implications of these very suggestive findings must be fully taken into consideration in any planned project for the renovation of wastewater for domestic consumption. Specific treatment processes having approved capability of removing toxic organics including the carcinogenic chemicals will certainly be required elements of any such treatment.

F. The Removability of Refractory Organics

While some of the complex, nonbiodegradable synthetic compounds under discussion may be harmful to health at concentrations at the parts per billion level, if ingested for long periods, advanced wastewater renovation technology still cannot normally reduce total organic carbon (T.O.C.) to an absolute zero concentration.

Such advance wastewater treatment processes as those used in the E.P.A.-Blue Plains Plant in Washington D.C. or at Lake Tahoe have successfully reduced the T.O.C. to 1-2 p.p.m. in the effluent by a series of biological and chemical-physical processes including passage through activated carbon columns and heavy ozonization. Current thinking in advance treatment technology is in the direction of even more extensive removal of organics to levels as low as 0.1 p.p.m. by the use of additional treatment processes such as chemical oxidation with ozone or hydrogen peroxide, resins, membranes and volatile stripping (E.P.A., 1975).

An experimental study which injected C.C.E. extracts of

Mississippi water suspected of causing cancer in humans into mice produced no evidence of carcinogenic properties in the water (Dunham, et al, 1967). The authors suggest that one possible explanation for the inconclusive results is that some of the potential carcinogens that may have been in the polluted river water were not adsorbed on activated carbon. This possibility must raise the question as to whether potentially hazardous organics are removed adequately even by activated carbon treatment which is certainly one of the most effective treatment processes available.

Bornoff and Fisher (1962) have shown that polynuclear aromatic hydrocarbons, potentially dangerous carcinogens found in wastewater, are poorly removed by conventional biological treatment and sand filtration but 99% are removed by activated carbon filtration if flow rates are low enough.

However, insufficient information is available today as to the ability of the various advanced wastewater treatment processes to remove the hundreds of different specific organics found in wastewater which may have a deleterious effect on persons exposed to low concentration of them in water over long periods of time (Ongerth et al, 1973).

The International Meeting on the Health Effects Relating to the Reuse of Wastewater for Human Consumption (W.H.O., 1975b) has recommended that this question be given the highest research priority in studies essential to evaluate the health effects of consuming renovated wastewater. Such information will be essential for planning water renovation and reuse for domestic purposes.

The same report emphasized that there is continued value in applying the use of a general test for total organics in water such as the T.O.C. They pointed out that good quality drinking water should usually contain no more than a few mg/l of total organic carbon.

They concluded that they "strongly felt that at this stage with the tremendous gap of knowledge concerning the toxic components in renovated water, the most prudent policy would be to provide for optimal removal of total organic carbon to the lowest feasible level. As a tentative goal, T.O.C. levels as low as 1 mg/l should be strived for in the case of either direct or indirect water reuse.

Whether or not T.O.C. removals to 0.1 or 1 p.p.m. can be fully achieved in practice is yet to be determined. The ability of available treatment processes to fully remove many known specific toxic chemicals is also a moot question.

III HEALTH ASPECTS OF VARIOUS TYPES OF REUSE

Each form of wastewater reuse presents its own specific health problems. This section will review the problems associated with each type of reuse.

The W.H.O. (1973) has presented a comprehensive and authoritative report on the health aspects of various forms of reuse. The W.H.O. report has suggested treatment processes to meet the given health criteria for wastewater reuse which is reproduced here as Table 1. The rationale behind the health criteria and the treatment processes required to meet them is presented in the following sections.

A. Agricultural Reuse

The application of human feces as an agricultural manure has been widely practiced in the Far East for many centuries, while the reuse of municipal wastewater for agricultural irrigation is one of the oldest forms of water reclamation. At the end of the last century, major land irrigation projects were developed in Germany and in England. It should be pointed out however, that the primary motivation of these early projects was essentially treatment and disposal of municipal wastewater rather than water conservation and recycling.

The first Royal Commission on sewage disposal in England concluded in its report in 1865 that "the right way to dispose of town sewage is to apply it continuously to the land, and it is by such application that the pollution of rivers can be avoided."

TABLE 1. SUGGESTED TREATMENT PROCESSES TO MEET THE GIVEN HEALTH CRITERIA FOR WASTEWATER REUSE *

	Irrigation			Recreation			Municipal reuse	
	Crops not for direct human consumption	Crops eaten cooked; fish culture	Crops eaten raw	No contact	Contact	Industrial reuse	Non potable	Potable
Health criteria (see below for explanation of symbols)	A + F	B + F or D + F	D + F	B	D + G	C or D	C	E
Primary treatment	●●●	●●●	●●●	●●●	●●●	●●●	●●●	●●●
Secondary treatment		●●●	●●●	●●●	●●●	●●●	●●●	●●●
Sand filtration or equivalent polishing methods		●	●		●●●	●	●●●	●●
Nitrification						●		●●●
Denitrification								●●
Chemical clarification						●		●●
Carbon adsorption								●●
Ion exchange or other means of removing ions						●		●●
Disinfection		●	●●●	●	●●●	●	●●●	●●●[a]

Health criteria:

A Freedom from gross solids; significant removal of parasite eggs.
B As A, plus significant removal of bacteria.
C As A, plus more effective removal of bacteria, plus some removal of viruses.
D Not more than 100 coliform organisms per 100 ml in 80% of samples.
E No faecal coliform organisms in 100 ml, plus no virus particles in 1000 ml, plus no toxic effects on man, and other drinking-water criteria.
F No chemicals that lead to undesirable residues in crops or fish.
G No chemicals that lead to irritation of mucous membranes and skin.

In order to meet the given health criteria, processes marked ●●● will be essential. In addition, one or more processes marked ●● will also be essential, and further processes marked ● may sometimes be required.

[a] Free chlorine after 1 hour.

*Reproduced with the permission of the W.H.O. from: Reuse of Effluents: Methods of Wastewater Treatment and Health Safeguards. W.H.O. Technical Report Series No. 517. Geneva, 1973.

It is important here to review the possible health risks associated with various forms of water reuse in agriculture. The degree of risk involved may vary greatly. Such reuse may be directed solely to the irrigation of industrial or other crops not for direct human consumption while on the other hand may involve highly health sensitive crops such as fruits or vegetable generally consumed uncooked. In either case, the health risks to agricultural workers must be evaluated as well as the possible dispersion of aerosolized pathogens by spray irrigation in the vicinity of residential areas.

1. Contamination of Crops with Pathogens

Although public health authorities have long ago pointed out the risks of using human feces as a manure on vegetable crops, systematic scientific studies on the survival enteric of pathogens in soil and on crops began to appear in the literature only in the 1920's. One of the earliest studies on survival of enteric pathogens in soil was made in 1921 by Kligler who was the founder of the Department of Hygiene at the Hebrew University of Jerusalem. He showed that typhoid bacilli could survive for months in moist subsoil contaminated with feces although the movement of the bacteria through the soil was very restricted. McClesky and Christopher (1941) studied pathogen survival on strawberries while Falk (1949) studied the survival of enteric bacteria sprayed on tomatoes. His work at Rutgers was followed up by the extensive classical study together with colleagues on the health risks associated with growing vegetables in sewage contaminated soil (Rudolfs, Falk and Ragotzkie, 1950, 1951).

These studies indicated that bacteria, protozoa and helminths do not penetrate healthy undamaged surfaces of vegetables, and die away rapidly on crop surfaces exposed to sunlight. However, pathogens can survive for extended periods inside leafy vegetables or in protected cracks or stem areas. We initiated our first studies with colleagues in Israel in the early 1950's on the health problems associated with sewage irrigation (Shuval, 1951). Our colleague Bergner-Rabinovitz (1956) detected numerous pathogens in the raw sewage of Jerusalem slated for irrigation including Salmonella sp., Shigella dysenteriae, parasitic eggs of Ascaris, Trichuris, Trichostrongylus, Taenia Hymonolepis and cysts of Giardia Lamblia and Endomoeba. In the effluent of a trickling filter plant used for one of our sewage irrigation studies (Rigby et al, 1956), the same pathogens were detected but less frequently and in smaller numbers.

In these studies the survival of Salmonella tennessee organisms inoculated into the effluent was studied in sewage irrigated soil. In the winter, the bacteria could not be detected by the 46th day on the surface of the soil, but only disappeared from the moist subsoil on the 70th day. While in the summer they disappeared on the 23rd day on the surface and 37th day at a depth of 8 inches.

In all cases, there was however, about a 99% reduction within the first week.

Dunlop et al (1951) found that although Salmonellae could be recovered from a large number of samples of sewage contaminated irrigation water, they were unable to recover these pathogens from samples of vegetables irrigated with this water.

In our own recent studies in Israel we have investigated the survival of poliovirus in sewage irrigated soil and on crops. We have been able to show that poliovirus inoculated into the sewage could be detected in the irrigated soil for 7 days and it was possible to recover the virus from the cucumbers irrigated 7 days after initial irrigation (See figure 1).*

It is quite clear from the many studies to date that pathogens are present in sewage in great quantity and variety and can survive for periods in the soil or on crops (Benarde, 1973). However, the viability of such microorganisms varies greatly depending on the type of organisms and various environmental factors such as climatic conditions, soil moisture, soil pH and the amount of protection provided by the crops.

In his review of this problem Dunlop (1952) went so far as to say that despite the known presence and viability of pathogens in wastewater and soil, he knew of no disease outbreaks or epidemics that had been related to or were known to be caused by irrigation

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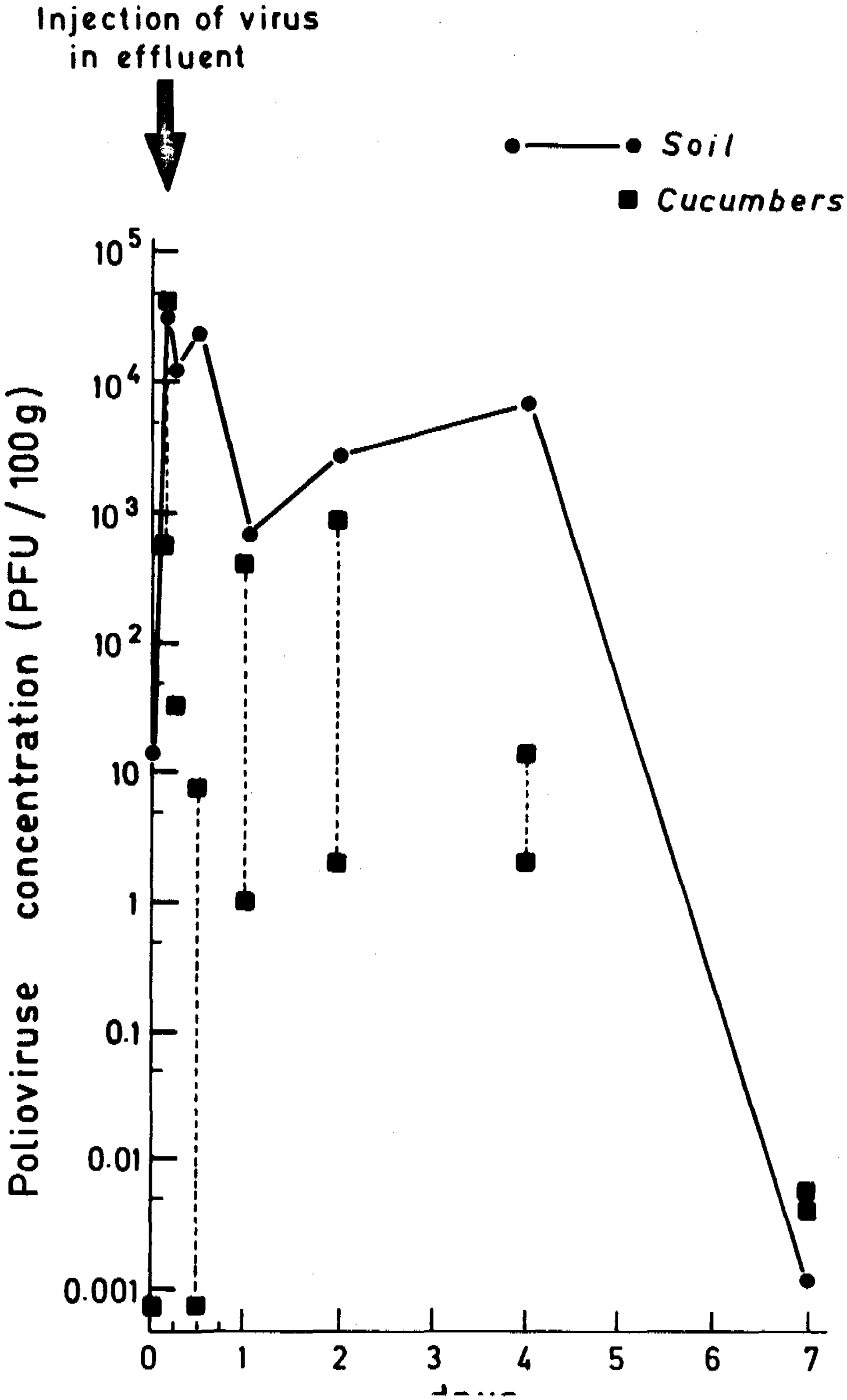
* Unpublished data. B. Fattal and E. Katzenelson, Environmental Health Laboratory - Hebrew University of Jerusalem

FIG. 1

VIRUS CONCENTRATION IN SOIL AND ON CUCUMBERS IRRIGATED WITH EFFLUENT INOCULATED WITH POLIOVIRUSES



with properly treated sewage. He concluded that if effluents were properly treated, it was safe from the microbial point of view to harvest crops for human consumption in 4 hours of irrigation. Dunlop did not define the degree of treatment or disinfection he would require.

A dramatic demonstration that pathogens in untreated sewage used for irrigation could in fact remain viable on the vegetables long enough to cause a cholera outbreak, occurred in Jerusalem in 1970 (Cohen et al, 1973). When cholera cases first appeared in the city, it was quickly ascertained that the drinking water supply derived from deep protected wells and chlorinated before distribution was not the vector. However, during the outbreak we demonstrated the presence of Cholera vibrio of the El tor type in the main sewage lines in various parts of the city and in the soil of some agricultural plots illegally irrigated with raw sewage used for growing vegetables supplied to the Jerusalem market. The pathogens were later recovered from the sewage irrigated vegetables. The illegal sewage irrigation was stopped and the epidemic quickly came to an end. The freshly sewage irrigated leafy vegetables widely sold in Jerusalem undoubtedly provided the main secondary pathway for the spread of the disease after a few carriers or clinical cases entered the city from neighboring countries where cholera outbreaks were in progress.

From the foregoing it is apparent that irrigation of health sensitive crops including fruits and vegetables eaten uncooked with raw or partially treated wastewater can present real health risks. Even effluent from conventional biological wastewater treatment plants cannot generally be considered safe. For these reasons health authorities in many countries have established regulations restricting the types of crops that can be grown with effluent that has not undergone a high degree of disinfection. Irrigation with non-disinfected effluent of industrial crops, seed crops, tree nurseries and other crops not desitned for direct human consumption are typically allowed.

The use of raw sewage is generally not allowed for irrigation of any kind, both for esthetic reasons and so as to avoid the presence of aggregates of fecal matter on the fields which may serve as a source of direct contamination of workers or mechanical transmission by flies and other vectors.

Even sewage irrigation of crops usually consumed cooked such as potatoes or beets should not be considered free of risks since the contaminated surfaces of the vegetables may introduce pathogens into kitchens where working surfaces and utensils may become contaminated thus infecting other foods.

2. Unrestricted Agricultural Utilization

Certain agricultural and economic conditions may warrant the treatment of wastewater to such an extent that it can be used for unrestricted irrigation of all agricultural crops. Farmers find some difficulty in carrying out normally required crop rotation regimes if restrictions on the types of crops that may be grown are too onerous. This also may become a serious public health problem since it is not administratively possibly in many cases to control the types of crops that are in fact grown by farmers who are supposedly required to limit themselves to certain "safe" crops. The economic temptation to grow high value salad crops even if the effluent quality does not warrant this is difficult to overcome. These considerations have led to the development of treatment procedures and standards to overcome such problems.

If wastewater is to be used for the irrigation of agricultural crops in an unrestricted manner, including fruits and vegetables usually consumed uncooked, a high degree of disinfection is necessary to inactivate the pathogens. Additional processes may be required to remove certain resistant protozoans or helminths. The State of California (1973) has established standards which require that reclaimed water for the irrigation of food crops be at all times adequately disinfected, filtered wastewater showing a median coliform count of no more than 2.2/100 ml. The World Health Organization (1973) meeting of experts on this subject has recommended that crops eaten raw should be irrigated only with biologically treated effluent that has been disinfected to achieve a

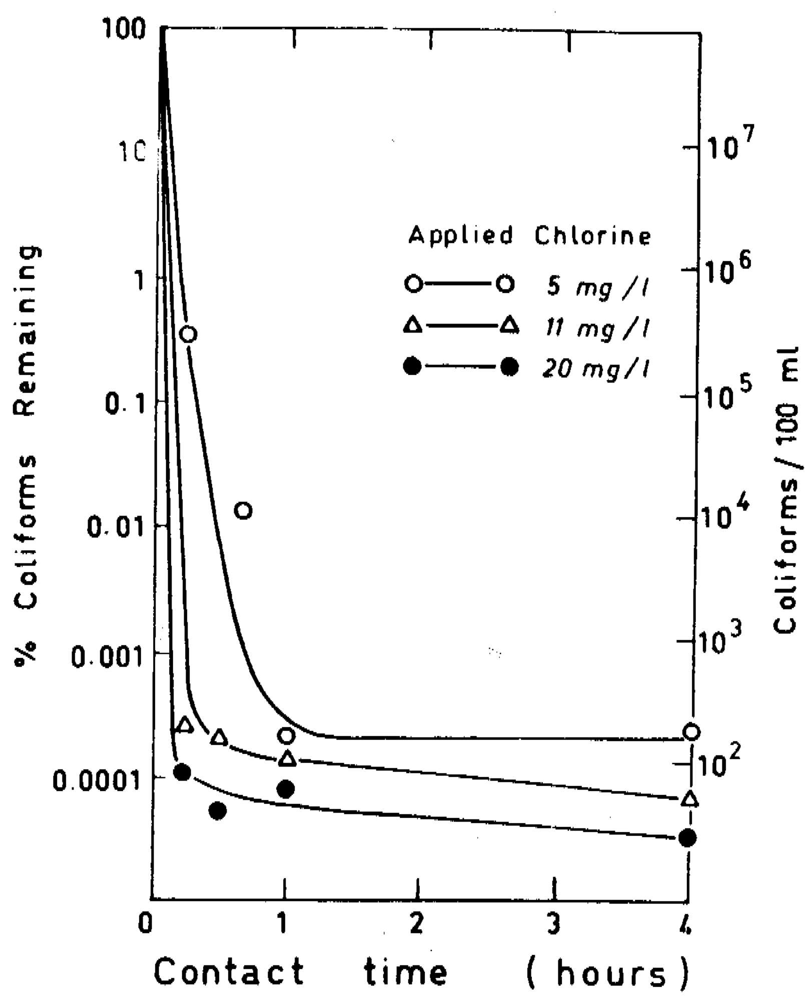


FIGURE 2

Inactivation of coliforms in sewage effluent at varying chlorine doses.

coliform level of not more than 100 organisms per 100 ml in 80% of the samples. It has been demonstrated that it is technically feasible to effectively disinfect a good quality wastewater effluent, so as to achieve such low coliform counts (Shuval, 1975a).

The W.H.O. also states that in certain situations, sand filtration or equivalent polishing methods may be required. This relates in particular to the need to remove helminths in those areas of the world where such parasitic diseases are endemic. A defined and tested technology for the removal of protozoans or helminths generally resistant to chemical disinfection is yet to be established but microstrainers have been proposed to meet this requirement. Slow sand filtration should be effective but may not be economically feasible in many situations.

A number of problems arise in meeting the objectives of effluent disinfection for unrestricted irrigation. The problem of the greater resistance of enteroviruses to chlorination has not been fully overcome. Our studies (Shuval, 1975a) have shown that it is quite feasible to achieve a coliform count of around 100/100 ml by applying as little as 5 mg per liter of chlorine to the effluent of a high rate biological filter plant (see figure 2). However, when we inoculated poliovirus type 1 into the same disinfection system, only about a 90% reduction was achieved in one hour (Figure 3). Figure 4 shows a comparison of concentration-time relationship required to achieve 99.9% inactivation of poliovirus,

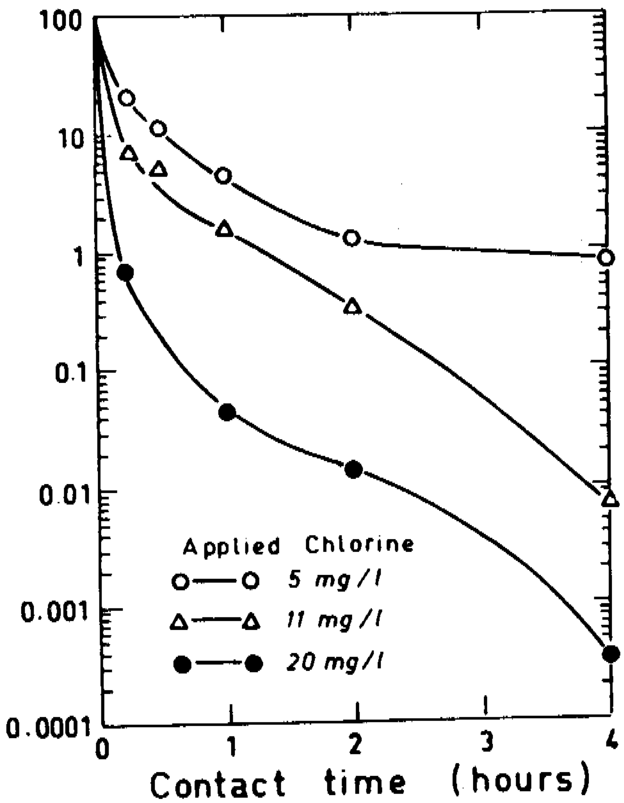


FIGURE 3

Inactivation of the 0.1% poliovirus suspension in sewage effluent by various concentrations of applied chlorine.

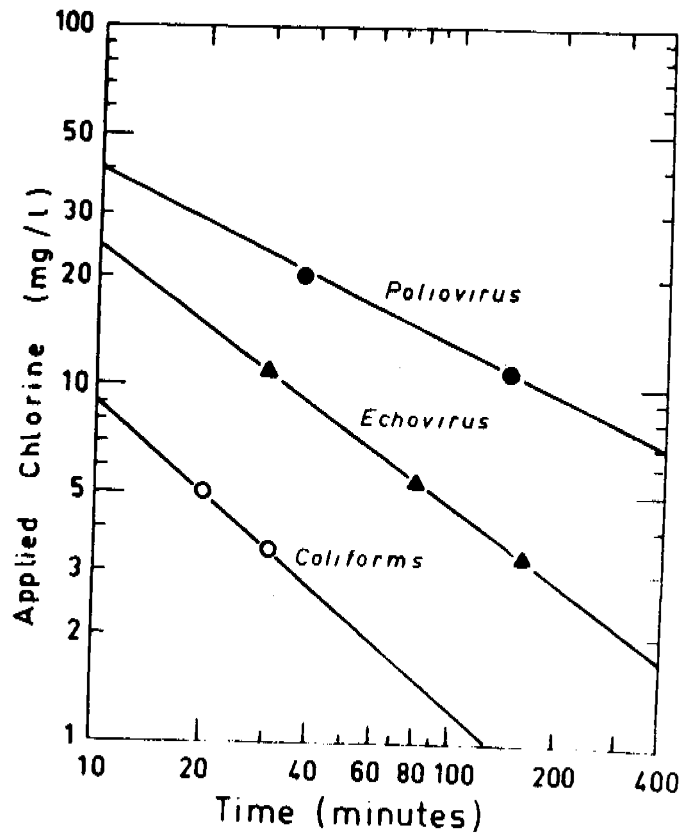


FIGURE 4

Concentration-time relationships for 99.9% inactivation of poliovirus, echovirus and coliforms in sewage effluent by chlorine at 20°C echovirus and coliforms in the effluent by chlorine. From this it can be seen that with a one hour contact time, about 10 times as much chlorine is required to achieve the same degree of disinfection for poliovirus as is required for coliforms. Besides the cost, the formation of potentially toxic organohalide compounds by such high doses of chlorine might rule out such treatment (Bellar et al, 1974).

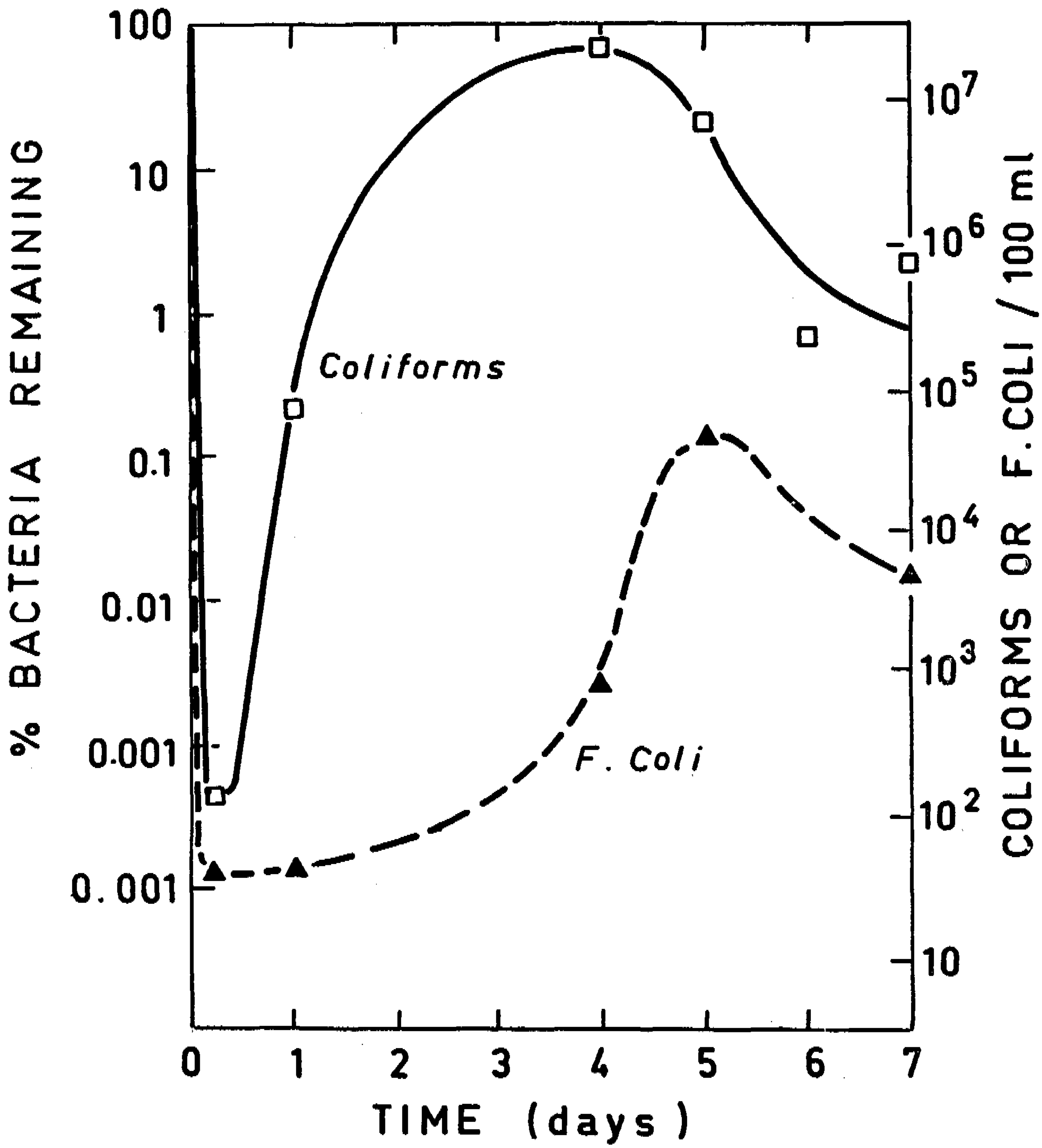
Our recent studies on ozone (Katzenelson et al, 1974) indicate that this powerful oxidant inactivates viruses many times more rapidly than chlorine and may hold promise in the effective disinfection required for reuse purposes.

Another problem is the regrowth of coliforms after chlorination. This phenomenon was first reported upon by Rudolfs and Gehm (1936). In our studies of disinfection of the effluent of a high rate biofiltration plant in Jerusalem we were able to show that coliform counts lower than 100/100 ml could be achieved on a regular basis within 15 minutes after chlorination at doses of some 10 mg/l. However after storage for 3 days in an operational reservoir prior to agricultural irrigation, coliform counts increased 10 fold on the average. In laboratory studies we were able to demonstrate massive regrowth of coliforms and fecal coli 3-4 days after chlorination (See Fig. 5). The hygienic significance of the high coliform counts in such cases is difficult to determine since it is not clear whether pathogens can regrow after initial partial disinfection in a like manner. The State Health Department in California (1973) has recognized this problem and specifically states that the

FIG. 5

REGROWTH OF COLIFORMS AND F. COLI IN SEWAGE EFFLUENT AFTER INACTIVATION WITH 5 mg/l CHLORINE

— NO DECHLORINATION —



bacteriological standard is considered to be fulfilled if "at some point in the treatment process" the required coliform count is achieved. However since information on the regrowth ability of enteric pathogenic bacteria in dilute substrates such as polluted water or wastewater effluent is lacking, the California formulation should be considered as a tentative one.

3. Health of Workers and Public

Little attention has been paid in the past to the potential health risks to workers in wastewater irrigation projects or to the public who may live in adjacent residential areas or who may pass through on public highways.

(a) Direct Contamination

One early study in the United States on the health of workers at sewage treatment plants did not reveal any excessive risks of communicable disease including infectious hepatitus in this group. However, it has been reported from India that hookworm infections are much more common among workers on sewage farms than among the farming population in general. The low levels of personal hygiene and the local custom of walking barefoot are undoubtedly major contributory factors to the disease situation among sewage farm workers in India.

The potential health risk to workers may be derived from direct contact with wastewater which may contaminate hands which later contaminate food. Appropriate sanitary facilities for washing and eating and good personal hygiene, can go a long way to reduce this risk.

(b) Dispersion of Aerosolized Microorganisms

Another problem is the possible inhalation of aerosolized sewage containing pathogens from spray irrigation. Our laboratory in Jerusalem has initiated studies to evaluate this problem (Katzenelson, 1975) and in preliminary studies have been able to recover enteric bacteria including Salmonella sp. 100-350 meters downwind of a field, spray irrigated with non-disinfected effluent. The size of the viable aerosol particles were determined with an Anderson Cascade Sampler and it could be shown that a significant percentage of the recovered bacteria were associated with particles in the 1-4 micron range which can be inhaled into the lungs and can be considered potentially infectious. Our estimates indicate that somewhere between 0.1% to 1% of the sewage sprayed into the air forms aerosols which are capable of being carried considerable distances by the wind. The rate of die-away and reduction in concentration of pathogens incorporated in the aerosols is a function of wind speed, temperature, relative humidity, u.v. radiation and local topographic features. Sorber et al (1974) made some theoretical calculations as to the potential dispersion of bacteria or viruses aerosolized by sewage spray and suggested that a buffer zone of up to one mile would be advisable to prevent infections in adjacent residential areas. Although there is as yet no sound scientific basis for establishing such buffer zones, there is already sufficient data to indicate that an area of some 500 meters from spray irrigation with sewage can carry infectious bacteria in the air. The limits of the buffer zone including some safety factor should surely be beyond this range.

The possible health risks from aerosols associated with the land disposal of the sewage of Muskegon, Michigan by spray irrigation over a 10,500 acre area, became a critical issue in the evaluation of that project by governmental authorities, and by the public (Chaiken et al, 1973).

Here one must also reconsider the health risks to workers moving directly in the zone of spray in such irrigation projects. Although no epidemiological evidence is available as yet it does appear from existing evidence that there is a real risk of inhalation of aerosolized pathogens. The regulations of Department of Health of the State of California (1973) previously cited have already related to this problem. They require the same rigorous disinfection standard for effluent used for spray irrigation as that required for the irrigation of edible food crops. There appears to be strong logic in their case and it would appear advisable to consider heavy disinfection of all effluents used for any form of spray irrigation regardless of crops irrigated or proximity of residential areas.

There is undoubtedly need for further investigation of this question and such studies are in progress in a number of places. Until such time as a more firm scientific basis is available, caution should prevail in the protection of agricultural workers and the public from any risks that may be associated with spray irrigation.

4. Irrigation of Pasture Land

The risks to the health of animals and potentially to man associated with cattle grazing on sewage irrigated pasture has been studied by a number of authors. Greenberg and Kupa (1957) in their review of the transmission of tuberculosis by wastewater point out that the wastes from institutions treating tuberculosis patients or industries such as dairies or slaughter houses handling tuberculous animals will almost always contain large numbers of tubercul bacilli. The baccilli will not be removed by conventional biological treatment but only after very heavy chlorination.

Animals grazing on sewage irrigated pasture or drinking such sewage can become infected. In another review Greenberg and Dean (1958) point out that a number of authors reported that cattle grazing on pastures irrigated with sewage often show significant increases in beef tapeworm infections of Cysticercus bovis which can infect persons who consume the beef with the adult stage of the tape worm called Taenia saginata.

The disease is widely distributed throughout the world in both animals and man, and is still considered a serious health problem in many areas. Reports indicate that conventional sewage treatment is inadequate to completely eliminate tapeworm eggs from sewage or sludge. Sand filtration or microstraining are suggested processes for effective removal of the eggs (Silverman and Griffiths, 1955). Micro straining has been shown to remove about 90% of the T. Saginata eggs.

Some regulations for sewage irrigation of pasture lands have recommended allowing the grazing of cattle after the fields are completely dry. The efficacy of this procedure is open to question in light of the findings of Jepsen and Roth (1952) who showed that the eggs of T. Saginata may remain viable under natural conditions for months, "long enough to permit protracted contamination of fields and crops."

It appears that in areas where this disease is endemic, sewage irrigation of pasture lands should be avoided unless special treatment facilities for removal of the pathogens are provided for.

5. Fish Ponds

Wastewater has been used to add nutrients to fish ponds which are used for growing fish for human consumption in some areas. In an area such as Israel, where this is practised, the potential danger of shistosomiasis transmission to pond workers exists. The parasitic eggs are excreted by persons infected and the larvae which hatch in the water infect specific species of snails which are often found in fish ponds. These snails serve as the intermediate hosts and from them go forth the second stage larvae which can infect the workers by directly penetrating through the unbroken skin. The treatment of sewage in oxidation ponds provides good removal as does conventional biological treatment (Rowan, 1964). In addition, the control of the snails in the fish ponds can prevent the transmission of the disease.

Although fish cannot become biologically infected with human pathogens, they can become mechanically contaminated and in that way introduce pathogens into kitchens and cause human infections. Although there is little epidemiological evidence on actual disease transmission, the risk exists. Holding fish in clean water ponds for some period before marketing may reduce the extent of contamination. The question of accumulation of toxic chemicals and off tastes in fish grown in wastewater has not been studied but reports on taste problems have been made.

B. Industrial Reuse

In many areas, the use of municipal waste water in industry has been successfully practised but a number of problems exist from a public health point of view. Possibly the main problem concerns the danger of cross-connecting pipelines carrying treated sewage and those carrying safe water for use in food processing or for human consumption. The careful color coding of pipes would be helpful in reducing such risks. Generally speaking, however, it would be sound policy to bring treated sewage into industrial plants only after it has been treated and disinfected to the highest possible degree, and has achieved a bacteriological quality approaching that of drinking water. Such a high level of treatment would reduce the risk of a major outbreak of disease occurring as the result of an accidental cross-connection. If wastewater is used for cooling purposes only, very few additional health problems exist, although there are a number of engineering and hydraulic problems that must be overcome. For example, unless the sewage is adequately treated, the problem of slime control might become critical.

If the reclaimed wastewater is to be used as process water in industry, special consideration must be given to possible public health implications, and particular care will have to be taken if treated wastewater is to be considered for use in industrial food-processing plants. In such cases only water meeting the strictest standards for drinking waters should be used. One of the most effective and economical ways of using wastewater in industry is in the intra-plant re-use of treated and recycled industrial effluents. Generally speaking, public health problems involved in recycling industrial effluents are less severe than those resulting from the use of municipal sewage.

Great care must be taken to prevent cross-connections with the general community water system which supplies industrial plants reusing wastewater. The arrangement of a total physical disconnection between the community water supply by an appropriate air-gap is the safest. There are a number of tested "fail safe" double check-valve arrangements which may be used if continuous maintenance and inspection can be assured.

Little consideration has been given to data on the possible health risks to the community through the use of wastewater for cooling towers. Studies have shown that spray and mist from large cooling towers can be carried for many miles. Little data is available on the possible dispersion of aerosolized bacteria and virus which can potentially be carried even greater distances. Cooling towers can in effect serve as huge aerosol generators which could spread pathogens still present in wastewater effluent used for cooling to nearby communities causing potential health hazards. Until this question has been fully evaluated the only prudent policy would be to require that effluent used for cooling towers be disinfected so as to reduce pathogenic bacteria and viruses to the lowest feasible levels. It should be pointed out here that the use of heavily polluted river water in cooling towers may present no less a health risk than the case of overt, direct wastewater reuse. There is every justification to require the same bacteriological standard for such cases of indirect wastewater reuse with polluted river water.

C. Reuse for Recreational Impoundments

Although many rivers and lakes contaminated with varying degrees of raw or treated wastewater have been used for recreational purposes including body contact sports, planned, direct reuse for such purposes is relatively recent. The Santee Recreation Project and a similar one at Lancaster both in California successfully developed treatment processes that produced renovated wastewater for recreational impoundments meeting the most rigorous microbiological criteria (Askew et al, 1965). After initial periods of careful monitoring for bacteria and viruses, body contact sports were tentatively approved under carefully supervised conditions with no deleterious health effects being detected. Public reaction at these desert locations suffering from a shortage of water sport recreational facilities, has been very favorable.

The evaluation of the health risks associated with bathing in polluted water has been a controversial subject for years, particularly since clear cut epidemiological evidence associating contaminated bathing water with overt enteric disease transmission has been sparse. Moore (Medical Research Council, 1959), who studied this question at English beaches concluded, ". . . the risk to health of bathing in sewage contaminated seawater can for all practical purposes be ignored." Many public health authorities have not accepted these conclusions, however.

The potential health hazards that may be associated with bathing in contaminated recreational water includes water-borne enteric infections, as well as upper respiratory ear, eye and nose infections.

Ingestion of toxic chemicals or skin or eye irritations due to chemical industrial wastes must also be considered in cases of wastewater reuse.

It must be assumed that persons bathing in a recreational impoundment filled with renovated water may ingest from 10-50 ml of water. It also must be assumed that for certain enteroviruses the ingestion of one virus infectious dose is sufficient to cause infection in some percentage of the persons so exposed (Shuval, 1975b).

The risk of infection will also increase at times of epidemics of enteric disease in the community when a higher percentage of pathogens are being shed by the population. The relative risk will also be greater in those communities with high endemic rates of enteric disease.

Taking all these factors into consideration a W.H.O. working group on guides and criteria for the Recreational Quality of Beaches and Coastal Waters (W.H.O., 1975a) concluded that there is indeed a potential health risk associated with bathing in sewage contaminated water which justifies establishing broad microbial standards of quality. They recommended that recreational water of good quality should show an E. coli count of under 100/100 ml while E. coli counts above 1000/100 ml indicate an unacceptable level of pollution. The State of California (1973) is apparently one of the few authorities to establish specific standards for recreational impoundments using reclaimed wastewater. They require that the reclaimed water used as a source of supply in a non-restricted recreational impoundment shall be at all times an adequately disinfected and filtered wastewater. Their regulations imply that an effective system of coagulation and filtration following secondary biological treatment precede the disinfection which should produce an effluent with a medium coliform MPN which does not exceed 2.2/100 ml. For restricted recreational uses. not involving body contact sports, the same bacterial standard is required but the requirement for additional filtration after biological treatment is dropped.

These requirements are indeed more stringent than those that may be required of naturally polluted recreational areas but are justified for both hygienic reasons and in light of the fact that the legal and moral responsibility in cases of direct wastewater reuse is a heavy one indeed and falls directly on the shoulders of those who operate or supervise such a project. Maximum feasible precautions should therefore be required.

D. Restricted Municipal Reuse

In this category there are two possibilities. One is the limited use of treated waste water for certain restricted municipal purpose such as fire-fighting, irrigation of parks, gardens and golf courses, and for street cleaning.

There is also the possibility of using treated wastewater in public buildings, or even in homes, for the purpose of flushing toilets. The cost of dual water systems might make this use uneconomical in existing built-up areas, but similar restricted utilization of waste water might be worthwhile in new areas suffering from a severe water shortage. It should be assumed that even for limited municipal use, wastewater would have to be treated and disinfected to such an extent that it would be safe from a microbiological point of view, although it might not meet all the chemical standards usually desirable for drinking water. The specifications for treatment and disinfection would be rather strict because the danger of cross-connections or the possibility of accidental use of treated water for drinking purposes is quite considerable.

Okun (1973) has argued in favor of a concept of a hierarchy of water quality uses based on the policy for planned water reuse of the UN Economic and Social Council (1958): "No higher quality water, unless there is a surplus of it, should be used for a purpose that can tolerate a lower grade." It suggests that, rather than treat wastewater to meet the standards of drinking water, dual supply systems for wastewater reuse deserves careful study, since a relatively small portion of the total community water supply is required for drinking, cooking or other uses that demand high purity. In most industrialized cities this portion is only about 10 percent, the remainder being used for industrial purposes, toilet and street flushing, public fountains, irrigation and other purposes not requiring high quality water.

Key (1967) has also supported this concept of preserving high quality water for domestic consumption while using lower quality water, including reuse of wastewater for other needs. Such dual water supply systems are in use in the Bahama Islands and in HongKong Island, where seawater is used for flushing toilets. Okun feels that the technology for safe management of dual water supply systems now exists and that in new communities or new sections of existing cities the cost of such a dual distribution system would be only about 20% higher than a conventional one. He argues that, in the long run the benefits may outweight the disadvantages.

Considering the many problems associated with direct reuse of reclaimed wastewater for unrestricted urban use to be discussed later, the concept of dual municipal water systems may indeed offer many important advantages and should be given careful study in new communities or even in existing communities suffering from very severe water shortages.

E. Unrestricted Municipal Reuse

The development of advanced wastewater treatment technology has in recent years brought the possibility of direct wastewater reuse for domestic consumption under active consideration in a number of water-short areas. In the process of developing wastewater treatment trains capable of removing much of the incremental inorganic and organic contaminants which might have deleterious effects on water courses it has become apparent that in certain cases the effluent produced could be of a quality comparable to recognized drinking water standards.

In Windhoek, South West Africa, wastewater processed by such advanced treatment trains has actually served as a source of municipal water supply (Van Vuuren et al, 1971) and the acequacy of treatment has been justified by comparing the effluent quality with drinking water standards. A number of other cities including Denver (Ogilvie, 1975), Dallas (Graeser, 1974) and Tel Aviv are in the preparatory stages for such planned reuse for domestic purposes.

At this point it is appropriate to ask whether our knowledge of the toxicological and epidemiological implications of wastewater renovation for domestic consumption is sufficient. Is the public health adequately protected if processed municipal and industrial wastewater can be brought in line with today's conventional drinking water standards? Were these standards conceived with such a possible application in mind? If not, what remains to be done prior to giving the final green light to total direct wastewater reuse for all purposes including human consumption? We shall attempt to discuss these questions and propose possible approaches to answering some of the yet unanswered questions.

1. Adequacy of Drinking Water Standards.

The U.S. Public Health Service Drinking Water Standards (1962) list 20 chemical parameters, only nine of which serve as absolute grounds for rejecting a supply as unsafe for human consumption. The World Health Organization Drinking Water Standards (1941) contain a few more chemical parameters which may serve as grounds for rejecting a supply. E.P.A. proposed drinking water standards will expand the list a bit further. None of these widely known and accepted standards list more than a few synthetic organic compounds despite the fact that hundreds of such chemicals may find their way into municipal and industrial wastewater, and many of them are known for their potential deleterious effects on human health. For that matter, neither do these standards exhaust the list of potential inorganic toxicants that may be found in industrial wastes.

It must be recognized that conventional drinking water standards were originally based on the assumption that water for human consumption would generally be drawn from groundwater sources or from the "best available" protected uncontaminated surface water sources, and that the limited number of chemical parameters included were adequate for most situations. This assumption is rarely true for most surface supplies of today. Can standards be developed to cover the wide range of contaminants that are actually found in wastewater destined for domestic consumption?

Several hundred threshold limit values for such industrial chemicals in air have been established in the field of industrial hygiene, while some 100 maximum allowable concentrations for pesticides and other toxic chemicals have been established under food regulations. Stokinger and Woodward (1958) have shown how certain of these accepted threshold limit values of substances in air may be used to arrive at approximate limiting concentration in water. Although there is still little direct evidence that such chemicals in water have produced or are now producing widespread deleterious effects on health, it is not difficult to envision such a possibility as the percentage of wastewater in many surface supplies increases or if renovated wastewater is used for drinking without proper removal and control of such toxic materials. The experience in New Orleans cited earlier points in that direction.

The drinking water standards of the U.S.S.R. Ministry of Health are the first to have recognized the scope of the problem and now include some 400 chemical parameters with more under study. Certainly not everyone of the 400 or so chemical standards for drinking water established by the U.S.S.R. health authorities is of toxicological importance since many were established on organoleptic grounds alone. Nevertheless, the differences between the large number of standards in the U.S.S.R. list and the very limited number of standards presently considered adequate in the U.S.A. and many other countries indicates the extent of the problem that will have to be faced when the wastewater of a city including industrial wastes has to be processed to the point of becoming fit for human consumption.

The mere comparison of the quality of the final effluent against those standards currently listed in the U.S.P.H.S., W.H.O. or E.P.A. drinking water standards leave too many questions unanswered, to be accepted as adequate evidence that such an effluent is completely safe from the public health point of view.

The W.H.O. International Working Group (W.H.O., 1975b) concluded ". . .that conventional drinking water standards alone cannot provide a sufficient basis for the health evaluation of reused water for domestic consumption."

Not enough is known about the true identification of the residual microchemical pollutants both inorganic and organic, which of course vary widely from one situation to the next depending on the nature of the industrial wastes which enter the sewerage system.

2. Microbiological Problems

While most bacterial pathogens can be effectively inactivated by chlorination and other conventional disinfection procedures there is ample evidence that some of the enterovirus are many fold more resistent than bacteria to the same disinfection processes

(Shuval, 1970). Infectious Hepatitus has been transmitted by sewage contaminated water on many occasions and in some cases the virus appears to have passed through conventional water treatment plants which have included disinfection by chlorine.

Enteric virus levels in raw sewage have been shown to be about 10-100 per ml while their concentration in heavily contaminated river water, which may lead to epidemics of Infectious Hepatitus may be 1 per liter or even less. Laboratory techniques for detecting such low levels of viruses in water are still in the developmental stage and present one of the major obstacles in studying the efficiency of wastewater renovation processes as to the virus removal. Methods for concentration of and detecting viruses in water have been reviewed by Shuval and Katzenelson (1972). Another problem arises from the fact that it may take five days or more before the results of a virus assay of water can be completed. Effective methods of assuring virus inactivation in wastewater treatment still leave something to be desired although there is growing evidence that advance waste treatment methods followed by disinfection with adequate concentrations of free available chlorine (HOCl) with sufficient contact time can be highly effective in reducing enteric viruses levels. Ozone is particularly promising as a virucidal agent for water and wastewater treatment (Katzenelson et al, 1974).

Nevertheless the effectiveness of any proposed wastewater renovation treatment train must be fully evaluated as to its virus removal efficiency and ways must be found to monitor routinely such plants to assure their continued effectiveness in removing viruses.

3. Toxicological Evaluation of Renovated Wastewater

There are two possible approaches to the toxicological evaluation of renovated wastewater to be used as drinking water (Shuval and Gruener, 1973). The first would require the establishment of maximum allowable concentrations or limits for each of the potentially hazardous chemicals that may be found in renovated wastewater. The approach that has been developed by toxicologists in setting tolerance limits for food additives and chemical contaminants in food has been to establish acceptable daily intake (ADI) levels for man. These ADI are based on all relevant toxicological data available at the time of evaluation including data from cases of human exposure which is usually very limited. After determining the "no effect" level and introducing a certain safety factor the ADI can be established. Such figures can then provide the toxicological basis for establishing tolerance levels or maximum allowable concentrations in food, water and air based on known consumption patterns and realistic levels of contamination which are unavoidable. In establishing such figures for water, due consideration must be given to the total body burden from all environmental sources. Although much of the basic data may be available to assist in setting such standards for water, important information is still missing. (W.H.O., 1973).

The toxicological evaluation of chemicals found in the environment cannot be simplified to take into account acute or subacute effects alone. Today such evaluation must include effects from long term exposure and studies for carcinogenicity, mutogenicity, teratogenicity and various biochemical and physiological effects. Even if the specific toxicity of defined industrial and agricultural chemicals is established, the possible toxic effects of their breakdown products may be more difficult to determine. Natural biodegradation or specific treatment processes such as chlorination may lead to the development of new compounds having toxic properties quite different from those of the parent compound. Work to identify these breakdown products and to study their toxic effects is required.

Another factor complicating the toxicological evaluation of heavily polluted water or renovated wastewater is the combined and possible synergistic effect resulting from the exposure to a mixture of toxic and non-toxic chemicals. Increased toxic impact of such combinations is known to occur under certain circumstances and the case of renovated wastewater must take into account such possibilities.

Although much is to be gained by establishing proper tolerance levels for many of the known toxicants that might appear in renovated water, this approach will take a long time to develop and even then will not cover all possible toxic effects as pointed out above.

For these reasons it is felt that a second approach is required. A full toxicological evaluation should be carried out on the actual finished renovated water intended for human consumption with its real mixture of known and unknown residual chemicals remaining after treatment.

Long term feeding experiments with more than one species of experimental animal should be required as well as other more rapid toxicological screening tests using bacteria or cell cultures. Such studies would include testing concentrates of the residual chemicals in the final processed water as well as the normal unconcentrated effluent. Concentration techniques used must avoid being selective as is the case with activated carbon and most not lead to the breakdown of the chemicals involved by overly harsh treatment such as high temperature distillation. Reverse osmosis and lyophilization might be possible approaches to this problem.

Most governments require the full toxicological evaluation of any new drug or food additive before allowing its commercial use. The requirements for evaluating renovated wastewater with its many and often complex unknowns should be at least as rigorous.

W.H.O. reports on this matter (W.H.O., 1973 and 1975b) have emphasized the need for complete toxicological evaluation of the actual water planned for reuse as an essential step in evaluating the safety of such reuse.

4. Build-up of Dissolved Solids in Recycling.

In multiple recycling of wastewater there will be a build-up of those dissolved solids not removed or only partially removed by the wastewater treatment plants unless specific demineralization processes are included. However the build-up in concentration will not be infinite since there will be fresh make-up water added in each cycle to compensate for water losses that do not appear as sewage flow. In most cities these losses normally range from 10-20% (Metzler et al, 1958).

Thus if we assume that in each recycle 90% of the water input into the community appears as wastewater which will be processed for recycling the concentration of, say, sodium chloride will increase with recycling until it reaches an equilibrium 10 times greater than its original concentration in the wastewater. (Long and Bell, 1972). The same might be true for certain refractory organics of potential public health danger. Under such conditions, partial demineralization would be necessary to keep dissolved inorganics at acceptable levels. The problem of toxic trace organics and inorganics may be more difficult to deal with since these substances may not all be removed with equal effectiveness by some demineralization processes which may be selective. The build-up of such toxic materials on multiple recycling would be very undesirable. Until such time as complete information is available on the removability of the various toxic organic and inorganic trace elements that may appear in the wastewater stream it might be prudent to reduce the possibilities of build-up by providing additional dilution from fresh water sources. For example, with a reuse factor of only 30% the maximum concentration at equilibrium of a chemical not removed at all will be only 40% greater than the original concentration in the wastewater stream while with a reuse factor of 50% equilibrium will be reached with the concentration of the refractory compound of twice its original concentration. Such dilution with fresh water would normally avoid the need to include an expensive demineralization step in the treatment and would provide an additional safety factor against the build-up of compounds whose removability by demineralization or other processes may not as yet be known.

5. Epidemiological Evaluation.

No matter how thorough a toxicological evaluation is made there always remains the problem of extrapolating the findings with laboratory animals to fit the human situation. With drugs of potentially great medical importance, human trials are held after completion of the toxicological evaluation. In the case of new food additives which are usually less essential to human welfare than drugs, negative findings in the toxicological evaluation do not automatically mean that the new chemical will be allowed for use in human food. It must be demonstrated that the chemical will make a significant contribution to improving the quality or preservation of the foods in which it will be used. In water short areas, water renovation can often be justified as being of potentially great importance to human welfare as well.

A considerable body of information has been built up concerning the effect on human health of many of the environmental contaminants discussed here as a result of direct exposure of humans under various industrial situations. Further information has been gained by the accidental exposure of humans of certain toxic materials.

If wastewater renovation for domestic consumption is ever to become widely accepted there will be a need at some stage to carry out a full scale epidemiological evaluation of the impact of such reuse on the health of the population exposed. It may be difficult to choose an appropriate population group for such a study but to the extent that certain communities in water short areas have already gone ahead with wastewater reuse for drinking water, every effort should be made to carry out a thorough epidemiological evaluation. Such a study should include baseline health evaluation of a sample population before the introduction of renovated water and then a follow-up of the same group, as a panel study, over a five or ten year period. Such opportunities will be few and far between and every effort should be made to gain as much data from each case as is possible.

A promising alternative to such a study with a population exposed to planned wastewater reuse, would be a series of studies of populations exposed to indirect or unintentional wastewater reuse. Such population groups are easier to identify than might be imagined since many millions of people throughout the world are, in fact, consuming renovated wastewater every day and have been doing so for years. Some 15-20% of the flow of the Ohio River is fully or partially treated municipal or industrial wastewater (Cleary et al, 1963). Millions of people in the Rhine River Basin consume water from the river which at times of low flow may contain as much as 40-50% of industrial and municipal wastewater.

It must be recognized that unintentional and in many respects uncontrolled reuse of wastewater is now very widely practised and provides a basis for evaluating the health impact of such use as well as the expected impact of fully engineered and carefully controlled direct wastewater reuse of the type under discussion.

Although such prospective epidemiological studies are expensive and take many years to complete it is essential that they be made even if planned direct wastewater reuse were not under consideration. Such studies are essential to evaluate present environmental exposure from consuming water from polluted sources which will become even more polluted in years to come. Epidemiological studies may shed light on the need to make major improvements in present day water treatment technology which has been demonstrated as being relatively ineffective in removing many of the refractory organic toxicants that appear in increasing quantities in polluted water. The New Orleans study with all of its limitations illustrates the importance of this approach. The findings will also be of vital importance in planning future wastewater renovation programs where it can surely be expected that treatment trains of demonstrated efficiency will be utilized to remove potentially hazardous chemicals or pathogenic microorganism to the lowest possible levels.

6. Monitoring the Reuse of Wastewater for Domestic Consumption.

The nature of a monitoring or quality control program for products produced for human consumption should vary according to the degree of risk to health involved. Conventional water supply monitoring programs have in the past assumed that the product is basically a safe one and that it can be supplied to the consumer directly after processing without waiting for the results of quality control tests. Bacteriological test results are usually available 24-48 hours after sampling while routine tests for toxic chemicals, when they are made, may be available only after many days. The water tested has usually been consumed by the population by that time.

Drugs and food additives and many processed foods are tested routinely and are released for use in batches only after the test results indicate no positive findings.

In the case of a plant for renovating wastewater for human consumption it would appear necessary to require a more rigorous monitoring and quality control regime than that currently practised by the water supply industry. Technical breakdowns and human failures at such a plant might lead to major health hazards. It would not be illogical to require that renovated water be fully tested and certified as safe before its release to the general water supply system. With improved bacteriological techniques results can be obtained in under 24 hours as can the results of most of the important chemical tests, many of which can be automated. Ways of carrying out rapid toxicological evaluation of the finished water with bioassay techniques should be developed. For the moment, virus assays require at least five days for completion but here too more rapid assay techniques are under study and may become available. Katzenelson (1975) has developed a rapid virus detection method using fluorescent antibodies that can provide a qualitative answer in 10 hours and a quantitive one in 24 hours.

Renovated water could be produced and held in batches until completion of the quality control tests, before being released. This will add additional costs to wastewater renovation plants but the additional safety obtained would justify the expenditure. Certainly such precautions should be practiced in all early plants until it can be demonstrated that less stringent quality control measures are adequate.

Many might agree that the proposed monitoring regime should be applied to any case where heavily polluted surface water is the source of drinking water supplies. Such supplies may be an even greater risk than planned direct reuse programs.

7. Policy Considerations.

The approach presented here concerning water reuse for domestic consumption may appear to be overly cautious and place too heavy a burden on future wastewater renovation programs. In answer, it must be stated that criticism of current drinking water standards applies to any case where polluted surface water serves as a source of drinking water no less than to the special case of direct wastewater renovation. In fact, indirect, unplanned wastewater reuse may well be a greater risk than planned direct reuse which would include treatment processes better capable of coping with the organic pollutants found in wastewater. Unplanned or covert wastewater reuse is far too widely practised today, with too few controls, to allow one to feel complacent.

However, planned direct wastewater reuse for domestic consumption carries a heavy responsibility with it, since it involves full engineering and health responsibility from the beginning to the end without the intervening hand of "nature". The fact that "nature" provides little protection in heavily polluted rivers whose self-purification capacity is over-taxed gives little justification for a similar lax approach in a planned direct reuse project. In such a project the designers, operators and health authorities which must give their approval must carry the full responsibility of any adverse health effects which may result, even if it can be shown that communities consuming polluted surface water are exposed to equal or greater risks.

Many such communities are indeed exposed to undesirable health risks and an equal tightening up of standards, treatment procedures and quality control for all cases of wastewater reuse whether direct or indirect is fully justified.

It still remains to be demonstrated that water treatment technology can overcome the many problems involved in processing wastewater with its many complex components and rapid fluctuations in quality to achieve a uniform end product meeting the health requirements for wholesome and safe drinking water. However, further work in this direction will be very important whether it be applied to direct water reuse projects or to the more urgent and widespread cases of indirect, covert wastewater reuse that exists so widely today in communities drawing water from the polluted lower reaches of the great rivers of the world.

Another consideration that cannot be overlooked is that of public attitudes towards water reuse for domestic purposes. The strong public opposition which in many cases thwarted efforts to introduce fluoridation despite strong technical evidence and the support of the scientific community is an illustration of the power of an aroused public opinion. Water reuse for domestic consumption may not be easily accepted by the public even if all the precautions outlined above are taken. The study of Bruvold and Ward (1972) in ten towns in California indicated that out of 25 forms of possible wastewater reuse 11 would be likely to receive no public opposition. These include such items as golf course irrigation, commercial air conditioning and hay, alfalfa or orchard irrigation, while over 50% of those interviewed opposed reuse for domestic purposes. Any planned programs for reuse must give careful consideration to this question from the very beginning or they may find years of scientific and technical effort vetoed by public opinion.

In the final analysis, direct planned wastewater reuse for human consumption may well become feasible through the development of advanced wastewater treatment systems with a demonstrated fail safe capability of removing the hundreds of potentially toxic inorganic and organic chemicals which appear in today's wastewater streams. A major combined effort of developing appropriate advanced technology and health effects evaluation will certainly be required to achieve this goal.

H. Groundwater Recharge

The use of treated wastewater for groundwater recharge is practiced in a number of areas. In some cases the sole objective has been to build up a barrier to prevent salt water intrusion into coastal areas where groundwater withdrawals have been excessive. If no direct withdrawal of the groundwater and with it, the recharged wastewater is practiced it will flow to the sea and create few if any public health problems. However if the recharge occurs in areas where groundwater pumping takes place the effects of the effluent on the quality of the groundwater withdrawn may be considerable. The main factors that must be considered are the nature of the aquifer, the mean residence time between recharge and withdrawal, withdrawal rates and finally the degree of dilution obtained with the surrounding groundwater.

In uniform sandy aquifers a high degree of microbial removal can be anticipated. Studies have shown that within a distance of a few hundred meters from the point of recharge, effective removal of viruses and bacteria can generally be achieved. Long residence times of several hundred days in the aquifer may also prove effective in the removal of viruses and bacteria through die-away. However, in the case of non-uniform aquifer formations of gravel or karst limestone there may be little or no microbial removals over extensive distances.

Inorganic and organic chemical removals will be a function of the adsorption and ion exchange characteristics of the aquifer which may under certain circumstances provide a considerable degree of removal while in other cases such chemicals may travel over great distances with little or no reduction in concentration. Even when studies indicate a degree of chemical removal by filtration through the aquifer there is the possibility that once the adsorptive or ion exchange capacity is exhausted there will be a breakthrough of chemical contaminants which may appear suddenly and possibly in high concentrations at the withdrawal wells. This can present a serious threat to the quality of the reclaimed water.

In areas where groundwater recharge with treated wastewater is planned a major factor in determining the degree of pretreatment required is the ultimate use of the water withdrawn. If only agricultural or industrial utilization is planned it will usually be possible to meet the health requirements for such use without too much difficulty or at most by additional disinfection of the pumped well water. However, if the water is destined, all or in part, for municipal use including domestic consumption, all of the limitations mentioned previously for unrestricted municipal reuse must be applied, unless very high rates of dilution with pure groundwater can be assured.

Effective removal of toxic organics and heavy metals must be assured prior to the recharge operation although some dilution effect and actual removal may be obtainable by aquifer filtration.

The water renovation project in Orange County, California (Cline, 1975) is based on full tertiary treatment including multiple stages of biological and physico-chemical treatment and disinfection prior to recharge in order to meet the strict State of California Health Department requirements for recharge with wastewater. Even with all of that the California Health Authorities are yet to approve of the reclaimed groundwater for domestic consumption. They will require that the safety of the reclaimed water be fully demonstrated by careful toxicological testing.

In Israel, the Dan Region Water Reclamation Project will provide full biological treatment including nitrification and denitrification as well as excess lime treatment prior to recharge of the effluent into the sand dune area south of Tel Aviv (Shuval, 1975c). The designers' original plans call for producing a reclaimed effluent withdrawn after recharged which could be used for all purposes including domestic consumption. The ~~mean~~ minimal retention time in the aquifer will be 400 days. The recharge area will be completely surrounded by a ring of recovery wells controlled and operated by a single authority. In the final stage the recovery wells will be pumping almost 100% recharged wastewater.

However, our preliminary studies have indicated that the sand dune filtration provides only partial removal of the dissolved organics.

The effluent before recharge contains about 40 ppm of T.O.C. while water withdrawn from the aquifer shows T.O.C. levels of about 10 ppm with high concentrations of A.B.S. detergents which reached the 13 ppm level. The Health Authorities in Israel have not approved this water for domestic consumption as yet and will most likely require considerable additional treatment to remove dissolved organics and any remaining traces of toxic chemicals prior to domestic use. For the moment the plan is to restrict the use of the reclaimed water to agricultural and industrial use until a procedure is developed which will provide potable water of a demonstrated safe quality.

Groundwater recharge prior to reuse for domestic consumption certainly provides many advantages and a considerable safety factor as a result of the buffering effect of long retention and groundwater dilution as well as a degree of removal of microbial and chemical pollutants. It also provides for an excellent opportunity to enable complete monitoring of water quality prior to withdrawal since observation wells between recharge areas and withdrawal wells can be used to test water quality months before it is withdrawn from the aquifer. Just such a monitoring program has been included in the Dan Region Water Reclamation Project.

The W.H.O. (1973) has pointed out in its report that "groundwater recharge involving extended periods of underground storage can provide a considerable safety factor in wastewater renovation." However, careful planning and control of such recharge programs is essential to ensure that the full benefits of such a strategy are obtained.

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SECRETARIA DE RECURSOS HIDRAULICOS
Subsecretaría de Planeación

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DIRECCION GENERAL DE USOS DEL AGUA Y PREVENCION DE LA CONTAMINACION

WATER RECLAMATION AND WASTEWATER REUSE FOR IRRIGATION OF AGRICULTURAL LANDS IN MEXICO

WATER RECLAMATION AND WASTEWATER REUSE FOR IRRIGATION OF AGRICULTURAL LANDS IN MEXICO

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I.- INTRODUCTION

Up until the begining of the sixteenth century, the Valley of Mexico was a closed basin with a relative abundance of water. Hydrologically, equilibrium was reached naturally through the increase of the lake areas during the wet years, slowly decreasing depending upon the length of the droughts. The risk of flooding has always been a problem, necessitating the construction of important projects very early in its history.

The Valley of Mexico is a watershed without a natural outlet to the sea. It used to have a great lake made up of the Zumpango, Xaltocan, - Texcoco, Xochimilco and Chalco lakes that covered an area of approxima tely 150,000 Has. During prehispanic time, one of the first flood control works to be constructed was a 16 km long dike that divided the freshwa- ter in the southwest from the brackishwater of the northeast.

Later on, after the Spanish conquest, it was decided that the solution to the flooding would be through the conveyance of the waters out of the - Valley of Mexico. This resulted in the drainage and reduction of the lake area to about 15,000 Has. In 1607 a cut was made in the mountains to a depth of 60 meters to let the Cuautitlan River out of the valley. Thus the flood problem was solved with the generation of another: that of water -- shortage.

The lack of a separate storm sewer system with sufficient capacity to remove storm runoff, led to the construction of the Gran Canal and the first tunnel of Tequisquiac towards the end of the 19th Century. This -

system safeguarded the valley from flooding during the rainy season; - however, it speeded up the drying of the lakes.

As a result of the water shortage, large quantities of groundwater have been withdrawn from the acquifer below the old lakebed. The subsidence of the soil in this area has caused the sinking of a major portion of downtown Mexico City. At the start of the century, Lake Texcoco was 3 meters below the city; while, at the present time, the lake bottom is - 3 to 4 meters above the streets.

A.- Water Availability in Mexico

It is estimated (1975) that Mexico City has a population size of - about 8.5 million inhabitants and an industrial capacity that generates 50% of the industrial production of the country. By the year 2000 it -- should have between 16 to 25 million inhabitants, depending on whether the present growth rate is maintained or if it is reduced by about 20%. The population growth problem is further complicated by the fact that - the greater metropolitan area of Mexico City has a total population of - about 11 million people.

The different sources, present and future, of water supply are analyzed in Table 1 and Figure 1, where one can note that at least 25% of the present supply comes from the acquifer directly beneath the city and another 20% from the Lerma River system. All of the nearby sources are already under utilization, adding up to about 40 m^3/s. Future growth of the city will require bringing in water from a radius of 200 km to meet the demands

TABLE 1.- Present and Future Water Supply Systems for Metropolitan Mexico City.

Systen	Q (m^3/s)	Wells (No.)	ACUEDUCTS (Km)	ELEVATION (m)
I.- Valley of Mexico - Basin				
A.- Groundwater				
1.- Urban Area*	9.0			2400
2.- Chalco*	3.0	30	40	2400
3.- Apan*	3.0	40	245	2540
4.- Cuautitlan*	1.8	40	100	2450
B.- Surface Water				
1.- San Mateo-Nopala-Nadim*	1.5		85	2600
2.- Guadalupe*	2.0		80	2600
II.- Outside Water Basins				
A.- Groundwater				
1.- Oriental*	7.0	70	465	2750
B.- Surface Water				
1.- Necaxa	20.5		250	
2.- Upper Balsas (Tenancingo)	9.0		100	2020
3.- Upper Balsas (Jojutla)	13.5		75	1544
C.- Combined				
1.- Tepeji-Cuautitlan*	2.5	40	135	2250
2.- Alto Amacuzac	16.0		180	1544
3.- Tecolutla	41.0		600	1000
4.- Lerma*	8.0			2500
5.- Alto Lerma	14.0	40	140	2700

* Systems Presently in Operation.

for the year 2000. The cost of such an enterprise makes entirely feasible wastewater reclamation practices for industrial and agricultural purposes.

The volume available would be the sewage generated within Mexico City, which amounts to about $30m^3/s$. Average values for the main water quality constituents of these wastewaters are given in Table 2. The dissolved solids, boron, MBAS and bacterial counts require special care - before these wastewaters can be indescriminately utilized for irrigation. The Metropolitan Wastewater Collection System is shown in Figure 2.

B.- Water Reuse Practices.

Water reuse is not a new topic in Mexico. Domestic wastewaters- are often utilized raw for the irrigation of farmland. This is done quite extensively throughout the country due to the fact that approximately - 77% of the territory is considered arrid or semi-arrid.

Since there is a water shortage, to a certain degree in the Valley of Mexico, the city has provided for the watering of parks and recreational areas with the disinfected effluent from three sewage treatment facilities and the irrigation of nearby agricultural areas from the effluent of two - other plants. A summary of the characteristics of these and seven other treatment plants that are located within the valley are presented in Table 3.

The first of the plants run by the Mexico City Government was built in 1955 to provide water for Chapultepec Park. Since this was the first -

TABLE 2.- Physical and Chemical Characteristics of the Mexico City Wastewaters.

Characteristic:	Concentration (mg/l)	Characteristic:	Concentration (mg/l)
Temperature (°C)	18	Heavy Metals:	
Conductivity (umho/cm)	1792	Lead	0.090
Total Solids	1590	Mercury	0.0015
Total Suspended Solids	1150	Cadmium	0.027
Total Coliform (MPN/ 100 ml)	$63x10^7$	Zinc	0.54
BOD	220	Copper	0.09
COD	500	Nickle	0.1
MBAS	8.2	Iron	2.40
Ammonia (as NH_3)	12.0	Manganese	0.17
Organic Nitrogen	6.0	Chromium (total)	0.1
Nitrate - N (as NO_3)	0.2	Potassium	50.0
Total - P (as PO_4)	23.0	Sodium	308.0
Ortho - P (as PO_4)	10.0	Boron	1.40
Chlorides	182.0		
Sulphates	147.0		
Hardness (total)	483.0		
Alkalinity (total)	433.0		
pH	7.7		

TABLE 3.- Wastewater Treatment Plants in Mexico City

N A M E	Capacity (l/s)	Type	Use of Effluent
1.- Chapultepec	160	Activated Sludge	Parks and lakes
2.- Cd. Deportiva	230	Conv.Act.Sludge	Parks and Sports Fields
3.- San Juan de Aragón	500	Conv.Act.Sludge	Parks and lakes
4.- Xochimilco	1250	Conv.Act.Sludge	Lakes and irrigation
5.- Cerro de la Estrella	2500	Conv.Act.Sludge	Lakes and irrigation
6.- Bosques de las Lomas	55	Extended aeration	Parks
7.- Acueducto Guadalupe	80	Conv.Act.Sludge	Parks and cemetery
8.- Unidad Ixtacalco	10	Act.Sludge + Nut. Rem.	Lake and irrigation
9.- San Juan Ixhuatepec	500	Conv.Act.Sludge	Industries
10.- Club Campestre	60	Biological Filters	Parks and Golf course
11.- Thermoelectric (CFE)	450	Conv.Act.Sludge	Industry
12.- Plan Texcoco	2000	Not Final	Parks, lakes and irrigation

plant of its kind in Mexico, sludge treatment facilities were provided. However, difficulties in the operation of the anaerobic digester soon - led to it being left idle and the solids (primary and excess secondary) disposed of directly in the local sewer system. The other plants built by the city, as well as those used by private enterprise, do not have any sludge disposal facilities.

Chapultepec Park covers 400 Ha with three artifical lakes; San - Juan de Aragón has an artificial lake, sports fields and a large wooded area. The wooded area in both of these parks is watered during the - dry season, with treated wastewaters from the 160 and 500 l/s treatment facilities, respectively. The sports fields of the Ciudad Deporti va, as well as gardens and small parks in the surrounding area are -- watered with the treated effluent from a 230 l/s treatment plant. There are several country and golfs clubs that reuse the effluent from small package treatment plants for watering the fairways.

Two such plants serve the Club Campestre and Club de Golf Hacien da with a capacity of 60 and 10 l/s, respectively and as all the others, provide secondary biological treatment with disinfection. As most of the other plants they take wastewaters directly from the municipal sewer -- system as their needs make it necessary, returning to it the waste solids. Some of the newer housing developments are being provided with similar facilities, primarily to use the effluent for watering parks and playgrounds. Near the Xochimilco lake and canals in the southeastern part of the city, a 1250 l/s treatment facility was installed to provide water during the dry season, so that the proper lake level could be maintained. The Canals of Xochimilco are well known for their floating gardens, where trees and plants can be obtained for the home, as well as a great many vegetables, grown in the vicinity.

In many of the recreational lakes, the large amount of nutrients - present in the treated wastewaters has given rise to nuisance growths, primarily in the way of algae and small aquatic plants. Both, Chapulte pec and Xochimilco lakes present this problem, which reaches its peak - during the hot months of the year, when mats of bluegreen algae decompose on the water surface. In the case of the treatment plant serving the Ixtacalco housing unit provisions were made for nutrient removal by --- physical-chemical means. The Ixtacalco lake has thus been maintained free of nuisance conditions during its first year of operation.

The lack of fresh water readily available for industrial growth in Me xico City has given rise to the installation of wastewater treatment facili ties in order to reuse the effluent for industrial purposes. With this in - mind, a group of 11 industries formed the Sociedad de Usuarios, Aguas de San Juan Ixhuatepec and built a treatment plant to take raw wastewaters - from the Río de los Remedios. After secondary treatment, the disinfected effluent is distributed to the industries where it might receive some form of terciary treatment in order to meet the individual requirements of each - manufacturing process.

In a similar fashion, the Thermoelectric Plant of the Valley of Mexico, treates wastewaters from the Gran Canal to reuse as cooling water. This - allowed them to use their freshwater allotment in their boilers for the ---

generation of steam. The treatment plant began operating in 1963 at a capacity of 150 l/s, which was increased to 450 l/s in 1974.

Finally, one of the newest and largest treatment plants in Mexico - City is the Cerro de la Estrella facility with a capacity for 2500 l/s. This plant was installed specifically to provide treated wastewaters for the - irrigation of vast tracts of land, that used to make up the old lake bed. It is presently providing the Tlalmac region with about 1 000 l/s for the ejidos located in this area. The rest of the plant capacity is available - for the exchange, in the future, of freshwater that is presently being - - utilized for irrigation of farmland near Mexico City and thus increase - the municipal water supply.

The future of water reuse in Mexico appears, if anything, to be -- growing in magnitud. Presently, a large portion of the Mexico City waste waters are used for irrigation of some 50,000 Ha in the Valley of Mezquital. This will probably be increased by the new trunk collector that has recently been put into operation. It is known as the Emisor Central, has a capacity of 200 m^3/s, reaches depths of up to 230 m below the ground level and takes the municipal wastewaters out to the Irrigation District No. 03 in the Mezquital. This region is extremely arid and was almost totally - sterile before it was provided with sewage for irrigation purposes.

The Lake Texcoco Plan comprises a group of experts that are studying the technical and economic feasibility of rehabilitating the old Texcoco lakebed. During the rainy season the lakebed is used to dampen

TABLE 4.- Water Consumption in the Irrigation District No. 03

FARMING CYCLE	YEARLY VOLUME (x 10^6 m^3)		
	Wastewater	Clean Water	Total
1969 - 1970	760.7	177.8	938.5
1970 - 1971	700.0	176.6	876.6
1971 - 1972	731.4	247.8	979.2
AVERAGE	730	201	931

TABLE 5.- Urban Population Within The Irrigation District No. 03.

TOWN	Sewered Population	Total Population
1.- Actopan	11,037	25,959
2.- Mixquiahuala	10,887	17,513
3.- Tula	10,720	38,685
4.- Progreso	8,694	9,959
5.- Ixmiquilpan	6,048	35,016
6.- Tlaxcoapan	5,883	10,912
7.- Tlahuelilpan	5,284	6,177
8.- Tetepango	3,337	4,339
9.- Ajacuba	3,070	8,981
10.- Tezontepec	2,762	4,930

out the rain fall runoff peaks and thus avoid flooding the downtown area; however, it soon dries out giving rise to large dust storms that are origi nated by the spring winds. This situation createsa health hazard, as -- well as a nuisance, since pathogenic organisms are easily transported - by the fine detritus particles raised by the wind.

The old Texcoco lakebed has a surface area of about 14,500 Ha, -- where a series of five recreational lakes is planned, along with parks and agricultural zones. A treatment plant with a 2000 l/s capacity will provide water for the lakes, parks and agricultural fields. The lakes will have progressively better quality water, with fishing and boating allowed in the last one of the series.

Another important project that is getting underway is the establishment of Water Reuse Districts within the city, whereby the Federal Govern ment will install treatment facilities in certain areas of the city to treate the municipal sewage and sell the treated effluent to the industries located within each region and thereby free enough fresh water for the city's inmediate population growth requirements. Along these same lines, it is thought that treated wastewaters can be exchanged for freshwater from - the farmers of the Apan, Chalco and Cuautitlan regions.

II.- WASTEWATER REUSE FOR AGRICULTURAL PURPOSES

The Ministry of Water Resources has funded a continuing research -- program to look into the development of water quality standards for the -- reclamation of wastewaters to be used in agriculture, industry and acquifer recharge. This project was initiated two years ago with a comprehensive study of an area that had been irrigated with raw sewage during the past-60 years. The area picked was the Irrigation District No. 03 located north of the Valley of Mexico in the Mezquital Valley (Figure 3) which afforded the unique opportunity of evaluating the effects of irrigating with different water qualities on different types of soil with a variety of crops being --- grown on them.

The irrigation district is run by the Ministry of Water Resources and has available a large amount of data on water use and agricultural yield. The district is feed primarily by the Tula and Salado rivers which drain a basin of about 11,000 km^2. Two reservoirs (Endho and Requena) are loca_ ted in the upper portion of the Tula River, which control the flow of fresh water into the district. The Salado River receives the Mexico City municipal wastewaters, as these exit through the Tunnels of Tequisquiac. The water available for irrigation purposes is made up of 24% freshwater from the upper Tula River and 76% wastewaters from the Salado River. This - flow insures the irrigation of about 42,000 Ha, with a remainder of about 10,000 Ha dependent upon rainfall.

Several studies were conducted to determine what risks, if any, deve_ loped to human health, soil conservation and productivity. These studies included: a) boron in soils, b) salinity, c) pathogens and d) productivity.

A.- Boron in Soils

Boron, an essential nutrient at trace concentrations can be toxic in -

varying degrees to different types of plants. A classification has been set up where plants are grouped together depending upon their tolerance level for boron, as shown in Table 6.

TABLE 6.- Boron Plant Tolerance Classification

Classification	Boron Concentration in Water (mg/l)
Highly Sensitive	0.4
Sensitive	0.4 - 1.25
Semi-tolerant	0.7 - 2.50
Tolerant	1.6 - 4.0

Soil characteristics are also important in determining whether the plant will be at the lower or upper end of each group. Soils with a saturation extract between 0.7 and 1.5 mg/l are considered marginal and anything above 1.5 mg/l is contaminated. Since the Mexico City wastewaters contain 1.4 mg/l of boron on the average, a very careful look was deemed necessary to determine if any toxic effects were apparent. Hence a series of experiments was designed to test the boron desorption capabilities of three soil types at three different depths and with four different amounts of water applications.

Soil samples were gathered from nine different locations in order to have three samples of each type of soil (clay - loam, sandy - clay - loam and clay). Each sample was separated into three different strata (0 to 20, 20 to 40 and 40 to 60 cm) obtaining about 45 kg. of soil per -

strata. The sample from each strata was then air dried and passed -- through a 0.5 cm sieve before being placed in plastic test columns 1 m tall and 10 cm diameter. The 60 cm soil depth was chosen since it relates to the maximum root penetration of the type of crops generally grown in this region.

Once the soil was placed in the columns it was saturated with water before starting the experimental runs. Four columns were prepared for each soil type to receive a different amount of water (15,30,45 and 60 cm) from the Requena Reservoir applied in increments of 2.5 cm. -- After the applied volume of water drained out of the columns the soil was removed carefully so as not to mix the three different strata. The soil - was then dried and analyzed. The boron analyses were performed on the saturation extract of the dried soil by the potentiometric method out -- lined in Standard Methods (1971).

The experiments were arranged according to a factorial design -- known as "Randomized Blocks" (Bernard, 1970). This type of experimental design was used based on the need to reduce the experimental error that may have been introduced by not packing all the soil columns in -- exactly the same manner. Three replicates were run in each case with approximately the same drainage velocity. A variance analysis was performed on the results to a significance level of 1%. Once significant - results were obtained, these were compared through a " least significant difference", defined as:

$$LSD = \tau (1 - \alpha)_{\nu} \, \delta_{\bar{y}_i - \bar{y}_j}$$

where:

$\tau(1-\alpha)_{\nu}$ = value for the Student τ at a significant level α with ν degrees of liberty.

$\delta_{\bar{y}_i \; \bar{y}_j}$ = standard error for the difference of two means.

Results

The initial boron concentrations found in the saturation extracts of the soils studied were, for the 0 to 20, 20 to 40 and 40 to 60 cm strata, respectively, as follows: a) clay-loam, 1.21, 1.66 and 1.54 ppm; b) - sandy-clay-loam, 1.60, 0.94 and 0.92 ppm; and c) clayey soil, 2.05, 2.05 and 2.3 ppm.

From the statistical analysis an evaluation was made that is summarized in the following points.

1.- Clay - Loam Soil.

a) All four applied water layers reduce significantly the initial boron concentration, independent of the three soil strata studied (Figures 5 and 8).

b) There were no significant boron removals between the 15 and 30 cm applied water layers, as well as between the 45 and 60 cm -- layers. The only significant differences are between the 45 cm layer and the two smaller layers.

c) The boron removal is significantly greater in the 0 - 20 cm stratum independent of the applied water layer.

2.- Sandy-Clay-Loam Soil.

a) The initial boron concentration is removed significantly by the applied water layers above 30 cm only in the 0 to 20 cm soil - stratum.

b) The initial boron concentration is not significantly different in - the three strata. Also, the boron concentration does not vary - significantly after the application of the 15 and 30 cm water la- yers. However, when the 45 and 60 cm water layers were appli ed, a significant increase was noted in the boron concentration of the 20 to 40 and 40 to 60 cm soil strata with respect to the - upper stratum (Figures 6 and 9).

3.- Clayey Soil.

The four water layers applied remove significantly the initial boron concentration of the 0 to 20 and 20 to 40 cm soil strata. There was no significant difference between the initial boron concentration of the 40 to 60 cm soil stratum and the concentration after the applica tion of the four water layers (Figures 7 and 10).

It is important to note that the boron concentration, as the applied water layers increase from 15 to 60 cm. decreases in the clay-loam soil in all three strata; while in the sandy-clay-loam soil, it only decreases - in the upper stratum (0 to 20 cm). In the clayey soil the boron concentra- tion decreases in the upper and middle strata, while it remains unchanged in the lower stratum.

The soils used in this study can be considered dangerous to sensi tive plants, since the intial boron concentrations tended to exceed the 1.5 ppm maximum limit for marginal soils (Richards, 1954).

The data obtained so far seem to indicate that the boron removal - capacity for each applied water layer is dependent upon the soil texture and the depth of the soil stratum to be washed. It is easier to remove - boron from the upper stratum since the concentration gradient between - the water and soil is greater, decreasing as the water passes from one stratum to another.

B.- Salinity

The Mexico City sewage falls within the C3-S2 classification, - corresponding to highly saline water with a medium content of sodium. It is not suitable for application to soils with a poor drainability. -- When applied to soils with good drainability salinity should be contro-lled with the aid of chemicals or organic matter (Samplón 1968). It -- should not be used on fine textured soils and, when used on rough tex tured soils, the plants should be of varieties highly tolerant of salts.

Since the texture of the soils studied was fine and the salt con-centration in the saturation extract low (1150 ppm), it is thought that the high content of organic matter (125 mg/l of BOD) in the water is - helping to lessen the salinity effects. This situation seems to be res ponsible for the good growth of salt sensitive crops, such as beans,- raddish, celery and orchards. They do not appear to be affected by salt contents of from 0 to 2500 ppm.

C.- Pathogens

The potential risk to human health from the irrigation of vegetables with municipal wastewaters is in the transport of pathogenic organisms - to the plant surface from where it can gain access to the human body if - the vegetables are eaten raw. However, the survival period outside the intestinal tract for the pathogenic microorganisms of fecal origen is from 3 to 72 hrs and from 9 to 30 days for amoebic cysts. Hence, there are - certain conditions which can be met in order to safeguard against this -- type of infection.

The overall objectives of this part of the research program are to determine what these conditions are and how they can be met.

It is not certain as yet, which is the predominant mechanism for fecal infection. Several mechanisms are under consideration, including: - a) irrigation with raw sewage, b) unsanitary produce handling practices and c) contamination of the water supply system. Of the aforementioned, it appears that unsanitary produce handling practices contribute to a great extent in the transmission of fecal pathogens. A recent study (Ordoñez, 1974) of the major Mexico City markets showed that most produce arrives at the market in a relatively uncontaminated condition; however, it becomes contaminated by the handling that it receives within the market. A survey was conducted, at the same time, of typical households and it was shown that a large increase in the number of fecal bacteria takes place — within the home. It was established that up to 70% of the increase in fecal organisms was attributable to poor handling practices.

Preliminary results of some of the work that is going on now indicate that produce irrigated with raw sewage will contain on the order of 3 organisms per 10 grams of vegetable (MPN of fecal coliform). Further research will determine survival times after each application of sewage, as well as the feasibility of the penetration of plant tissues by the patho genic organisms.

This work will establish what vegetables can and cannot be grown with sewage and to what degree must the sewage be treated before applica tion to the fields, as well as what length of time must be allowed to trans pire before harvesting.

D.- Productivity

At first it was throught that the Mexico City sewage might contain substances that could be toxic to the crops, specifically: salinity and - boron. However, although the salt and boron content is high, a difference could not be seen between the productivity of plots watered with raw --- sewage and those watered with clean water. It seems that most of the - crops grown in the irrigation district are semi-tolerant of salinity and -- boron, except for beans and alphalpha that are considered tolerant. It is also significant that soils within a semi-arid zone give yields comparable to those of fertile zones (86 Ton/Ha of alphalpha). This is quite feasible if one consideres that these soils have been under irrigation with sewage for more than 60 years; during which time the organic matter in the waste waters has been able to condition the soils, as well as the accumulation of nutrients, which to a great extent precludes the use of fertilizers. - Table 6 presents the yearly contribution of nitrogen, phosphorus and -- potassium to the irrigation district from the municipal wastewaters. The clean waters are somewhat deficient in nutrients and require the use of fertilizers wherever they are the only source of water. Table 7 illustra tes the nutrient requirements of some of the most widely grown crops in the study area.

The differences between mean productivities for each crop with -- different types of irrigation water were subjected to a null hypothesis - with a significance level of 1% to reject the hypothesis. The results of this analysis did not reject the hypothesis in any one of the cases stu- died, thereby concluding that the observed differences in productivity for alphalpha, barley, oats, tomato and corn are not significant.

III.- CONCLUSIONS

A summary of the preliminary findings of this study is given in the form of the following conclusions.

1.- Within Mexico City, at the present time, 20% of the municipal -- wastewaters generated are already being reused for watering parks and lakes. Since industry uses up about 28% of the water supply, the percentaje of water reuse can be easily increased to about 50%, as is the case in the city of Monterrey.

2.- One of the limiting factors for the urban and industrial growth of - the Valley of Mexico is the availability of water. The acquifers -

TABLE 7.- Nutrient Input to the Irrigation District No. 03

Type of Water	Yearly Nutrient Input + (Kg/Ha - yr)		
	N	$P-PO_4$	K
CLEAN WATER	42	42	208
COMBINED WATER	303	199	810
SEWAGE	320	490	913

TABLE 8.- Nutrient Requirements By Different Crops

CROP	Optimum N : P : K Relationship (Kg/Ha-yr)		
	N :	P - PO_4 :	K
Alphalpha	20	200	300
Wheat	420	39	24
Corn	145	221	95
Barley	70	47	34

below the old lakebed are being mined, even through it has been established that such a practice will accelerate the sinking of - the downtown area.

3.- For the past ten years all possible water supply sources have been under study by the authorities. All these sources combined could provide 126 m^3/s of water to the city; however, they have not -- been deemed the most economical solution, since water is still- withdrawn from the lakebed acquifer.

4.- For the type of soils present in the Irrigation District No. 03, dangerous concentrations of boron can be avoided by applying water layers of 30 cm for crops with roots under 20 cm, and water layers of 60 cm for crops with roots under 60 cm.

5.- The available data indicates, that for the case of the Irrigation District No. 03, the use of raw sewage has not presented any ill effects on productivity from the high salt content of the water nor from a - bacteriological stand point. However, the health effects of irrigation with raw wastewaters have not been determined.

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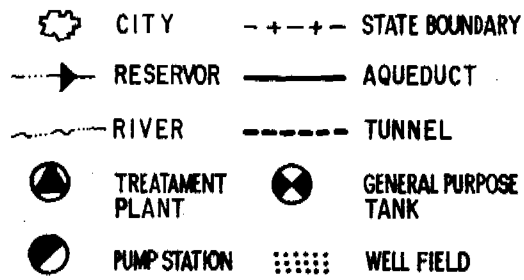
FIGURE 1.-SOURCES OF WATER SUPPLY TO MEXICO CITY

LOCATION

SYMBOLS



SOURCES UNDER STUDY

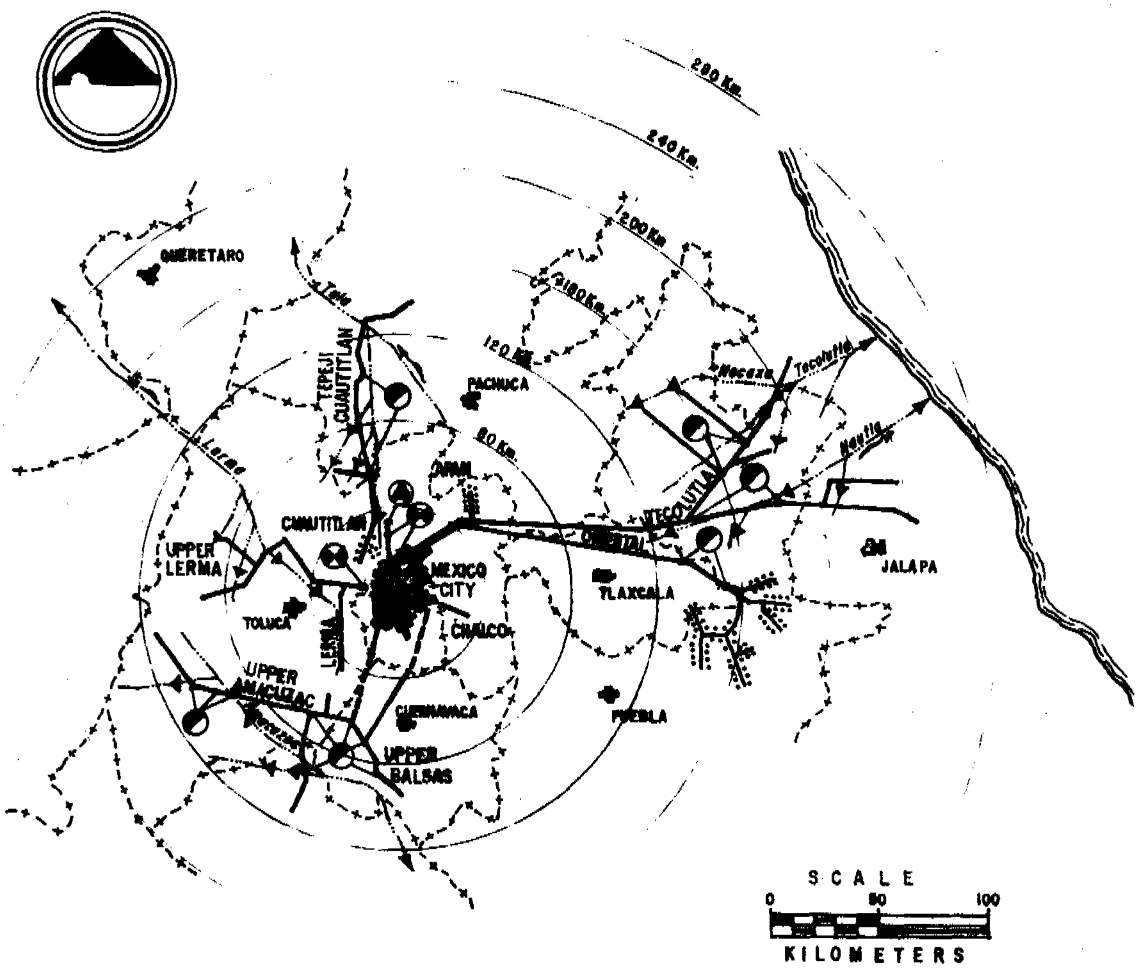


FIGURE 2.- WASTEWATER COLLECTION SYSTEMS IN MEXICO CITY

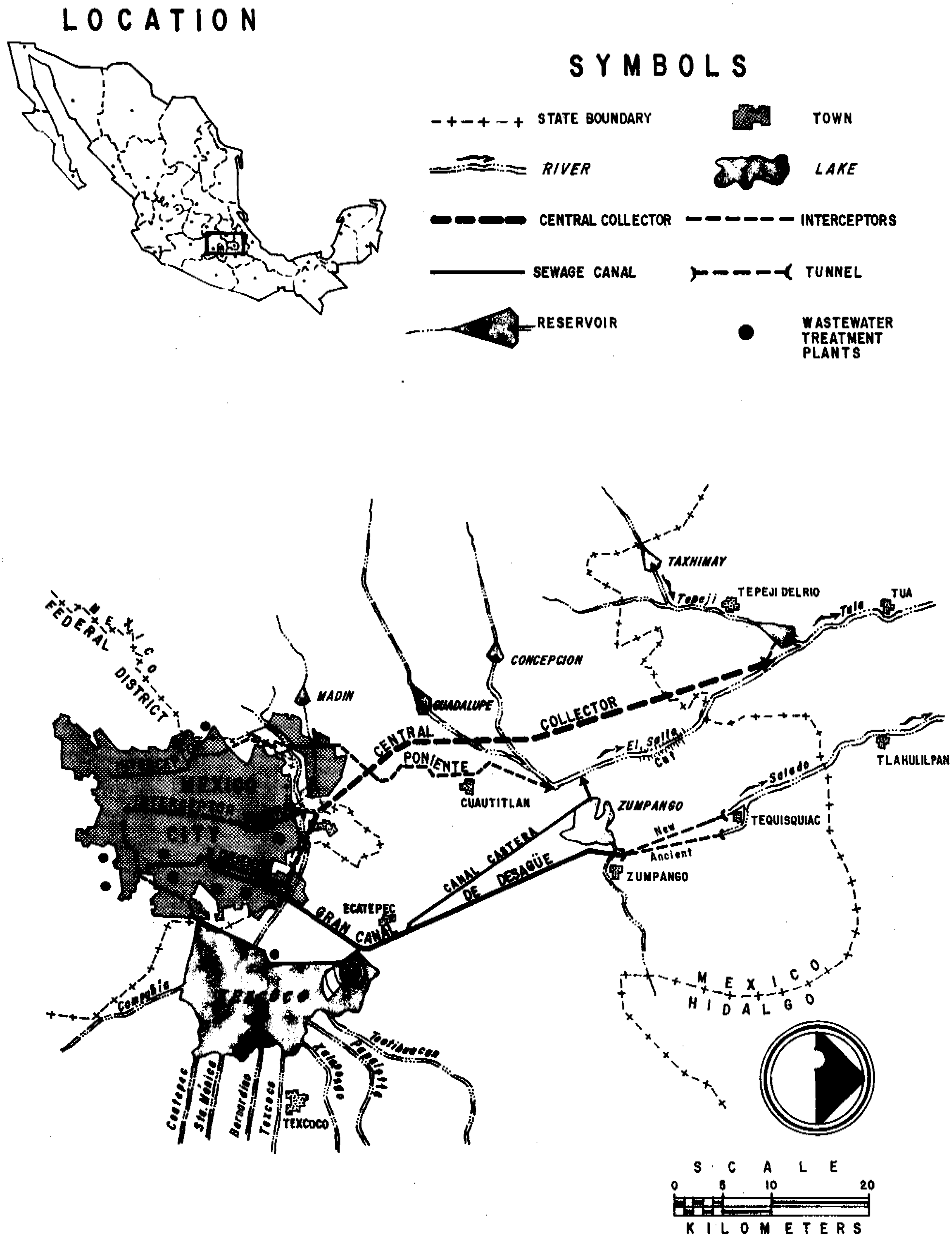
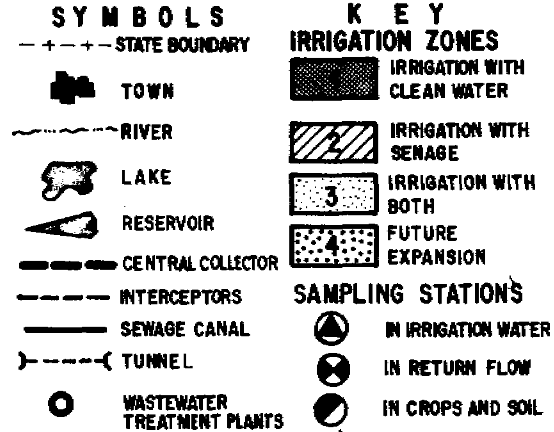


FIGURE 3.– IRRIGATION DISTRICT 03, TULA, HIDALGO

URBAN POPULATION IN THE STUDY AREA OF THE IRRIGATION DISTRICT Nº 03

TOWN	POPULATION WITH WATER SUPPLY AND SEWERAGE	TOTAL POPULATION
ACTOPAN	11 037	25 959
MIXQUIAHUALA	10 887	17 513
TULA	10 720	38 685
PROGRESO	8 694	9 959
IXMIQUILPAN	6 048	35 016
TLAXCOAPAN	5 883	10 912
TLAHUELILPAN	5 284	6 177
TETEPANGO	3 337	4 339
AJACUBA	3 070	8 981
TEZONTEPEC	2 762	4 930



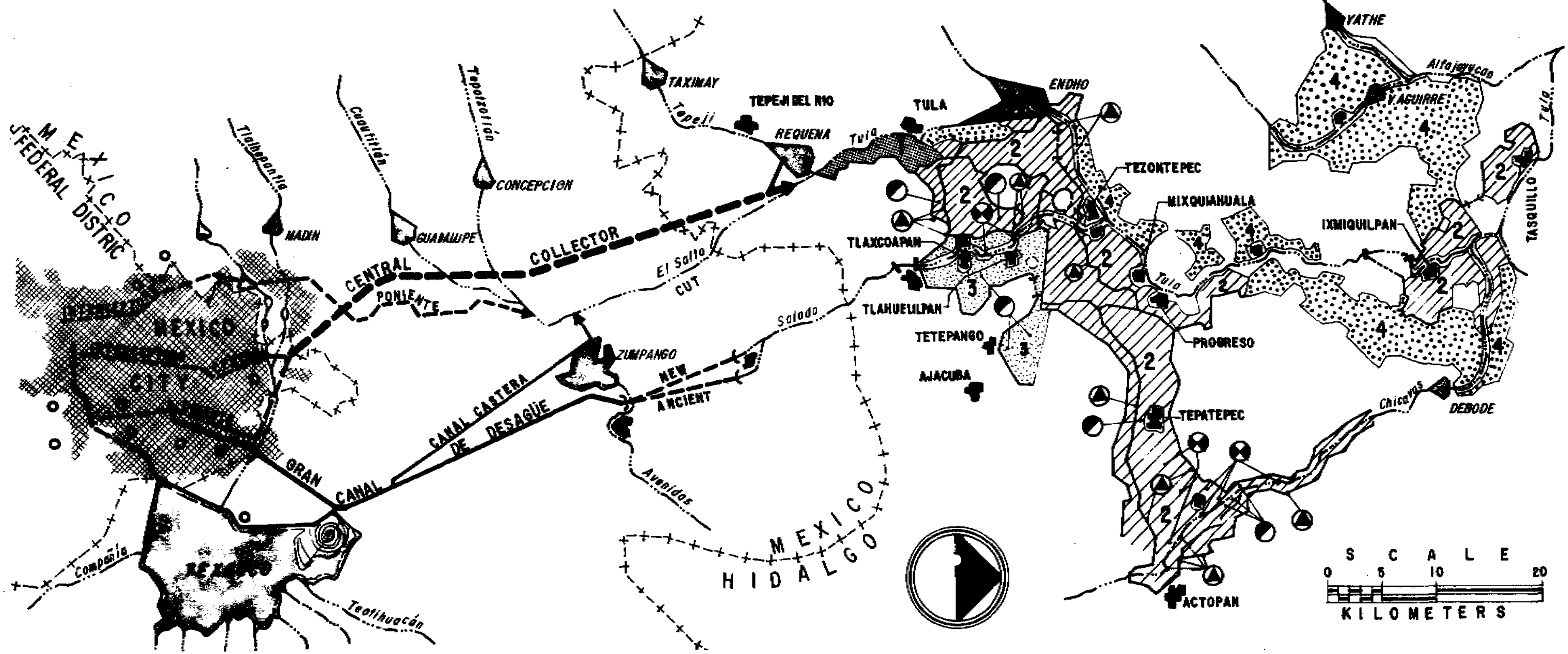


FIGURE 4.- MODEL COLUMN FOR BORON DESORPTION STUDIES

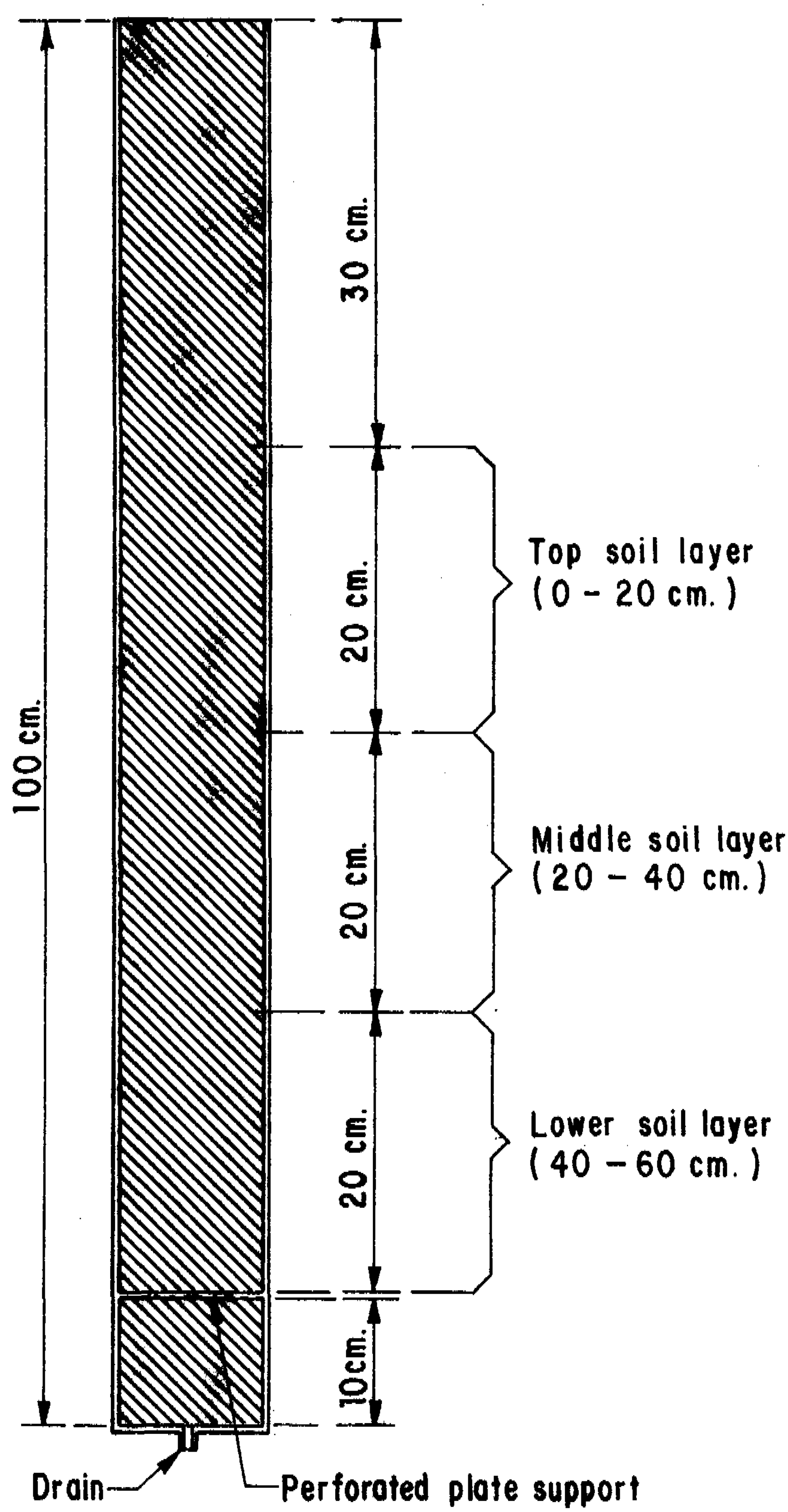


FIGURE 5.- BORON DESORPTION FOR CLAY-LOAM SOIL

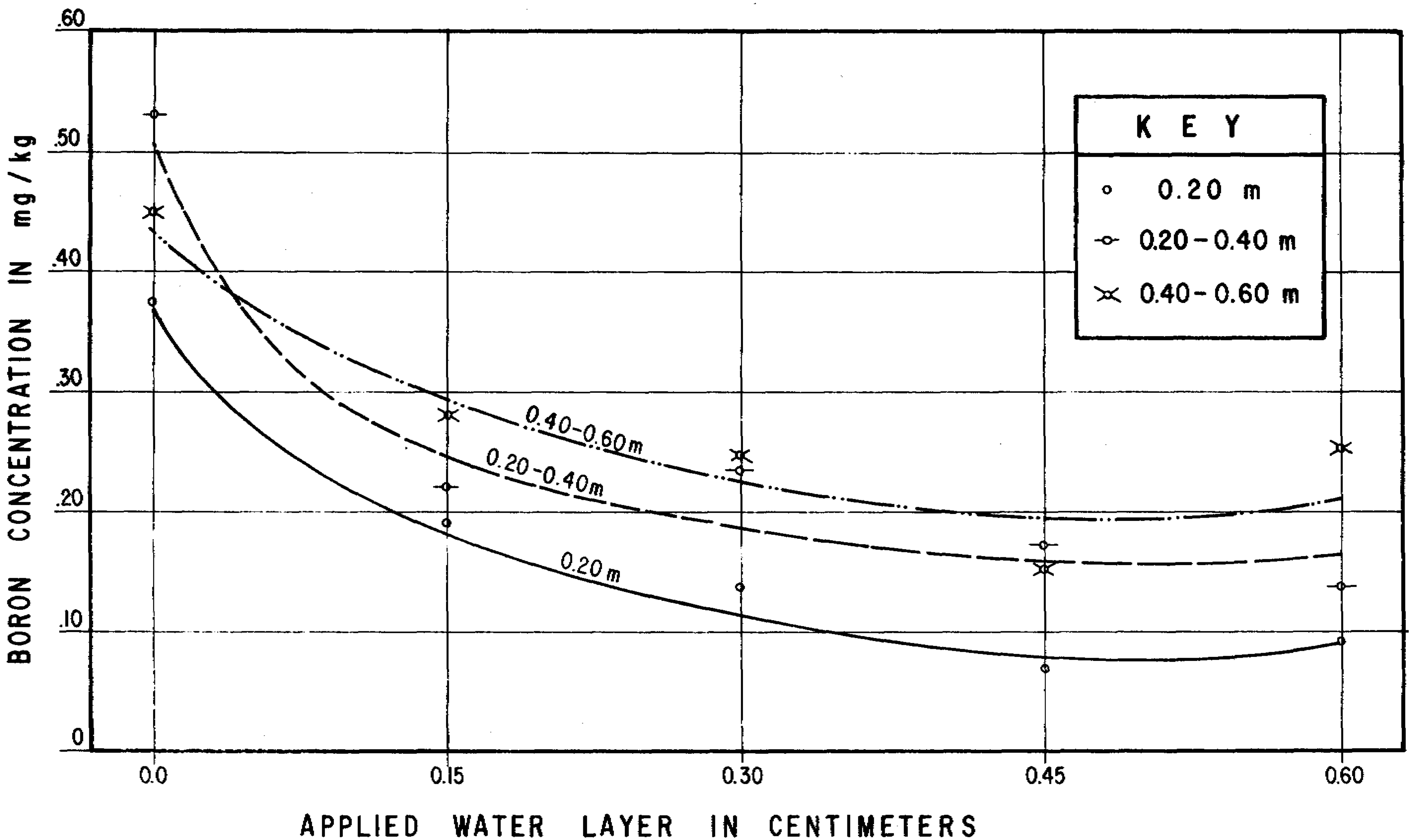


FIGURE 6.- BORON DESORPTION FOR SANDY-CLAY-LOAM SOIL.

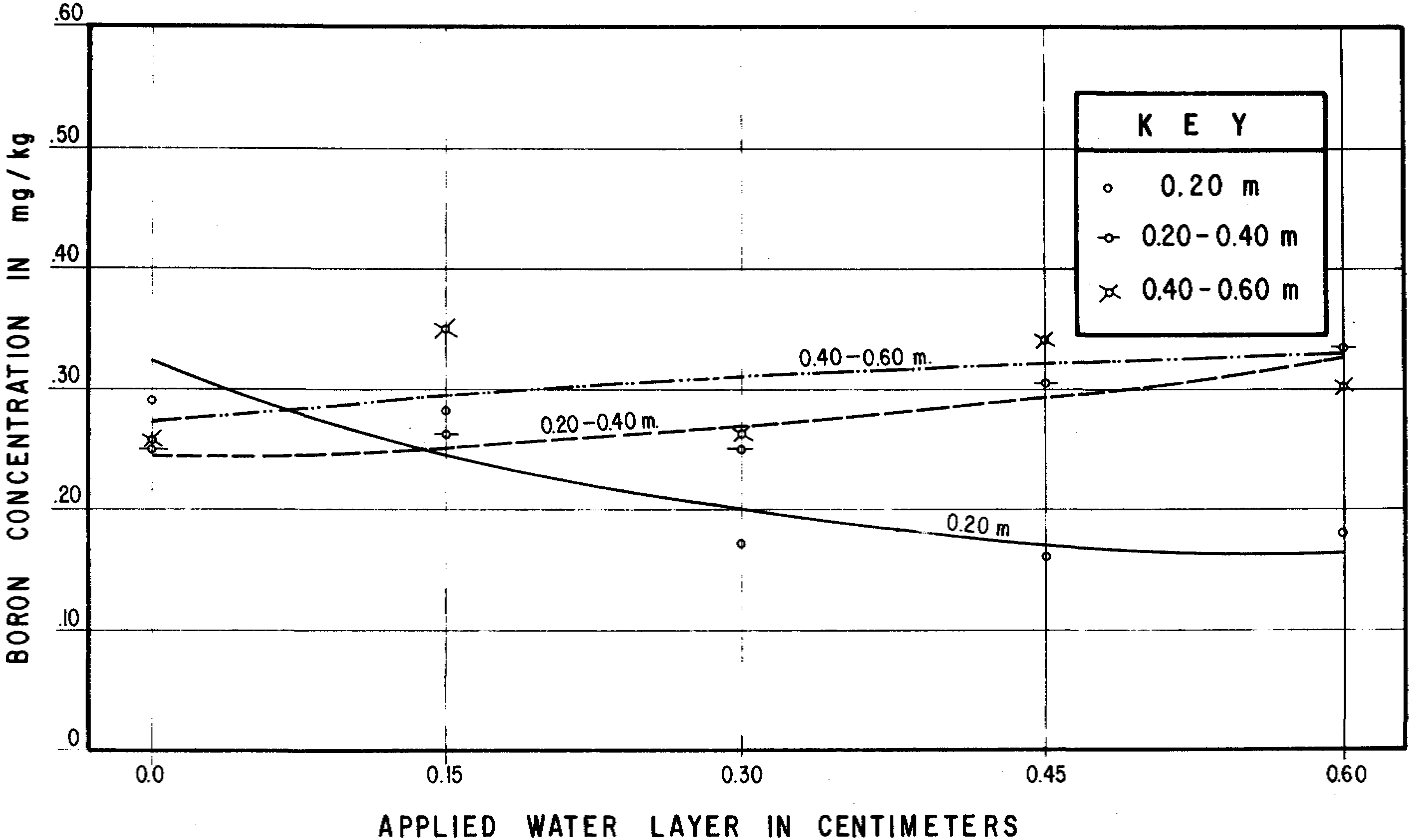


FIGURE 7.- BORON DESORPTION FOR CLAYEY SOIL.

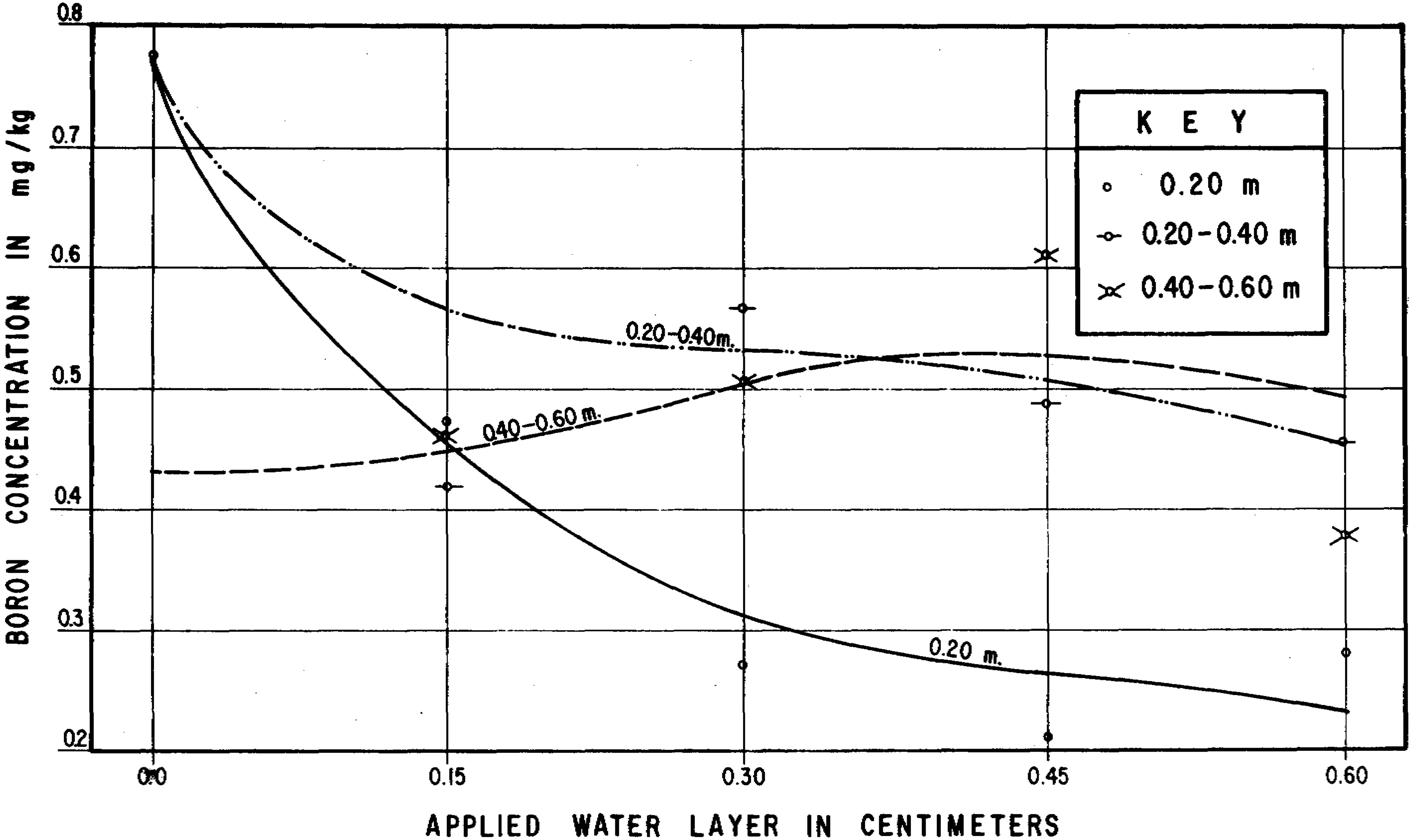


FIGURE 8.- BORON CONCENTRATION IN THE SOIL STRATA FOR THE CLAY-LOAM SOIL.

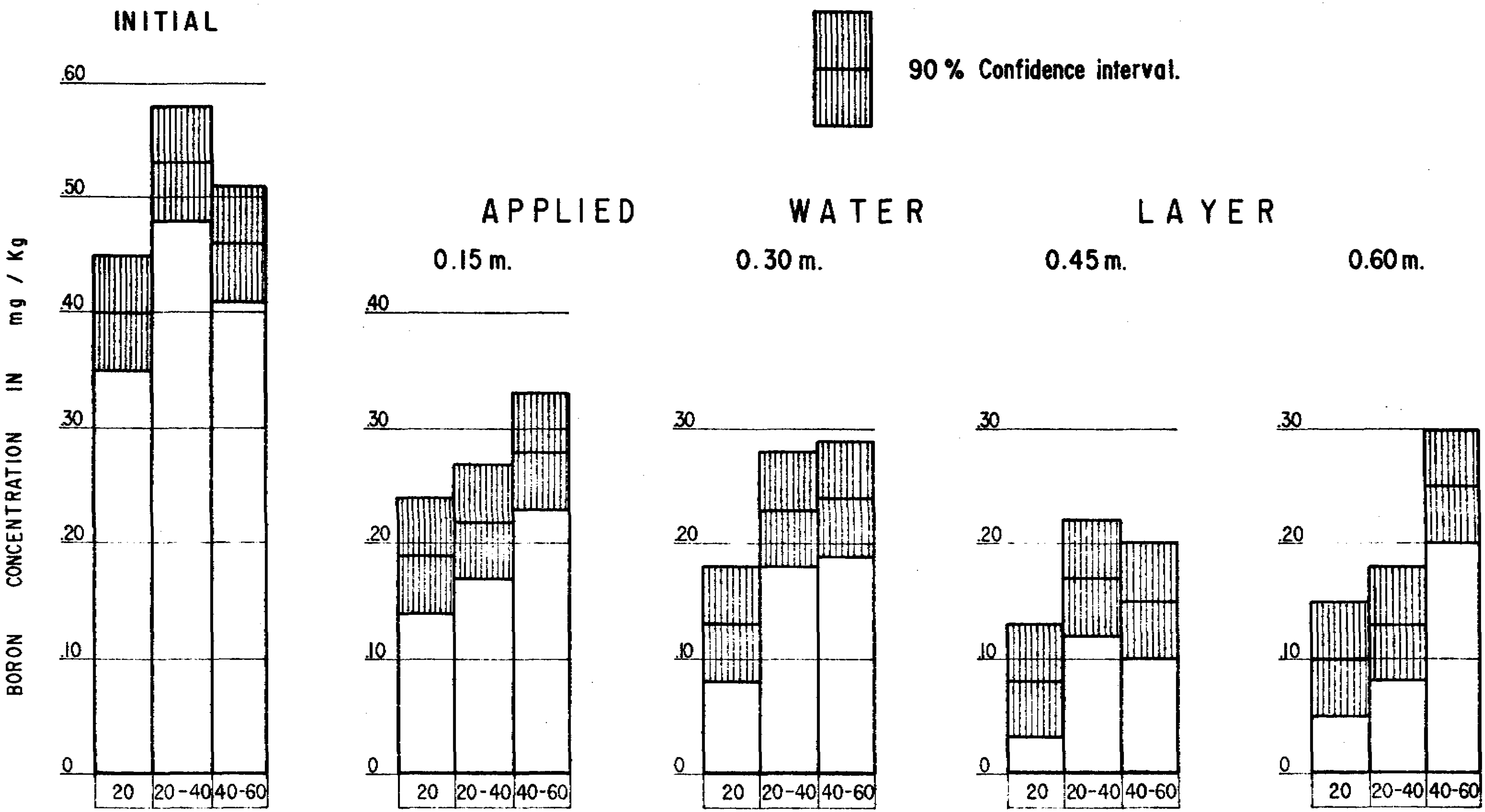


FIGURE 9.- BORON CONCENTRATION IN THE SOIL STRATA FOR THE SANDY-CLAY-LOAM SOIL

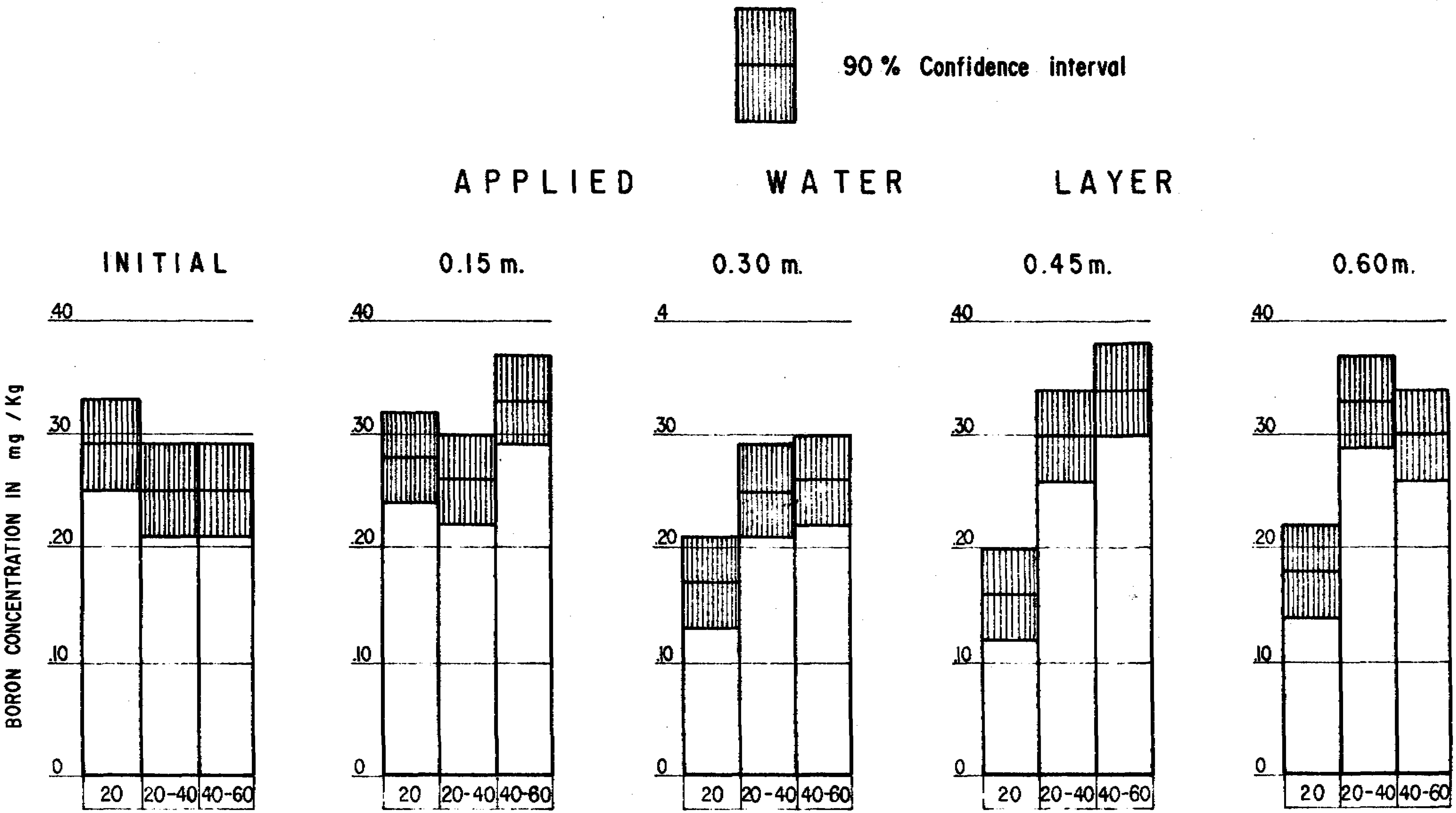
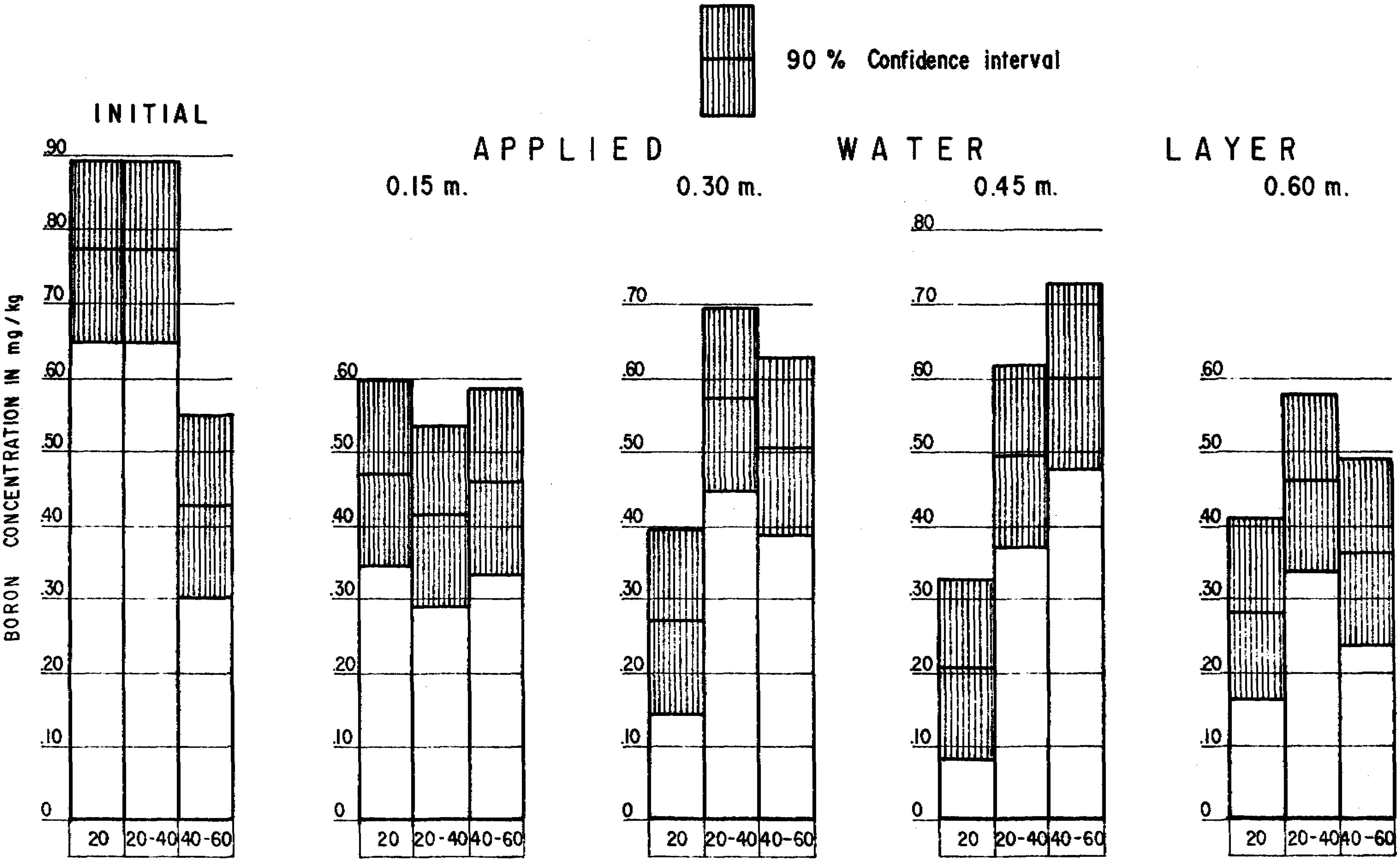


FIGURE 10.- BORON CONCENTRATION IN THE SOIL STRATA FOR THE CLAYEY-SOIL



THE DEBRECEN SEWAGE RESEARCH FARM PROJECT

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INTRODUCTION

The idea of utilizing wastewaters is as old as urban water supply and canalization. In each of the ancient cultures there are signs of attempts to utilize sewages in the agriculture by irrigation.

This practice has a centuries long past in many European countries. Following the renewed attempts in the second half of the 19th century, the use of sewages for irrigation has also been started in Hungary. The primary purpose of this sewage irrigation has been to utilize sewages collected by urban sewerage systems. In most places meadows and pastures have been irrigated, but soon - in the surroundings of some towns - also the irrigation of horticultural plants started for the best possible utilization of the yield-increasing effect of wastewaters. The irrigation of plants for direct human consumption with untreated domestic sewage involved health hazards /infections/ in some places and therefore irrigation of horticultural plants with sewages has been prohibited by the sanitary authorities.

Later the sewage irrigation farms were constructed to serve also the purpose of wastewater disposal. A common

~~failor~~ FAILURE of these first plants was overloading, which resulted in the neglection of the points of utilization and the agricultural demands were not satisfied either. After the second world war the rapid industrial development and urbanization increased the amount of sewages. In this situation the question of sewage irrigation has been raised again. The up-to-date sewage irrigation techniques naturally should eliminate the former deficiencies and provide satisfactory solution for both disposal and utilization of wastewaters. According to our directives a primary /mechanical/ treatment is always compulsory, but under special conditions a secondary /biological/ treatment is also necessary before wastewater irrigation. The application of this method could be especially useful in the case of towns, situated in a definitely agricultural surrounding and far from water-courses, or having no appropriate recipients at all.

<u>Debrecen</u>, the third largest town in Hungary, with more than 2oo ooo inhabitants, is far from the great rivers and it's sewage treatment problems aren't solved. The daily produced sewage is about 5o ooo m^3 which is led - after a simple and unsatisfactory primary treatment /sedimentation/ - by a small local water-course towards the Körös catchment area. The town has an agricultural surrounding with a definite demand on irrigation, but there isn't enough water for irrigation. In consequence of these the utilization of the sewages had already been

planed formerly, and in the future the same efforts will be made, after a required pretreatment of the sewages. Our first experimental sewage farm was established in 1959 just in Debrecen for the promotion of these efforts.

DESCRIPTION OF THE RESEARCH FARM

The Debrecen Sewage Research Farm is situated in the neighbourhood of the temporary sewage treatment plant of the town, on a total area of 14,37 ha. The type of it's soil is a meadow-chernozem created on a loessial-sand subsoil; according to the physical properties of the soil it is loamy-sand /adobial-sand/, with a rather good permeability. Chemically it has a hydrocarbonatic character, it is a calciferous soil with a medium natural nutrient content. The soil exploration shows that the surface layer is heteroganeous, it's quality varies spot by spot, because the experimental plant is situated on the boundary of two different areas: the loessial land of the Debrecen territory and the sandy zone of the Nyir-county. According to this the soil of the research farm represents well the soil of the surroundings, where the high-scale use and disposal of the Debrecen sewages is planed.

The meteorological data show that the many years' average number of sunny hours is over 2ooo hours/year and it was steadily as high during the time of the experiments, which was favourable both for the effectivity of crop production and for the disinfection of irrigated sewage. The many years' average of temperature is 1o,2 °C and the yearly precipitation is 599 mm. During the research period these data were more variable than the data of sunny hours, but all their combinations occur during the examined years. The direction of the prevailing winds is N-NE, the effect of which was considerably reduced by the forest belt fencing the research station.

(F. 1) The general layout of the research farm can be seen on Figure 1. The plant is bordered from East and from West by the branches of the local water-course, called Tocó, and the main sewage channel crosses the farm in direction N-SW. On the greater part /84 % - ~~XXXXX~~ 11,2o ha/ of the arable land forage and technical crops were cultivated, on the other part /16 % - 2,14 ha/ the disposal lands /filter-fields, special forests/ and the forest belts were situated.

The irrigation was carried out both with surface /furrow and streaming/ and sprinkling irrigation methods. Owing to the sewage disposal demand a continuous, whole-year watersupply was realized from the begining of 1963. Yearly 12o ooo m^3, dayly 33o-4oo m^3 sewages - less than 1 % of the total amount of the Debrecen sewages - were applied for a constant irrigation partly on the arable land, partly on the disposal lands. Considering the whole-year-round irrigation, estimating the results we took all the used sewage into account, which was given out on the parcels from the last harvest till the next one.

In 1967 an artificially introduced pasture was settled on the one part of the arable land /4,8 ha/ and from this time grazing investigations have been started with young cattles /heifers/ as well.

Because of the heterogeneous soil-condition and the many channels and diches crossing through and through the research farm, it wasn't possible to do there any correct, comparing experiments with small parcels; it was only possible to carry out experiments with middle-size parcels in two repetition, without a statistical evaluation of the results.

The used sewage was collected in the town Debrecen by a separated sewer-system. The total amount of the daily sewage conteins at about 3o-35 % industrial wastes and also artesian hot water coming from deep wells. Before using the sewage flows through the plain and hardly over-loaded sedimentation tanks of the temporary treatment plant. After the unsatisfactory mechanical /primary/ treatment the quality of the water is till like a sewage, and it's quality components exceed the literary average values of municipal wastes /Table I/.

T. I

The total organic content /399,o - 1789,6 mg/l/ is sutable high from the view-point of utilization; the total nitrogen content varies between 42 and 81 mg/l, the potassium content between 42 and 63 mg/l, and the phosphorus content between o,7 - 7,o mg/l. The total dissolved material fluctuates between loll and 1285 mg/l, the sodium-procent /Na %*/ between 36 and 61 %, the magnesium-procent /Mg %[xx]/ between 21 and 46. As the figures show among the plant-nutrient elements only the phosphorus isn't enough, therefor 350 kg/ha super-phosphate fertilizer was given under the row crops, twice during the examined years. Besides neither any other artificial fertilizers, nor organical dungs were used on the experimental farm during the research period.

AIMS OF THE RESEARCHWORK

At the Debrecen Sewage Research Farm regular and complex investigations have been performed, which include all the connected professions /water management, agronomy, water quality, soil research, public and veterinary hygiene, etc/. The aims of the researchwork can be summarised as follows:

- investigation of the urban sewage quality,
- determination of the agrotechnical, animal husbandrial and hygienical effects of continuous, constant irrigation with urban sewages,
- investigation of the possibilities of a whole-yearlong, continuous sewage-irrigation,
- determination of sewage-doses, by the application of which an economical crop production is possible,
- getting basical data for the development of natio-

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[x] $Na\ \% = \frac{Na\ mg.equ./l}{/Na+K+Ca+Mg/\ mg.equ./l} \cdot 100$ [xx] $Mg\ \% = \frac{Mg\ mg.equ./l}{/Mg+Ca/\ mg.equ./l} \cdot 100$

nal prescriptions and for planing of sewage-irrigation.

Research workers of other institutions take part in the investigations carried out on the experimental farm under the control and coordination of the Research Institute for Water Resources Development /VITUKI/.

RESULTS

The given extent of my paper doesn't make possible for me to analyse all the results of the 15-years-long work of the research farm in details. I will sum up the most important determinations as follows and I will show some of the numerical results on the tables.

T. II

1. The results of the experimental station proved unambiguously that the sewages of Debrecen can be used for irrigation without any harmful effect. With most of the plants irrigated with sewage - adding only phosphorus as fertilizer - a significant excess yield was obtained compared to the yield averages in the neighbourhood without irrigation /Table II/. The many years' average yields of corn, maize for silage and lucerne surpassed by 6o-15o % the non-irrigated yields of the surroundings. The best return is made in general by plants the vegetative parts of which are presenting the useful products. It is, namely, the green parts of the plants that grow better on the effect of sewage-irrigation. With corn and other grain crops, however, also the grain produce increased on the effect of sewage-irri-

T. III

gation /Table III/.

2. Based on results obtained so far it can be stated that generally a water-cover of 300-600 mm/year in average can be applied for the irrigation of fodder- and industrial plants on medium adobe /loamy sand/ soils in Hungery. This water quantity in a year-round distribution doesn't cause any over-irrigation damages, and at the same time provides for the plants a suitable water- and nutrient supply to be complemented only with chemical fertilizers containing certain nutrients /for example phosphorus/.
3. On filter fields and forests with border levees serving for continuous year-round wastewater disposal only water depths below 3000 mm/year can be applied to produce an appropriate filtering effect. The best results were given by poplar forests planted on fields with border levees.
4. The poplars irrigated with sewage grow fast and strongly. According to calculations the period for felling the poplars irrigated with sewage decreased to one half or third the non-irrigated ones. Experiments under way have shown that of the varieties the giant poplar /Populus robusta/ developed best, but the black poplar /P. nigra/ was also growing quickly. It is easy to estimate the fertilizing effect of irrigation with sewage, and the cleaning effect of the soil as well on the timber-trees of the poplar forest with border levees; the dia-

meters and heights of the stems of the trees in the rows near the irrigation furrows - and in the upper third of the rows - exceeded those in the rows near the intersepting diches to which - due to the soil's filtrating effect and settling - less nutrients were transported.

T. IV

Table IV shows the results of measurements of the trees in the third and seventh year after their plantation.

5. The soil analyses carried out for eight years showed that the humus, nitrogen and potassium content of the soil - in spite of the large plant production - increased comparing to the initial state. Only the phosphorus content decreased significantly during these years, but it was balanced by the addition of chemical fertilizers. It is worthy of note that although the total dissolved matter and sodium content of the sewage was high, the year-round irrigation for eight years didn't cause any significant alkalinization of the soil in the irrigation field. No alkalinization was manifest in either increase of salt content of the soil, or the sodification of the adsorption complex. According to the soil explorations about 6o % of the exchangeable kations is calcium, 25 % is magnesium, only 2-7 % is sodium, and this ratio hasn't altered during the 8-years-long irrigation.

6. Bacteriological investigations showed that the pollution grade of sewage-irrigated plants is the highest within three days after irrigation. At the 1o-14 days after irrigation the plants didn't show any differences com-

pared to the control, and only on the surface of the soil was more polluted in the irrigated field. During the whole period of two-weeks-long observation the soil of the surface irrigated fields was more polluted than those irrigated by sprinkling. The last conclusion of the investigations was that the Debrecen sewage can be used for irrigation on fodder- and industrial plants, if the last irrigation is finished 2-4 weeks before harvesting. From the view-point of public health the least dangerous way of disposing of both untreated and only mechanically treated sewages is the irrigation of trees and special forests.

7. Animal health protection experiments showed that there were no pathogens impairing animals demonstrable in fodder samples from sewage-irrigated fields after the required expectation time. The dried fodder plants /hays/ contained less bacteria - due to the effect of sunshine and dry air - than the green ones. Experimental results showed that the health of animals wasn't impaired even if they were fed with fodder exceptionable for number of non-pathogen saprophytes or fungi.
8. Our investigations carried out for fullfilling the requirements of continuous sewage disposal showed that it wasn't so much the weather as certain problems of organization and differences of attitude as well as agrotechnical causes /e.g. cultivation, harvesting/ that prevented adhering to the rules of sewage distribution

providing for the continuity of irrigation. The investigations also showed that with a proper attitude and some practice good progress can be made within one year in the practice of the exact execution of water distribution rules and the continuous disposal of a given amount of water.

9. Favourable results were reached by the grazing investigations carried out on an artificially introduced pasture distributed in 6-6 sections, irrigated by surface and sprinkling irrigation methods. On the pasture two groups of young cattles /heifers/ were kept in the same age and weight, with the help of an 'electrical shepherd'. During the experimental period the animals were allowed only to graze without any additional fodder. Despite of this the increase of weight and meat production was significant on the pastures /Table V/. At the end of the experimental period the muscles, organs, bones and connective tissues of compulsory slaughtered animals were similar to those of traditionally kept ones. Only animals free of infectious parasitic diseases were kept on the experimental pastures. During the experimental period no infectious or parasitic diseases or other infections occured in the closed herd under periodic veterinarian controls. After the examined period every tuberculin-test proved to be negative. 2o-21 days after irrigation with sewage microorganism pathogenic for animals wasn't found in bacteriological investigations

What are 6-6 sections

T.V

of grass and hay samples of the pastures grazed and irrigated in sections. Great number of non-pathogenic bacteria was observed in the half of the samples, but in spite of this such grass or hay caused no gastroenteric disease. Under animal health control cattle breeding - even without additional fodder - proved economic from May until October on sewage-irrigated pasture. The many-sided evaluation of the grazing investigations is in progress, on the result of which will be prepared the grazing technology of wastewater-irrigated pastures.

For completion of the results of the Debrecen Sewage Research Farm two other experimental plants were established: one in 1963 at a suburb of Budapest /Pesterzsébet/ and another in 1972 at Kecskemét. At the latter place started to work in 1973 the first up-to-date and high-scale sewage farm as well, for the disposal and utilization of the total sewage of the town.

Based on our results and operational experiences the design and establishment of further wastewater irrigation plants are in process. Design aids and directives composed recently on the basis of experimental results will make possible and facilitate these goals.

SUMMARY

In this paper - after a short introduction - a brief information is given about the soil- and weather conditions and the scheme of the Debrecen Sewage Research Farm /Fig.I/, about the irrigation methods applied, the water-consumption, the quality of sewage used /Table I/ and the aims of the researchwork. Results of the complex experiments over the last 15 years are discussed in detail: yields /Table II and III/, average water-cover, loading of filter-fields and disposal lands, results of sewage-irrigated poplar-plantation /Table IV/, soil-, bacteriological- and veterinar hygienical investigations, observations of continuous sewage disposal and results of the grazing experiment on sewage-irrigated pasture /Table V/.

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TABLE I

CHARACTERISTIC QUALITY DATA OF THE DEBRECEN SEWAGE

Examined components		Limit values
pH		7,8 - 8,1
Alkalinity W°		1o,4 - 14,o
Total hardness in German degree		29,12 - 39,2o
Carbonate hardness	- " -	2o,16 - 31,36
Calcium	mg/l	1o8,21 - 148,29
Magnesium	- " -	21,87 - 46,17
Sodium	- " -	185,oo - 3oo,oo
Potassium	- " -	42,oo - 63,oo
Iron		trace
Chloride	- " -	212,76 - 319,14
Sulfate	- " -	169,oo - 289,o5
Hydrocarbonate	- " -	634,5o - 854,1o
Phosphate	- " -	o,7 - 6,9
Ammonia	- " -	16,5 - 55,o
Nitrite	- " -	o,3 - 6,3
Nitrate	- " -	o,6 - 2,5
Oxygen consumption	- " -	14o,o - 5o9,4
BOD_5	- " -	76,2 - 2o4,7
Total dry matter	- " -	119o,o - 1937,1
Total dissolved matter	- " -	1o11,o - 1285,o
Total suspended matter	- " -	1o9,o - 261,o
Total organics	- " -	399,o - 1789,6
Dissolved organics	- " -	257,o - 8o2,o
Suspended organics	- " -	54,o - 449,o
Total minerals	- " -	923,o - 1o84,o
Dissolved minerals	- " -	765,o - 1o53,o
Suspended minerals	- " -	28,o - 124,o
Total nitrogen	- " -	42,5 - 81,2
Na %		35,8 - 61,2
Mg %		21,o - 46,1

TABLE II

COMPARISON OF THE SEWAGE-IRRIGATED AND NON-IRRIGATED YIELDS

/DEBRECEN, HUNGARY/

Plant	Examined year or period	Average sewage depth mm	Type of produce	Irrigated average yield /kg/ha/	Non-irrigated average yield /kg/ha/
Sugar beet	1961-67	392	root	36 324	36 300
Corn	1961-66	368	grain	5 850	2 249
Maize for silage	1961-67	290	green	34 619	21 725
Lucerne 1-3 years old	1961-66	399	hay	9 925	3 658
Sunflower	1965	490	grain	1 487	417
Potato /industrial/	1965	275	tuber	11 980	5 973

TABLE III

AVERAGE YIELDS OF PLANTS IRRIGATED WITH URBAN SEWAGE AND THE AVERAGE SEWAGE QUANTITIES USED

/DEBRECEN, HUNGARY/

Plant	Examined year or period	Type of produce	Average yield kg/ha	Average sewage depth mm
Sugar beet	1961-67	root	36 324	392
Corn	1961-66	grain	5 85o	368
Maize for silage	1961-67	green	34 619	29o
Lucerne, 1-year-old	1961-64	hay	9 o72	577
Lucerne, 2-years-old	1963-65	hay	12 o29	3o9
Lucerne, 3-years-old	1964-66	hay	8 5o7	115
Lucerne, 1-3 y.-old	1961-66	hay	9 925	399
Red clower	1966	hay	12 337	598
Alexandrian clower	1966	hay	9 143	2o1
Grass-papil. mix.	1961	hay	11.15o	3o8
Sudangrass /main sowing/	1962-66	green	29 268	254
Sudangrass /after seed/	1963-65	green	15 184	83
Vetch with barley	1963-65	green	15 236	248
Vetch with oats	1962	green	19 8o8	91
Fodder peas	1963	grain	2 991	268
Broom-corn /brown/	1964-66	grain	2 493	267
Spring vetch /intercultural/	1967	grain	662	38
White mustard /intercultural/	1967	grain	245	38
Sunflower	1965	grain	1 487	49o
Squash /for seed/	1966-67	seed	316	281
Castor-bean	1963-64	seed	1 92o	93o
Solanum	1963-64	green	16 469	777
Digitalis	1967	green	1 637	281
Hemp	1961	stalk	8 377	34o
Potato /industrial/	1965	tuber	11 98o	275

TABLE IV

COMPARISON OF THE TREES BEING BESIDE THE IRRIGATION FURROWS AND THE INTERSEPTING DICHES IN A FOREST WITH BORDER LEVEES IRRIGATED WITH URBAN SEWAGE

/DEBRECEN, HUNGARY/

Year	Difference in favour of the trees being beside the irrigation furrows	
	average diameter cm	average height m
1964	0	0
1967	1,7	1,o
1971	3,5	1,3

TABLE V

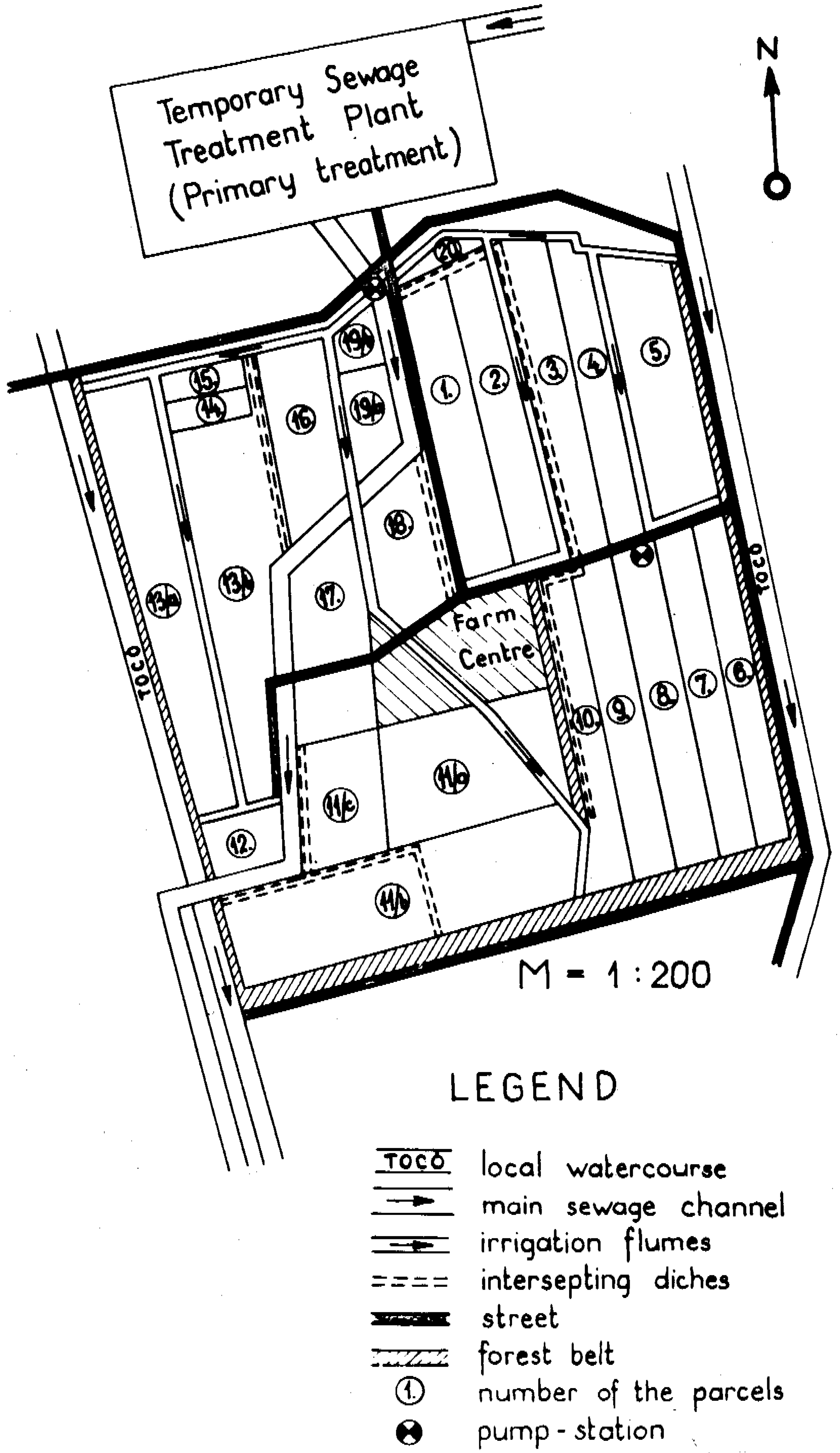
SEWAGE QUANTITIES USED AND THE MOST IMPORTANT PRODUCTION DATA ON THE EXPERIMENTAL SEWAGE-IRRIGATED PASTURE FOR YOUNG CATTLES /DEBRECEN, HUNGARY/

No.	Naming	1969		1970		1971		1972	
		I+	II+	I	II	I	II	I	II
1.	Pasture field, ha	2,4	2,4	2,4	2,4	2,4	2,4	2,4	2,4
2.	Average sewage depth, mm	545	428	305	193	530	386	861	558
3.	Average grass yield /green/, q/ha+	528	523	591	544	824	818	951	961
4.	Pasture period, days	176	176	182	182	179	179	175	175
5.	Number of animals, piece	16	16	28	28	20	20	20	20
6.	Average increase of weight per animal, kg/piece	120	122	85	89	101	116	102	106
7.	Average meat production, kg/ha	800	813	991	1038	841	966	850	883

+Note: I = surface irrigated pasture
II = sprinkling irrigated pasture
1 q/ha = 100 kg/ha

FIGURE I

THE DEBRECEN SEWAGE RESEARCH FARM



NITRIFICATION AND DENITRIFICATION IN SOILS RECEIVING MUNICIPAL WASTEWATER

by

F. E. Broadbent and D. Pal

INTRODUCTION

Most of the soluble nitrogen in municipal wastewater applied to land is in the form of ammonium or nitrate ions. Of these two, ammonium usually predominates even though the sewage treatment may have included one or more aerobic stages. Some organic nitrogen is also usually present, of which a small part is soluble and readily convertible to ammonium since it is easily attacked and mineralized by a large number of micro-organisms. The greater part of the organic nitrogen is likely to be associated with the particulate fraction and is somewhat lower on the scale of availability, but will gradually be converted to ammonium over a period of time. When ammonium nitrogen comes into contact with the soil, it is quickly adsorbed on cation exchange sites of the soil colloids near the surface. In soils which have clay minerals of the expanding lattice type, that is, those which tend to swell when wet and to shrink upon drying, ammonium ions may be trapped within the crystal lattice in a relatively inaccessible condition and are said to be fixed. This fixed ammonium is not easily utilized either by roots of growing plants or by nitrifying bacteria for conversion into nitrate. Exchangeable ammonium, on the other hand, may be displaced from the exchange complex by other cations in the wastewater such as calcium, magnesium, and sodium and subsequently through activities of nitrifying bacteria in soil.

NITRIFICATION

Nitrifiers are abundantly present in almost all soils and they have the capability of remaining active over a wide range of moisture and temperature conditions. Although these bacteria are considered to be obligate aerobes, they have been shown to function at oxygen concentrations substantially lower than that of the atmosphere. Their activities may be inhibited in acid soils and may cease altogether in locations where the pH is 4-4.5 or below. Nitrifying bacteria are autotrophic and require no organic matter as a source of energy since they derive their energy from oxidation of ammonium or nitrite. It appears, however, that their activities are stimulated by trace amounts of certain organic substances. They do require several inorganic ions in order to function properly including micronutrients. Occasionally, wastewater treatment may have resulted in removal of certain essential micronutrients, the lack of which tends to retard the nitrification process in the sewage treatment plant, but after application to soil there is seldom, if ever, a deficiency of essential nutrients for nitrification.

Although several intermediates occur in the oxidation of ammonium to nitrate, these do not normally accumulate in soils, although there may be temporary accumulation during the wastewater treatment process. The reactions may be written as shown below:

$$NH_4^+ + 2\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+$$

$$NO_2^- + 1/2\ O_2 \rightarrow NO_3^-$$

The first of these reactions is carried out by bacteria of the genera *Nitrosomonas* and *Nitrosococcus*, and the second by *Nitrobacter* species.

DENITRIFICATION

In most soils nitrate is not adsorbed and moves readily in the soil solution. If large quantities of wastewater are applied to land, nitrate will move downward and may eventually reach the groundwater. However, whether nitrate is formed from input ammonium or is initially present in the wastewater, it is subject to transformations which may prevent at least some of it from moving downward in undiminished concentration. Of these other transformations, uptake by plant roots is important and advantageous in the overall nitrogen economy in that a valuable nutrient can be utilized for the production of crops. This aspect takes on increased significance if we consider also the energy savings realized when nitrogen is recycled and the requirement for synthetic fertilizer nitrogen decreased. Another important transformation of nitrate is that of denitrification. If nitrate is initially present in the wastewater denitrification may occur immediately, provided environmental conditions are favorable for this process. If, however, nitrogen in the wastewater is predominantly in the ammonium or organic form as is often the case, then nitrification must precede denitrification. Denitrifying bacteria, like the nitrifiers, are abundant in most soils, but they differ from nitrifying bacteria in several respects.

Whereas nitrifying bacteria are obligate aerobes, denitrifiers can utilize nitrate as a terminal electron acceptor in the absence of gaseous oxygen. Denitrification normally does not take place in the presence of significant concentrations of dissolved oxygen. This condition may not be so restrictive as might at first appear when one considers the microenvironment in which many soil organisms live. This is the thin moisture film surrounding soil particles, some of which are very small in size. The habitat of the denitrifying bacteria may be in what can be termed a micropore in which the immediate environment is much different from that of the macropores where water and soil gases can move more freely. If, in the microenvironment of the bacteria, oxygen is consumed more rapidly than it is diffusing to the microsite, then a deficiency of oxygen will develop and the environment becomes an anaerobic one. It is quite possible to have in the same soil fully aerobic conditions in the macropores where nitrification is proceeding if ammonium is available as substrate, while a short distance away in the micropores oxygen is deficient and denitrification is taking place.

In wastewater application, denitrification is often desirable because this process has the capacity to convert nitrate to an innocuous gas which is a normal atmospheric constituent and which is readily volatilized from the soil. In this sense, denitrification is an ideal decontamination process. In another sense, denitrification is undesirable in that it represents the loss of a valuable nutrient with the additional energy loss implications referred to previously.

The denitrification reaction may be written

$$C_6H_{12}O_6 + 4\ NO_3^- \rightarrow 6\ CO_2 + 6\ H_2O + 2\ N_2$$

where glucose is used as an example of a readily decomposible organic substance which can be used as a source of energy by denitrifying bacteria. The necessary organic matter may be already present in the soil, may be carried in the wastewater, or may be produced by the roots of plants growing on the soil. Organic matter indigenous to the soil tends to be present in greater amounts in soil horizons near the surface and typically decreases with increasing depth. Since deep soil layers often contain little organic matter, denitrification may occur only at insignificant rates even though the soil is saturated with water.

NITRIFICATION IN WASTEWATER PRIOR TO APPLICATION ON LAND

One of the peculiarities of the nitrifying bacteria is that the nitrite oxidizers, in particular, are sensitive to ammonium ion and even more so to free ammonia, with the result that in aqueous systems there is frequently an accumulation of nitrite until the ammonium concentration has become very low, at which point the nitrite oxidizers find conditions more to their liking and increase in activity. The typical course of nitrification in municipal wastewater during aeration is shown in Figure 1. First there is a lag phase of a few days before nitrite appears in any concentration, then a period of nitrite accumulation and finally, after ammonium concentration has reached low values, nitrate begins to appear and eventually most of the inorganic nitrogen is converted to the nitrate form.

Figure 1 →

This pattern is altered somewhat when wastewaters come into contact with soil. Ammonium and free ammonia are adsorbed on cation retention sites of the organic and inorganic soil colloids so that the concentration of these species in solution becomes quite low. Consequently their inhibitory effect on *Nitrobacter* is eliminated and nitrite rarely accumulates.

EFFECT OF ENVIRONMENTAL FACTORS ON NITRIFICATION

The influence of environmental variables on nitrification in soils has been studied very thoroughly over a long period of time and will not be reviewed in detail here. Of the variables likely to be limiting in wastewater application, temperature is important because of the desirability in many situations of disposing of wastewater on land throughout the year. The optimum temperature for nitrification usually is within the range between 25 and 35°C depending on the location. There is some evidence that nitrifying bacteria are able to adapt themselves to the climatic circumstances in which they occur (Mahendrappa et al, 1966). Rates of nitrification are retarded by decreasing the temperature, but measureable oxidation of ammonium and nitrite has been reported as low as 21°C (Frederick, 1956).

The pH optimum for nitrifying bacteria usually is in the neutral to slightly alkaline range. Under acid conditions activity falls off quite sharply. The well known stimulating effect of calcium carbonate on nitrification is probably due largely to maintenance of the soil pH within an optimum range during the process, which generates some acid. The pH of municipal wastewater usually is in the range which is optimum for nitrification and during nitrification out of soil, wastewater typically becomes more alkaline by one or more pH units. This is illustrated by the data of Table 1 which shows pH changes during aerobic incubation of five municipal wastewaters. Even the atypical wastewater from the Sunkist plant which had an initial pH of 4.65 increased more than three full pH units during 31 days.

Table 1 →

When wastewaters are applied to soil the pH of the soil is likely to dominate the situation, because soils are normally much more buffered than are wastewaters. However, with prolonged application of wastewater the tendency is for acidic soils to be made neutral or even alkaline as has been shown in the application of wastewater on extremely acidic soil (Sopper, 1973). In some instances where the pH is initially too low even to permit the growth of plants, application of wastewater not only increases the pH but contributes nutrients to the soil which makes growth of plants possible.

Mineral Nutrition

Nitrifying bacteria require among other elements, magnesium, potassium, phosphorus, sulfur, and iron. Molybdenum and copper have been shown to stimulate the activity of Nitrobacter (Kiesow, 1962). Of these essential nutrients, one which is likely to become deficient in anaerobic wastewater treatment is iron because of its precipitation as the sulfide or its chelation by organic components of sludge resulting in an effluent which is very deficient in iron. In particular, the oxidation of nitrite is sensitive to iron deficiency. Aleem and Alexander (1960) reported the tripling of nitrite oxidation rate by addition of only 7 ppb of iron in solution culture. In wastewater nitrifying activity responds to substantially higher levels of iron. For example, Figure 2 shows nitrite oxidation curves of a wastewater in which there was a response up to 75 ppm iron added as ferric chloride. The role of iron in the reaction appears to be associated with the cytochrome electron transport system.

Figure 2 →

Another change which accompanies nitrification in wastewaters is alteration in the bicarbonate concentration and the buffering capacity of the wastewater. Figure 3 shows titration curves of two municipal wastewaters initially and after 10 days of aerobic incubation. It is clear that the buffer capacity of the wastewater has decreased substantially. These changes, together with the associated increase in pH, suggest precipitation of phosphates and decomposition of organic acids, leaving the associated cations such as sodium remaining in solution, in addition to a decrease in bicarbonate.

Figure 3 →

Effect of Size of Nitrifying Population

Although most soils are abundantly supplied with bacteria capable of carrying out nitrification, there may be some soils to which wastewater is applied where the nitrifying population is initially small. This may occur in situations where soil has been subjected to prolonged periods of drying or where topsoil has been removed and the subsoil has a low bacterial count. In such cases the effect of application of ammonium containing wastewater is a temporary accumulation of ammonium ion near the soil surface during a period when nitrifying bacteria are proliferating. The result is a lag period of a few days or a few weeks before nitrate is formed in appreciable concentration, followed by a wave of nitrate substantially higher in concentration than the ammonium concentration of the input sewage, owing to the influence of the soil colloids in accumulating ammonium in a localized region of the soil. This phenomenon is illustrated in Figure 4 showing results from an experiment in which sewage containing 41 ppm ammonium nitrogen was applied to a calcareous subsoil at weekly intervals in amounts of 4.5 cm per week. Nitrate concen-

Figure 4 →

trations in the effluent from the soil columns were initially essentially zero in the columns receiving ammonium nitrogen, but after a period of about 5 weeks nitrate began to increase very rapidly, reaching a concentration in excess of 100 ppm, but later subsiding to a value of about 50 ppm which was slightly above that of the input wastewater. The occurrence of some denitrification shortly after application of wastewater undoubtedly had an influence on the initially low nitrate concentrations observed in this experiment as will be discussed later.

EFFECT OF ENVIRONMENTAL VARIABLES ON DENITRIFICATION

The rate and extent of denitrification during application of wastewater on land are likely to be governed in large measure by the quantity of available substrate or organic matter and the supply of oxygen in the microsites in which denitrifying bacteria function. Wastewaters typically contain some dissolved organic matter, but normally are applied after having been stabilized, with a consequence that the BOD is relatively low. On this account, the organic matter status of the soil to which the water is applied is likely to be more important than that of the wastewater itself. Woldendorp et al (1966) have pointed out the important role that the roots of growing plants may play in relation to denitrification rates. This role is a two-fold one in which substrate may be contributed through root secretions or sloughed off root tissue. Plants may also play a secondary role by utilizing available oxygen and increasing the concentration of carbon dioxide in the soil, thereby favoring the activities of the denitrifiers.

Oxygen concentration is inversely related to the moisture content of soil and the degree to which denitrification occurs will be altered to a substantial degree by the manner in which wastewater is applied. Continuous application of wastewater resulting in a saturated soil condition may initially tend to favor the denitrification process, but if continued for prolonged periods nitrification of the input ammonium may be severely inhibited, with the result that denitrification is also of minor significance. The relative balance between nitrification and denitrification can be modified by management of wastewater application. For example, if wastewater is applied intermittently rather than continuously, cycles which are alternately aerobic and anaerobic can develop in the soil. During the aerobic cycle between periods of wastewater application, soil pores tend to fill with air and nitrification can take place. After the nitrate is produced, if the soil is then flooded to eliminate oxygen and to provide conditions favorable for denitrification, a considerable part of the nitrate may disappear through denitrification.

This is illustrated in the work of Lance and Whisler (1972) who found that short cycles of flooding soil columns with secondary sewage effluent caused no net removal of nitrogen but transformed almost all the nitrogen to nitrate. With longer cycles during which the soils were flooded from 9-23 days and then allowed to dry for 5 days net nitrogen removal from the system was 30%.

Measurement of Denitrification Rates

In the application of wastewater to soil it usually can be safely assumed that all of the input ammonium will eventually be nitrified. However, it is very difficult to make any general statements about the extent to which denitrification will occur. Some of the reasons are obvious. Considerable difficulties attend even the experimental measurement of denitrification in soil to which wastewater has been applied. Some of these difficulties are illustrated in an experiment with a California soil, Solado fine sandy loam. This soil is noncalcareous at the surface, having a pH of 6.5, and in the surface 15 cm contains 1.14% organic carbon and .1% total nitrogen. The subsoil is calcareous and contains only .54% organic carbon and .034% total nitrogen. Wastewater which had received an addition of sufficient ^{15}N-labeled ammonium chloride to bring the ammonium concentration to 3 meq per liter was applied to 1 meter columns of the Solado surface soil and subsoil at the rate of 4.5 cm per week. The wastewater was applied in a single weekly application, after which the columns were allowed to drain freely so that the soils were unsaturated much of the time. Effluents from these columns were analyzed weekly or biweekly over a period of 42 weeks. Most of the nitrogen in the effluent was present as nitrate but ammonium nitrogen was also analyzed and the isotopic composition of the combined inorganic

Figure 5 →

forms determined in a mass spectrometer to permit calculation of the quantity of effluent nitrogen which was derived from the input wastewater. A plot of input vs. output of nitrogen during the period of wastewater application is shown in Figure 5. This curve has several interesting features. For a considerable period there was essentially no output of nitrogen from the column although the input continued on a weekly basis. This lasted for 9 or 10 weeks corresponding to an input of 54 cm of wastewater or about 30% of the total input. After this initial period, output and input were related in linear fashion with the regression line having a slope of 1.31. In other words, the quantity of nitrogen, mostly nitrate in the effluent, was 31% higher than the ammonium concentration of the input wastewater. The obvious explanation is that mineralization of organic nitrogen in the soil contributed significantly to the nitrate concentration of the effluent. If one makes the reasonable assumption that some denitrification occurred in the column, the impossibility of measuring the contribution of wastewater nitrogen to nitrate in the effluent based simply on the input and output measurements of nitrogen becomes clear. The plot of input vs. output of labeled nitrogen in this experiment, also shown in Figure 5, provides an interesting comparison with that for total inorganic N. Again, there was a lag of some weeks before nitrate appeared in the effluent in significant amounts, and before the relationship between input and output became linear 15 weeks had elapsed, corresponding to a wastewater input of 76 cm, or about 40% of total input. The slope of the line was 0.88. This curve indicates that during the first several weeks of wastewater application nearly quantitative removal of nitrate through denitrification took place, but after depletion of available carbon in the soil an equilibrium situation was established with about 12% of the input nitrogen being lost. After taking into consideration the labeled nitrogen remaining in the soil columns after 42 weeks, including exchangeable ammonium, nitrate in the soil solution, and the immobilized nitrogen in the organic fraction an overall recovery of the input labeled nitrogen of 68% was obtained.

Figure 6 →

An interesting comparison with the data of Figure 5 is afforded by the data presented in Figure 6 showing corresponding values for columns of the calcareous Solado subsoil, which contained much lower quantities of organic matter. Here it will be noted that a plot of input vs. output N had a shorter time lag before the linear relationship became established and the slope of the line in this case was 0.94 compared to 1.31 for the surface soil. This indicates very little contribution of nitrogen to the column effluent from mineralization or organic nitrogen in the soil. The ^{15}N data plotted in Figure 6 are virtually identical with those for the total inorganic N, with a similar slope, 0.92, indicating about 8% loss through denitrification. The shorter time lag for the emergence of nitrate from the column after the first application of wastewater and the steeper slope of the regression line both reflect the limited amounts of available organic carbon in the subsoil. A mass balance including labeled N remaining in the soil at the end of the experiment showed an overall recovery of labeled N of 87%, compared to 68% in the surface soil.

The magnitude of denitrification illustrated in the data cited probably is relatively low in comparison to what might be achieved by deliberately managing wastewater input in such a way as to maximize removal of nitrate. In the foregoing experiments the soil was maintained in an aerobic condition most of the time and the input level of ammonium was somewhat higher than would be found in many wastewaters. Even under these conditions, which are relatively unfavorable for the denitrification process, a substantial increase in the fraction of nitrate removed through denitrifcation can be achieved in soils having a higher level of available organic matter, as is illustrated in Figure 7 showing data from Panoche sandy loam. This experiment was performed in much the same

Figure 7 →

fashion as previously described for the Solado soils but the input level of ammonium in the wastewater applied to the Panoche soil was only 1.17 meq per liter. The application rate was 4.5 cm per week over a period of 24 weeks. The plots of output vs. input shown in Figure 7 divide into two straight line segments, with an abrupt change in slope at about 70% of the total input. The striking feature of these data is the small magnitude of the slopes, indicating on the basis of the tracer data virtually complete removal of input N through denitrification during input of 148 cm of wastewater. It may be noted that the ratio of output/input for chloride in the Panoche sandy loam was 1.01, showing that there was no holdup in the column which might have affected figures for denitrification. These data suggest that at low input concentrations which do not exceed the denitrification capacity of a soil most of the nitrate in wastewater may be removed over a long period of time. The inaccuracy of using ratios of nitrate to chloride in the column effluent as an index of denitrification in this situation would be very large, whereas in a case such as that illustrated in Figure 6 such ratios could be used with a considerable degree of confidence. In a field situation samples of soil solution for the purpose of measuring NO_3^-/Cl^- ratios could be obtained without disturbing the soil by use of previously placed suction probes. Neither nitrate nor chloride is adsorbed to any significant extent by the ceramic probes used for these devices.

A mass balance for labeled nitrogen in the experiment with Panoche sandy loam showed 19.3% overall recovery of ^{15}N after taking into consideration the quantity of labeled nitrogen residual in the soil. After a long period of wastewater application it is conceivable that the supply of available energy material in the soil would be sufficiently depleted so that nitrate losses of this magnitude might not be maintained. Long-term field experiments are needed to establish whether the BOD of the input sewage itself is sufficient to maintain a high denitrification rate in wastewater having relatively low input ammonium levels.

Secondary Effects of Nitrification and Denitrification

In the conversion of ammonium to nitrate, two hydrogen ions are produced for each ammonium ion oxidized. Similarly one hydrogen ion is produced for each organic nitrogen converted to nitrate. This formation of acid by nitrifying bacteria has resulted in some concern that soils receiving a constant input of wastewater containing ammonium salts and some organic nitrogen would become more acid. The potential result of this acidification process might be the increased leaching of calcium and magnesium, thereby contributing to the hardness of groundwater. While most attention has been centered on the nitrification process it is important to keep in mind that denitrification may have the opposite effect of diminishing soil acidity. The denitrification reaction may be written in the following way:

$$H^+ + NO_3^- \rightarrow 1/2\ N_2 + 1/2\ H_2O + 5/4\ O_2$$

It may be seen that one hydrogen ion is neutralized for each nitrate ion reduced. Consequently, the relative magnitudes of the nitrification and denitrification processes will have an important bearing on whether soil acidification occurs as a result of wastewater application.

Another important consideration is the composition of the wastewater itself, as was illustrated in Table 1 which showed that the pH of wastewater typically increases under conditions favoring nitrification. In this connection it is of interest to consider the report of Kardos and Sopper (1973) on soil properties in areas receiving wastewater at rates of either 2.5 or 5 cm per week over a period of 6 years. In a corn rotation area, there were no significant effects on exchangeable calcium, but exchangeable sodium and magnesium both increased over the control as a result of wastewater treatment. In addition, the soil pH, which was initially about 5.8 in the surface 30 cm and more acidic at lower depths, increased down to a depth of 120 cm as a result of wastewater application.

Figure 8 →

Some data bearing on the question of leaching of calcium and other bases as a result of the nitrification process during wastewater application are presented in Figure 8. These data were obtained in an experiment with columns of Solado sandy loam to which synthetic sewage containing all the nitrogen either in the ammonium or the nitrate form was applied at the rate of 4.5 cm per week for 41 weeks. The composition of the input wastewater was based on an average of effluents from five different sewage treatment plants with the exception that only inorganic substances were included. Where nitrogen was present in the ammonium form, an equivalent amount of chloride was added and where nitrogen was solely present as nitrate the balancing cation was potassium. Otherwise, the two synthetic wastewaters were identical in composition. It is clear that where the nitrogen was present in the ammonium form there was an increase in calcium concentration but the magnitude of this increase was not major in terms of the total quantity of ions leached. Presumably the differences would be smaller if the concentrations of input ammonium were lower than the 3 meq used in this experiment, and in fact other experiments with other soils at lower ammonium levels have shown equal or higher quantities of calcium in the leachate from soils where input nitrogen was solely in the nitrate form.

Soil Properties After Prolonged Wastewater Application

If nitrification of ammonium in input wastewater has a significant effect in acidifying soil, these influences should be clearly evident in soils which have received wastewaters over a long period of time. Soils from one such site were sampled near the city of Bakersfield, California, where municipal wastewater has been used for irrigation of cotton land since 1938. For reference purposes samples were taken from an adjacent site where the soil was in the virgin condition, never having been brought under cultivation. In addition, samples of the same soil series were obtained from a nearby ranch where crops had been irrigated with well water. The soil was a saline alkali soil in the virgin condition, having not only a high pH but also an excess of salt with the cation exchange complex dominated by sodium. Figure 9 shows the distribution of

Figure 9 →

exchangeable calcium in the soil down to a depth of 150 cm at the 3 sites. If the virgin soil may be considered to represent the original condition it is clear that the application of wastewater has done an excellent job of reclaiming the soil, displacing much of the exchangeable sodium and replacing it with calcium. These changes are further reflected in Figure 10 which gives values for exchangeable sodium. Some properties of 1:5

Figure 10 →

soil:water extracts are given in Table 2. There was some reduction of

Table 2 →

pH in the soil as a result of wastewater application, which was desirable in this instance because of the excessively high pH of the virgin soil. However, even after 36 years of wastewater application, the pH in wastewater treated soils was higher than that in comparable soil where well water was used for irrigation. These results are in harmony with those of Kardos and Sopper (1973) in showing that other constituents of wastewaters exert a greater influence on soil pH than does the acid produced in nitrification.

Nitrogen in Wastewater--Nutrient or Pollutant?

In view of the probability that nitrification is seldom a limiting consideration in the application of wastewater on land, the question of the ultimate fate of nitrate produced rests predominantly on the efficiency of plant uptake and the rate of denitrification. Obviously it is advantageous to recycle as much of the nitrogen as possible. A consideration of plant uptake efficiency is beyond the scope of this paper, but it should be noted that optimum efficiency may not be compatible with maximum disposal of wastewater. Uptake efficiencies in field experiments typically are not much above 50%. Lower values result from excessive application, while higher values are difficult to achieve, even with good management.

The other major factor, denitrification, is influenced by a variety of soil properties as well as being subject to some degree of manipulation by water management. A sandy soil containing little organic matter provides a less favorable environment for denitrification than does a clay soil of higher organic content. The presence of layers of low permeability in a soil of otherwise porous nature may exert a pronounced influence on denitrification rates through formation of perched water tables of a temporary nature.

Whether input nitrogen becomes primarily a nutrient or a pollutant depends in large measure on the rate of denitrification. The potential for managed denitrification as a means of minimizing the pollution hazard in wastewater application appears to be great, but at present is still not a practical reality in any precise sense.

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TABLE 1

pH values of five secondary sewage samples during aerobic incubation.

Sewage	Days Incubation					
	0	4	10	17	24	31
Riverside	7.30	8.60	8.80	8.75	8.72	8.55
Ontario	7.45	8.75	8.78	8.80	9.00	8.50
Redlands	7.45	8.80	8.58	8.60	8.40	8.30
Sun City	6.45	8.65	8.15	8.20	8.00	7.80
Sunkist	4.65	7.85	8.03	8.65	8.60	8.75

TABLE 2

Effect of irrigation with wastewater and well water on some properties of 1:5 soil:water extracts of Kimberlina soil near Bakersfield, California.

Depth, cm	pH	E.C., mmhos/cm	Cl, ppm	NO_3-N, ppm
		Uncultivated Virgin Soil		
0-15	8.48	0.83	142	0
15-30	9.23	2.00	501	0
30-60	9.22	1.04	227	0
60-90	8.73	0.43	87	0
90-120	8.68	0.77	190	0
120-150	8.40	0.49	100	0
		36 Years Wastewater		
0-15	7.29	0.43	64	16.8
15-30	7.38	0.25	58	8.3
30-60	7.43	0.28	62	8.3
60-90	7.61	0.30	66	9.2
90-120	7.59	0.25	43	7.4
120-150	7.79	0.18	42	4.0
		Irrigated With Well Water		
0-15	6.71	0.15	26	0.4
15-30	6.58	0.17	29	1.4
30-60	7.01	0.13	22	--
60-90	7.13	0.10	27	--
90-120	7.12	0.11	25	--
120-150	7.15	0.13	24	--

ILLUSTRATION LEGENDS

Figure 1. Changes of ammonium, nitrite and nitrate during aerobic incubation of secondary sewage.

Figure 2. Effect of iron added as ferric chloride on nitrite oxidation in aerated municipal wastewater.

Figure 3. Titration curves of two secondary sewage samples, showing effect of aerobic incubation.

Figure 4. Nitrate concentrations in effluent from a column of calcareous subsoil receiving input wastewater containing 41 ppm NH_4-N.

Figure 5. Input vs. output of inorganic N and labeled N from one-meter columns of Solado surface soil receiving wastewater containing 3 meq/l NH_4^+.

Figure 6. Input vs. output of inorganic N and labeled N from one-meter columns of Solado subsoil receiving wastewater containing 3 meq/l NH_4^+.

Figure 7. Input vs. output of inorganic N and labeled N from one-meter columns of Panoche sandy loam receiving wastewater containing 1.17 meq/l NH_4^+.

Figure 8. Calcium concentrations in effluents from columns of Solado sandy loam receiving inputs of synthetic sewage containing 3 meq/l of NH_4^+ or NO_3^-.

Figure 9. Distribution of exchangeable calcium in three soil profiles, showing effects of 36 years of wastewater application.

Figure 10. Distribution of exchangeable sodium in three soil profiles, showing effects of 36 years of wastewater application.

Figure I

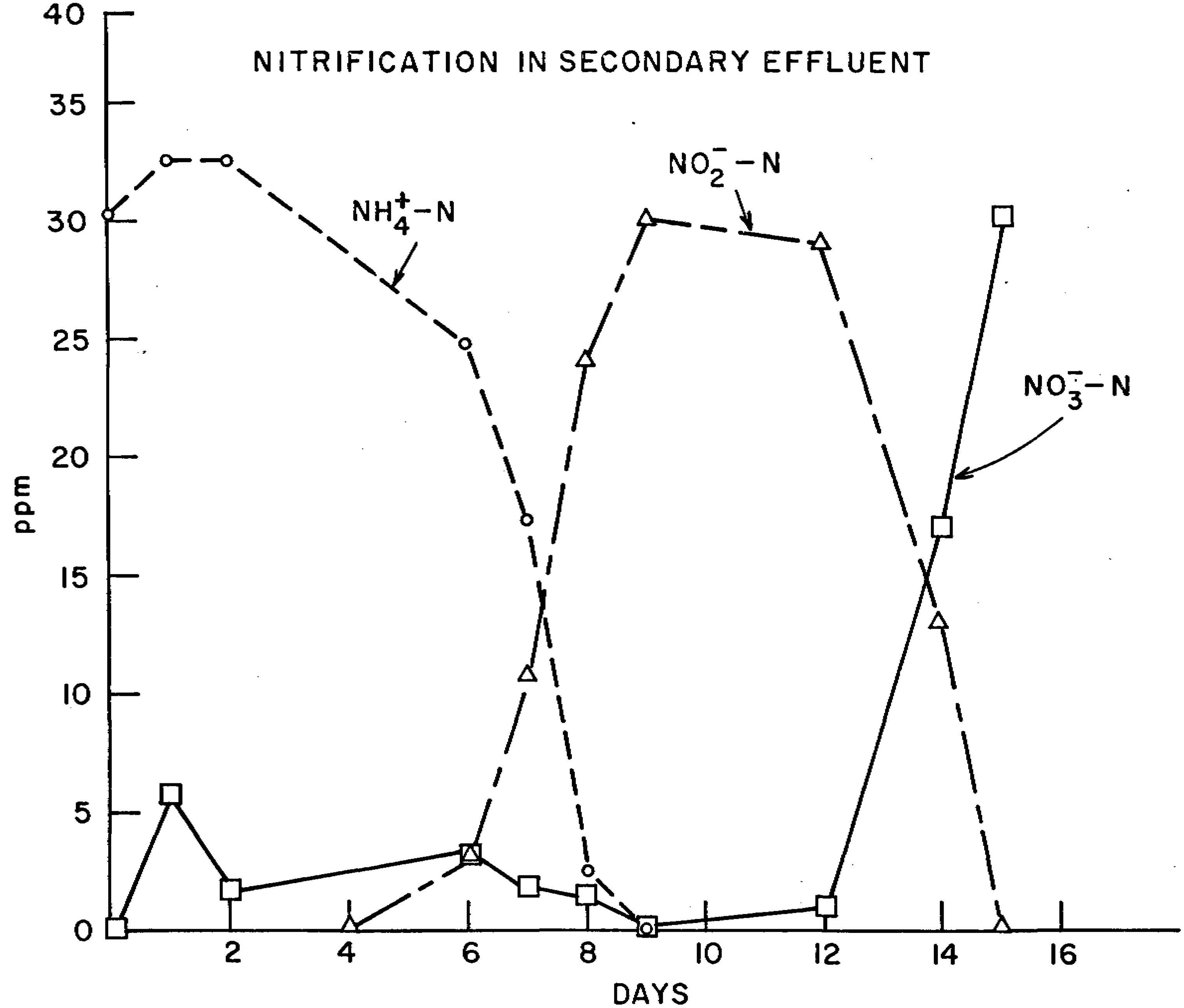

NITRIFICATION IN SECONDARY EFFLUENT
40
35
30
25
20
15
10
5
0
ppm
2
4
6
8
10
12
14
16
DAYS
NH_4^+-N
NO_2^--N
NO_3^--N

Figure II

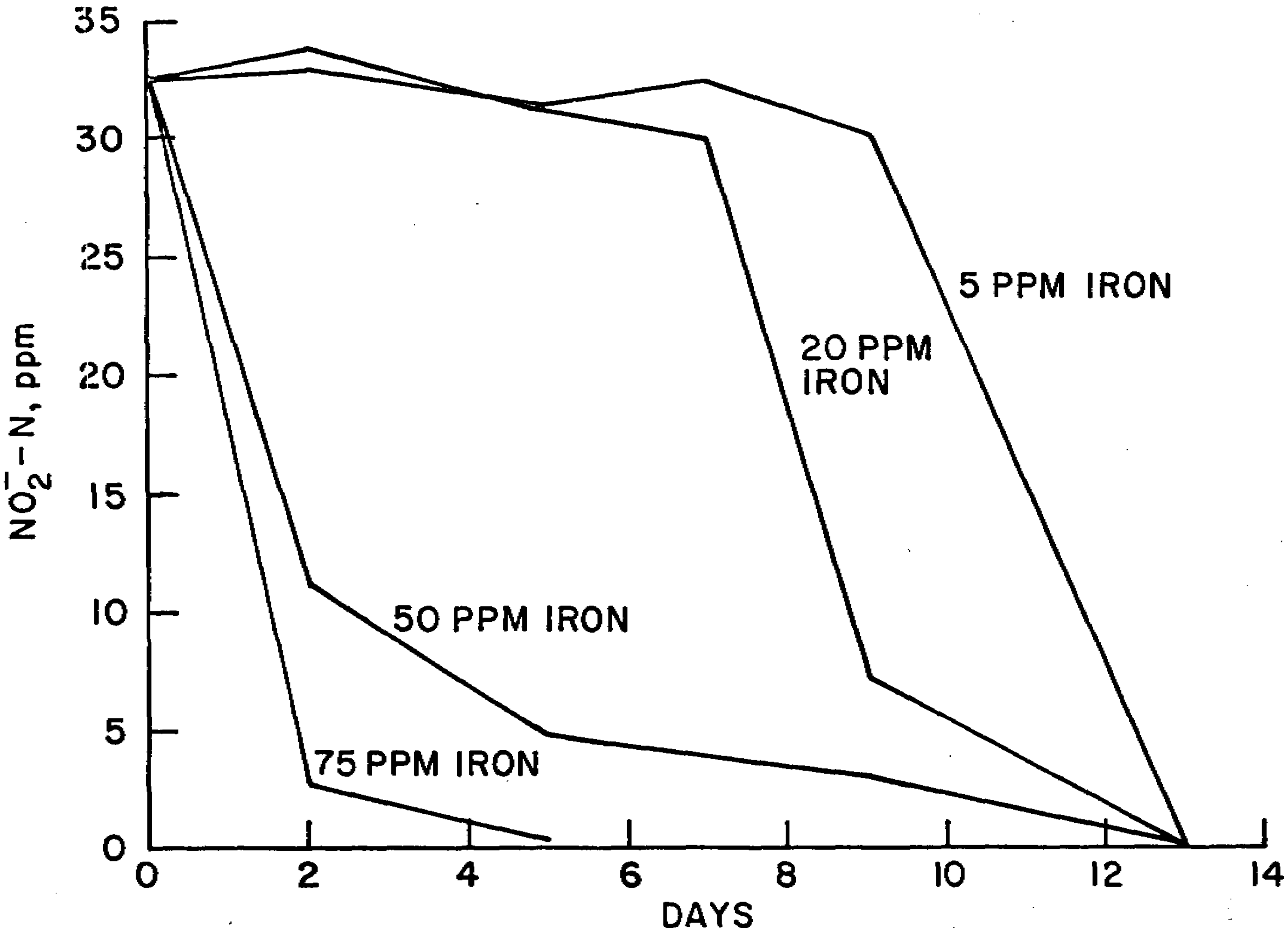

35
30
25
20
15
10
5
0
NO_2^--N, ppm
0
2
4
6
8
10
12
14
DAYS
5 PPM IRON
20 PPM
IRON
50 PPM IRON
75 PPM IRON

Figure III

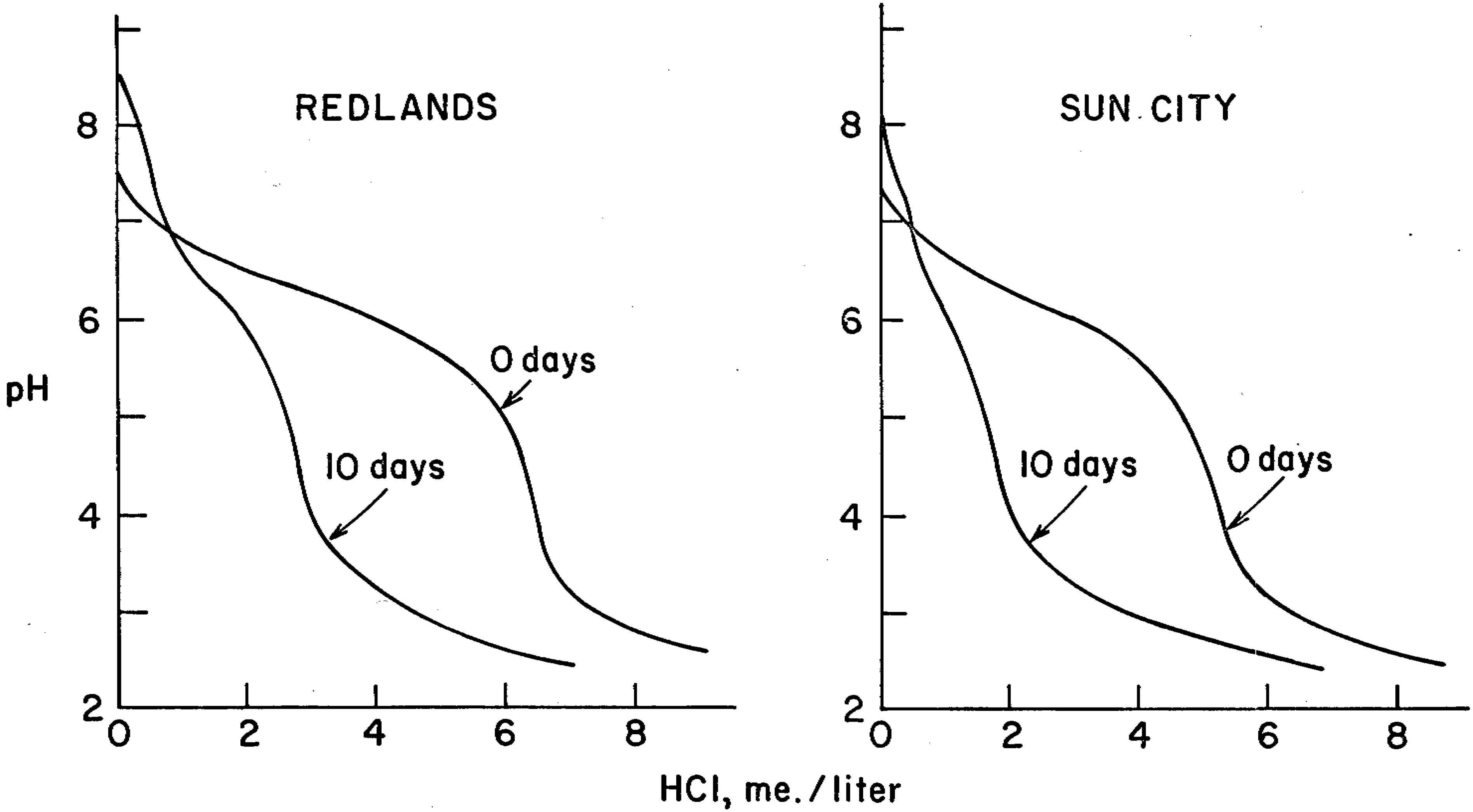


Figure IV

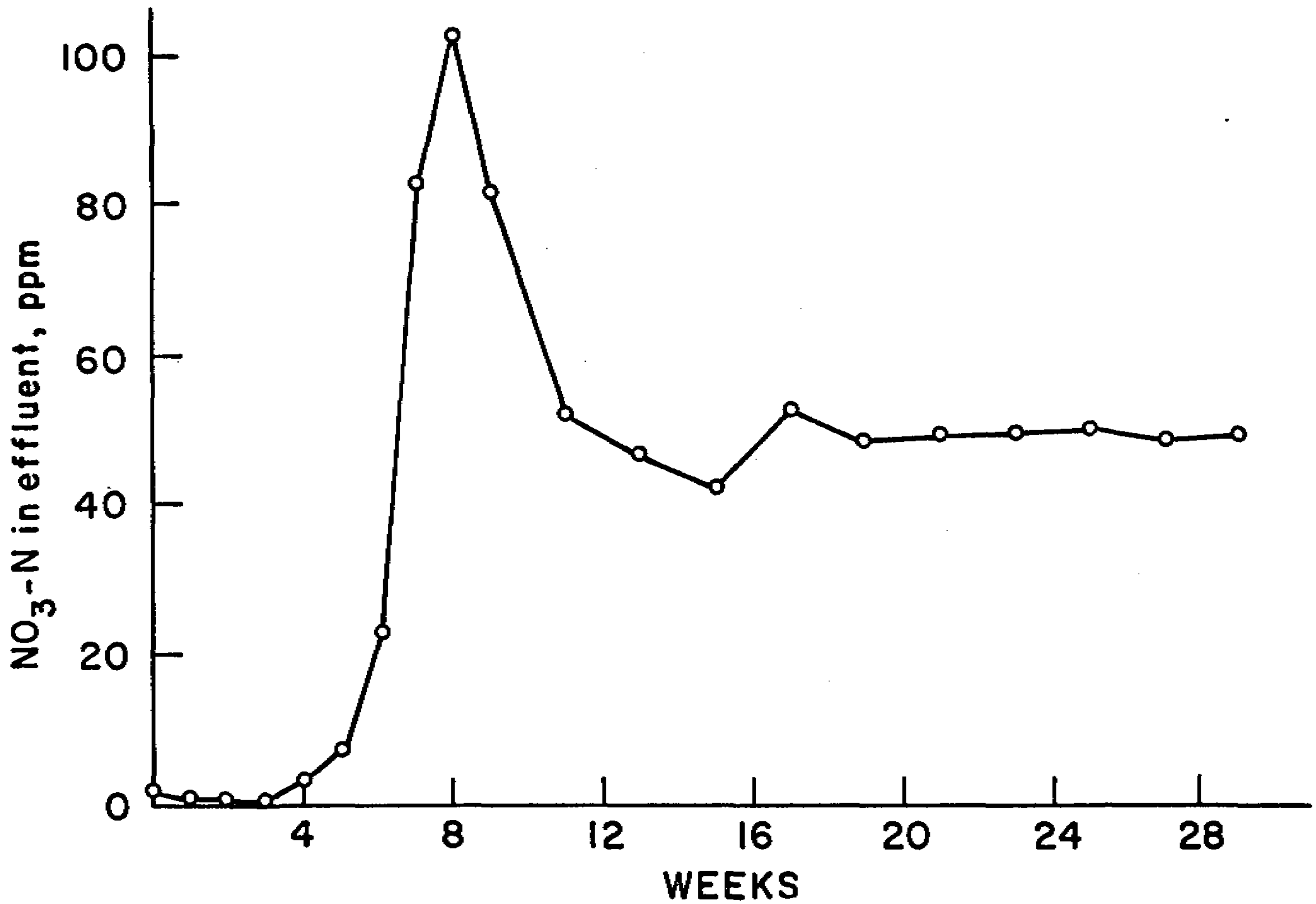
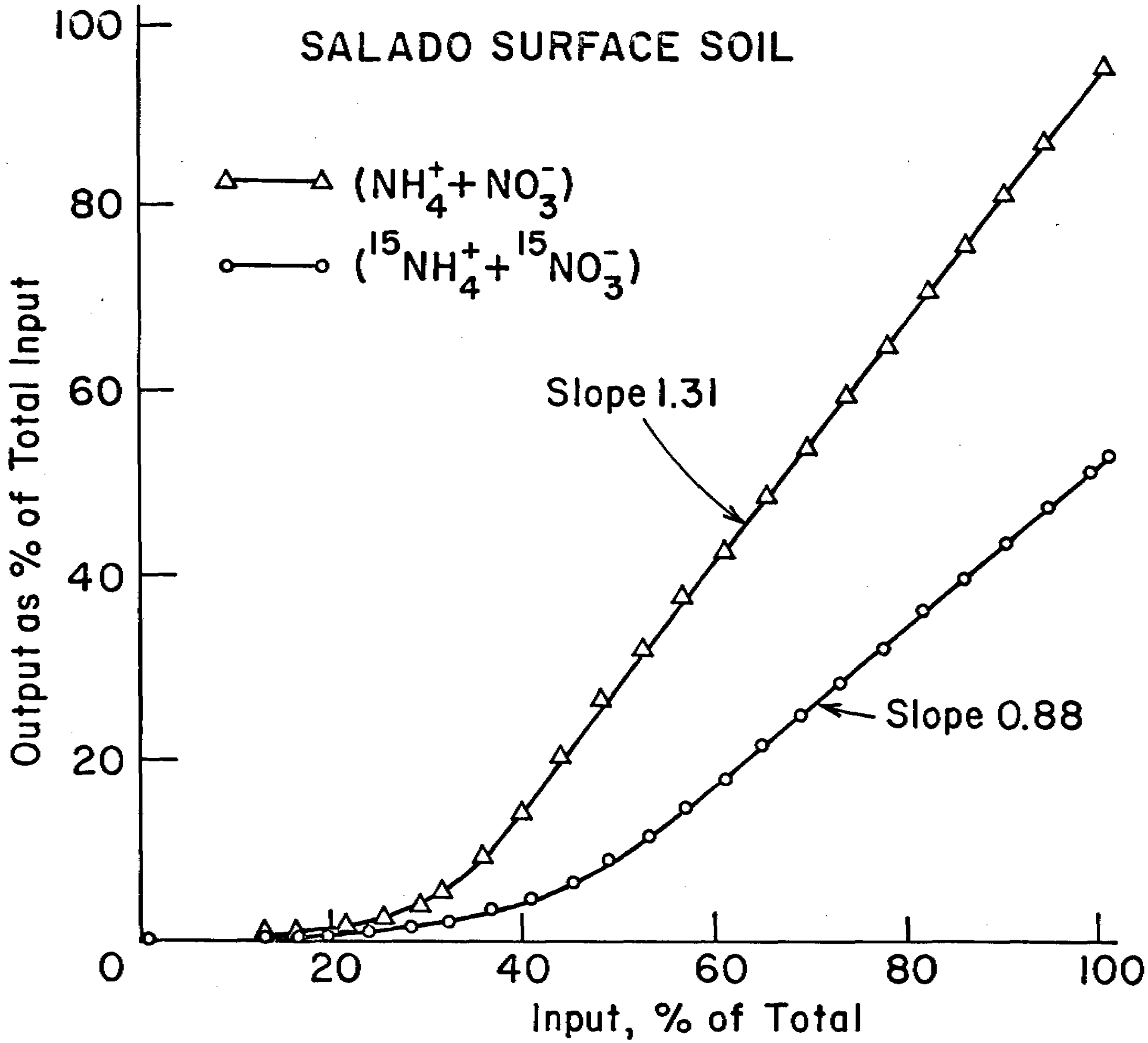
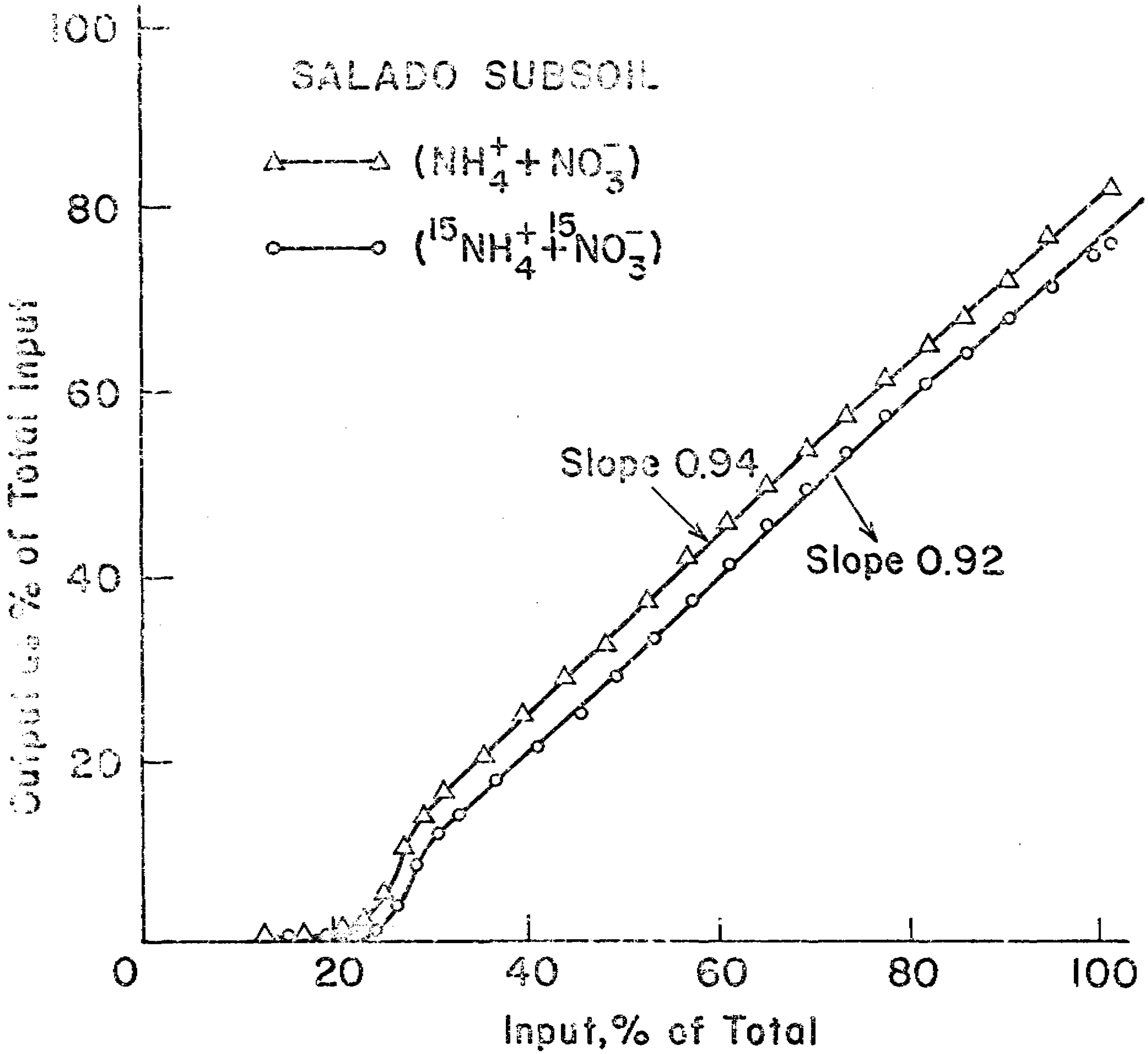


Figure V



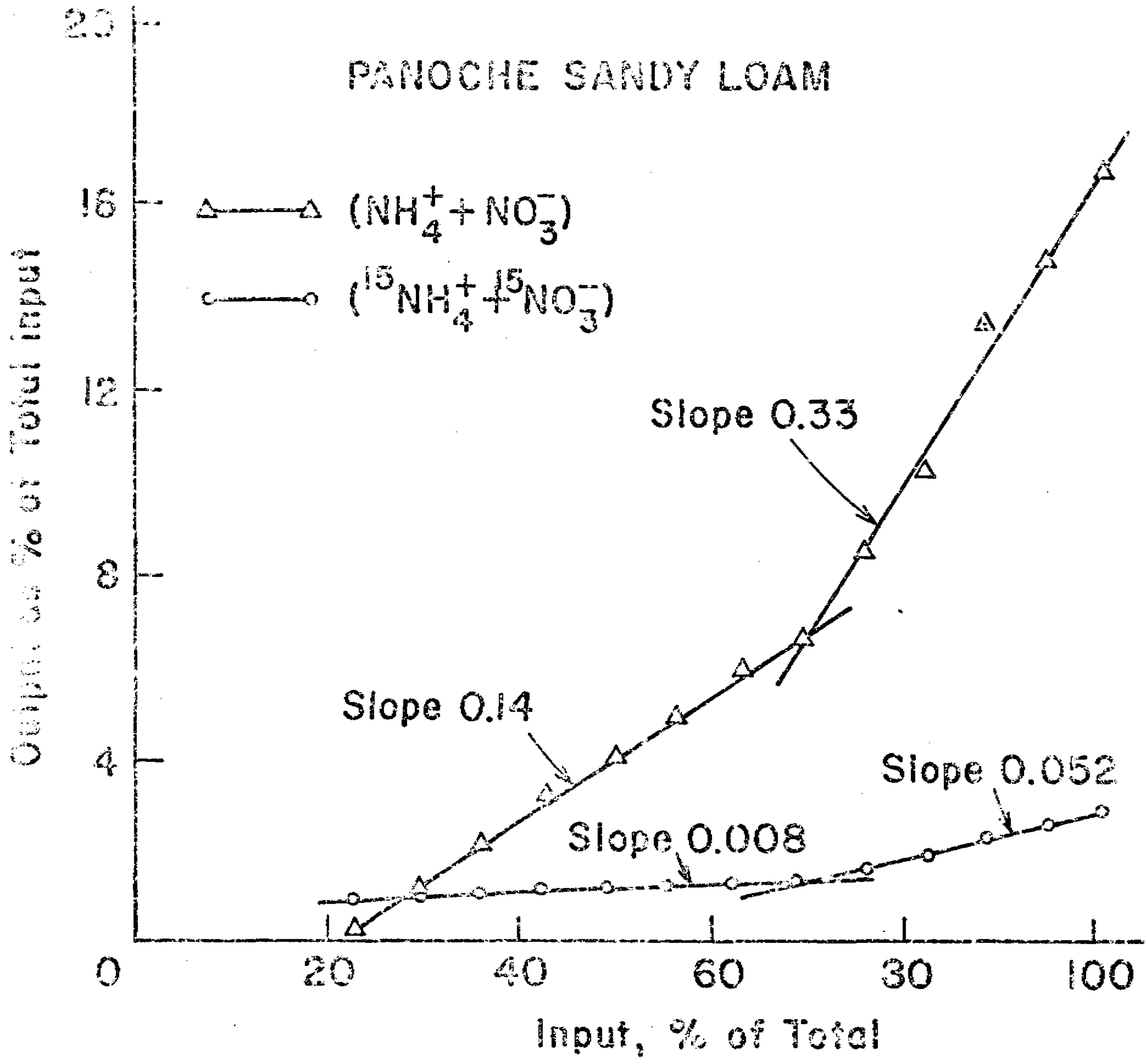
SALADO SURFACE SOIL
$(NH_4^+ + NO_3^-)$
$(^{15}NH_4^+ + ^{15}NO_3^-)$
Slope 1.31
Slope 0.88
Output as % of Total Input
Input, % of Total
0
20
40
60
80
100

Figure VI



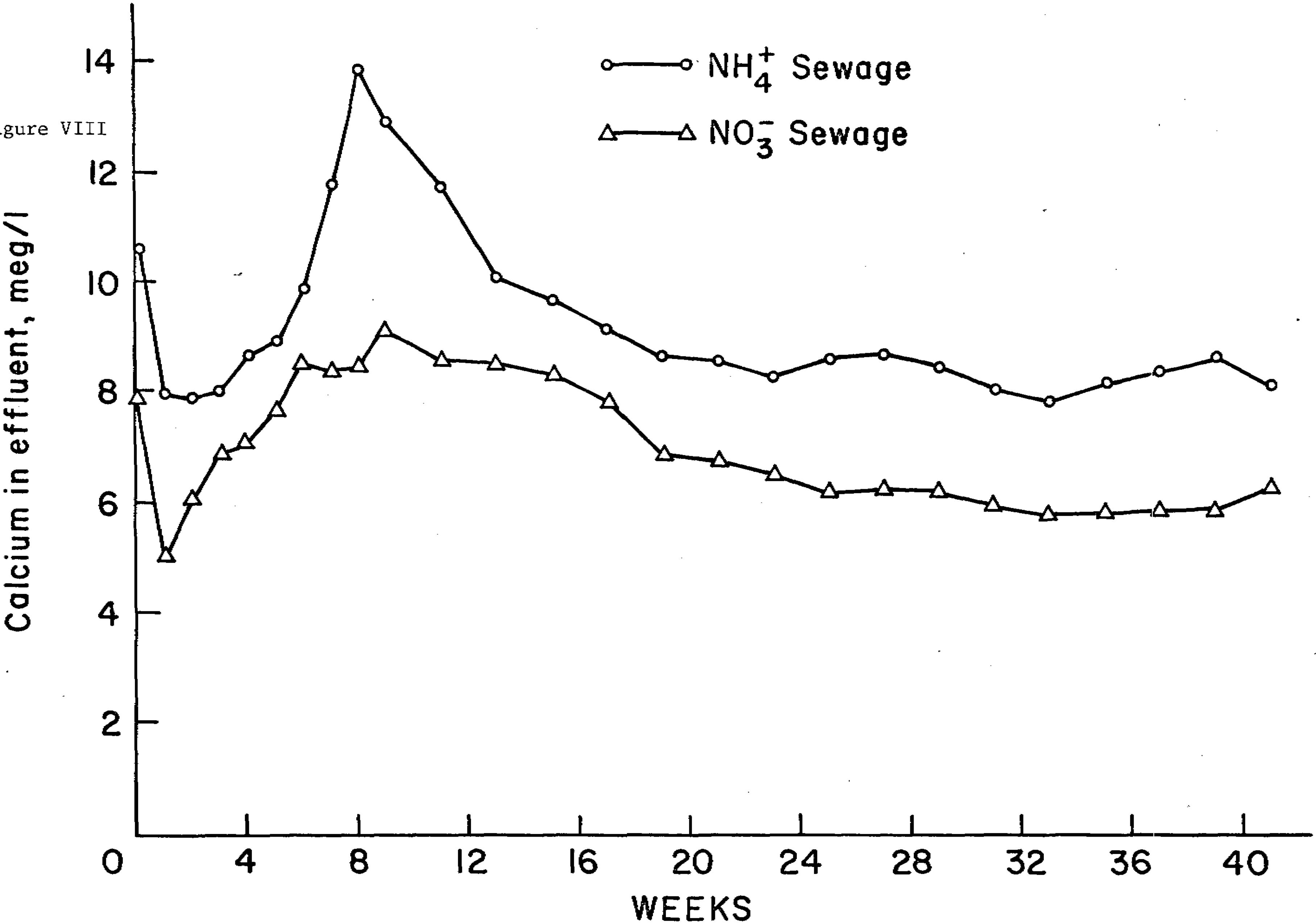
SALADO SUBSOIL
$(NH_4^+ + NO_3^-)$
$(^{15}NH_4^+ + ^{15}NO_3^-)$
Slope 0.94
Slope 0.92
Output as % of Total Input
Input, % of Total
0
20
40
60
80
100

Figure VII



PANOCHE SANDY LOAM
(NH4+ + NO3-)
(15NH4+ + 15NO3-)
Slope 0.33
Slope 0.14
Slope 0.052
Slope 0.008
Output as % of Total Input
Input, % of Total
0
20
40
60
80
100
4
8
12
16
20

Figure VIII



NH4+ Sewage
NO3- Sewage
Calcium in effluent, meg/l
14
12
10
8
6
4
2
0
4
8
12
16
20
24
28
32
36
40
WEEKS

Figure VIIII

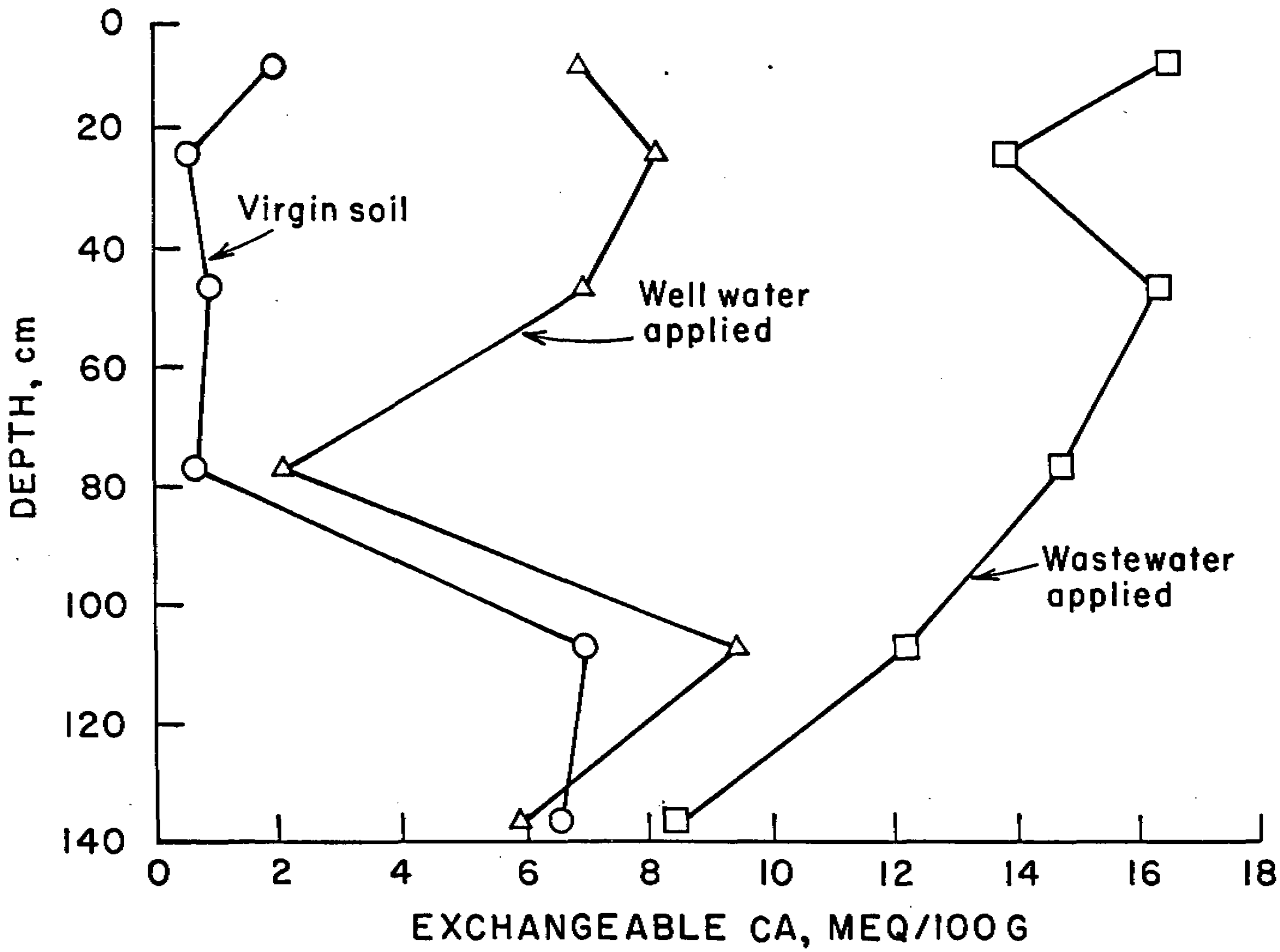
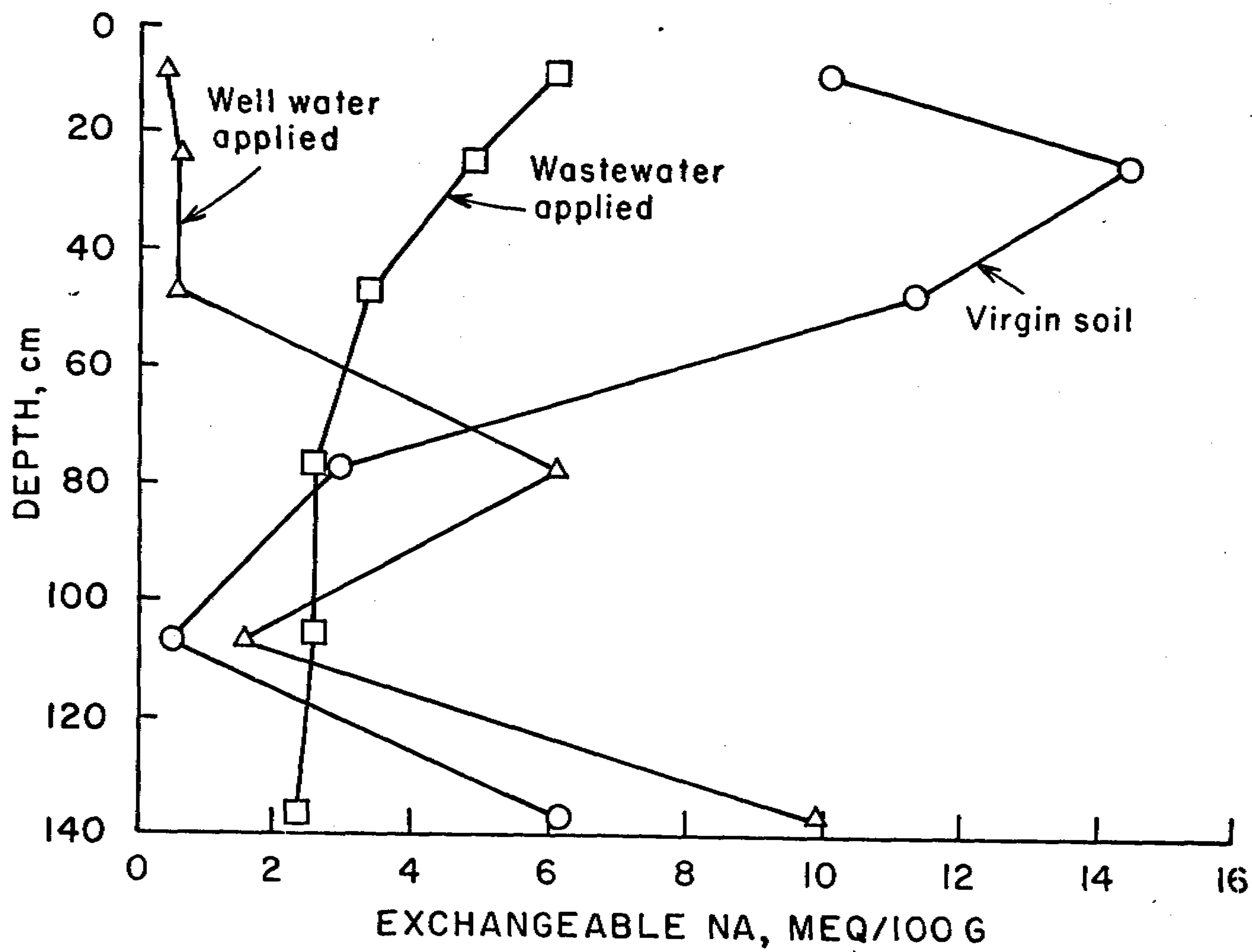


Figure X



Well water applied
Wastewater applied
Virgin soil
DEPTH, cm
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
14
16
EXCHANGEABLE NA, MEQ/100 G