Faecal Contamination of a Fish Culture Farm where Hospital Wastewater Grown Duckweeds are Used as Fish Feed

Dr. Sirajul Islam

International Centre for Diarrhoeal Disease Research, (ICDDR,B), GPO Box 128, Dhaka-1000, Bangladesh
Phone: 880-2-8811751-60 Ext. 2405/2407
Fax: 880-2-8823116, 880-2-8812529
mail: sislam@icddrb.org

Co-authors: M.J. Alam[1], M.A. Sadique[1], G.B. Nair[1], D.A. Sack[1], M.A. Rahman[1], M.S. Kabir[2], S.I. Khan[2], M. Ekramullah[3], , R.B. Sack[4], A. Huq[5] and R.R. Colwell[6]

Introduction

Duckweeds are tiny, fragile, free-floating, aquatic plants. Reproduction of duckweed is by vegetative means. An individual frond may produce as many as 10 generations of progeny over a period of 10 days to several weeks before dying. Duckweed fronds can double their mass in two days. Under experimental conditions their production rate can approach an extrapolated yield of 4 metric tons/ha/day of fresh plant biomass (Anonymous, 1976).

Duckweeds have long been recognized for their potential as a source of high protein feed for animals. Studies conducted in various countries of the world e.g. USSR, USA, Canada and others have demonstrated the nutritional benefits of duckweed for both livestock and fish. Animals grew better on duckweed-supplemented diets than they did on traditional diets using either soyameal or fish meal (Culley and Epps, 1973).

A project of duckweed based wastewater treatment has been undertaken at Mirzapur, Tangail, Bangladesh. "Duckweeds farming" is being done on agricultural land using either organic fertilizer or wastewater collected from Kumudini Hospital Complex (a 500 bed general hospital). In the Mirzapur duckweed project, there are two sets of ponds where the duckweed is grown. In one set of ponds inside the hospital complex, the duckweed is grown using artificial fertilizer. These are control ponds in non-wastewater area. The other set of ponds are in the wastewater area, situated 0.5 km away from the hospital complex. These are study ponds in wastewater area.

The hospital wastewater is "treated" with duckweed by having it grown in a series of wastewater lagoons (waste stabilization ponds). Duckweed, when grown in these ponds, convert substantial amount of organic material into plant biomass; they convert nutrients and dissolved minerals into plant biomass. When plants are harvested, nutrients and trace minerals are removed from the system and a dynamic nutrient and mineral sink is established. This forms the basis for a highly effective wastewater treatment technology. Waste stabilization ponds (lagoons) are

1 International Centre for Diarrhoeal Disease Research, (ICDDR,B), GPO Box 128, Dhaka-1000, Bangladesh
2 Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh
3 PRISM Bangladesh, Projects in Agriculture, Rural Industry, Science and Medicine, House#49, Road#4A, Dhanmondi R/A, Dhaka, Bangladesh
4 Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, 550 North Broadway, Suite 1001, Baltimore, MD 21205, USA
5 Center of Marine Biotechnology, University of Maryland Biotechnology Institute,701 East Pratt Street, Baltimore, Maryland 21202, USA
6 Department of Cell Biology and Molecular Biology (CBMB), University of Maryland, College Park, Maryland 20742, USA

generally the method of domestic wastewater treatment of first choice in developing countries (Mara, 1976), The duckweed which grow in wastewater lagoons are harvested and used as the only source of fish feed. Therefore, duckweed is utilised for two purposes; one is for treating wastewater and the other is as fish-feed.

Two sets of workers were involved in harvesting the duckweed from control ponds and wastewater ponds. The duckweed which were grown in beds using artificial fertilizer were used as fish-feed in fish ponds of non-wastewater areas. The duckweed grown in wastewater lagoons were used as fish feed in fish ponds of wastewater areas. Therefore, this project provided an unique opportunity of a comparative microbiological investigation of a wastewater grown duckweed based fish culture project.

The role of duckweed in treating wastewater bacteriologically by reducing bacteria is not known. The level of contamination of fish due to use of wastewater grown duckweed as feed is also not known. Faecal coliform has long been used as an indicator organism in the aquatic environment. The coliform group of organism consists of *E. coli, Klebsiella* spp., *Citrobacter* spp., and *Enterobacter* spp. Among these bacteria, *E. coli* is the dominant genera which produces various toxins (Sack *et al.*, 1975; Sack, 1980) and can cause diarrhoea (Sack *et al.*, 1975a; 1975b; 1978). If the faecal coliform load can be reduced, that can also help to reduce the diarrhoeal disease. Therefore, the present study was designed to find out the efficacy of removal of faecal coliform by duckweed and fish grown in wastewater and non-wastewater ponds.

Materials and Methods

Sampling spots and schedule: The sampling spot was located within the Kumudini Hospital Campus of Mirzapur thana, under Tangail district of Bangladesh. The spot is approximately 60 km to the north of Dhaka, the capital city of Bangladesh. In the first week of every month, duckweed (DW), water and fish samples were collected for a period of 12 months (from May 1994 through April 1995) from the wastewater and non-wastewater areas for analysis of faecal coliforms. From the wastewate area, DW and water samples were collected from three of six wastewater ponds and three fish growing ponds. Only water sample was collected from the raw sewage. Tilapia (*Oreochromis nilotica*) was collected from fish pond no.1 and silver carp (*Hypothalamichthys molitri*) was caught from fish pond no. 3 of the wastewater area. From the non-wastewater area, DW, and water samples were collected from two DW growing beds and two of four fish growing ponds. Telapia and grass-carp (*Ctenopharyngodon idella*) were caught from pond no.1 and 2 respectively of the non-wastewater area. The sampling sites are shown in fig. 1. The abbreviation B1 and B2 stand for duckweed growing beds in which artifical fertilizer are used as nutrients. The control pond numbers 3 and 4 are designated as CP3 and CP4. All these sampling sites are situated in the non-waterwater area within the Hospital complex. In wastewater area, L5 stand for wastewater lagoon 5 where the raw sewage is collected and no duckweed is grown. From L5 the raw sewage is pumped to a zig zag pattern wastewater lagoons in which the duckweeds are grown. The sampling sites in wastewater lagoons are designated as L6 and L7. The study fish ponds in wastewater area are designated as P8, P9 and P10. All the above mentioned sampling sites are shown in fig. 1.

Processing of samples

Water: When the count in water was high, either 0.1 ml was taken directly from the collected sample or 10 fold dilutions were prepared and then 0.1 ml wastewater was inoculated on MFC agar plate following drop plate technique (Hoben and Somasegoran, 1982). When the counts were low, membrane filtration technique was followed to concentrate faecal coliform. One ml water sample was passed through a membrane filter (0.22 µm pore size, 47 mm diameter, Millipore) and the filter with retained bacteria was placed on mFC agar plate which was incubated at 44^{0}C overnight. Following incubation characteristic blue coloured colonies were counted and expressed as faecal coliform/ml of water samples following standard procedures (APHA, 1992).

Duckweed: Ten grams of DW was washed 3 times with normal physiological saline for removing loosely attached bacteria. Then the washed duckweed was homogenized into a sterile electrical blender for one minute in 90 ml normal saline. Ten fold dilutions of the homogenate in normal saline were made. From each dilution, 25 µl sample was plated directly on mFC agar following drop plate techique (Hoben and Somasegoran, 1982) incubated at 44oC overnight. Typical FC were counted and expressed as CFU/g.

Fish: Fish gills were taken out and the exposed gills were washed in normal saline to eliminate unattached bacteria. Then ventral part of the fish was cut open with flamed sterile scissors and washed with sterile normal saline. Ten g each of washed gills and intestinal contents were separately homogenized in 90 ml normal saline in electrical blender for one min. Homogenized gills and intestinal contents of fish were processed for fecal coliforms following the same procedure as described for duckweed.

Stool samples: Stool samples were collected from the duckweed handlers every month during environmental sampling. Attempts were made to isolate common diarrhoeal bacterial pathogens e.g., pathogenic vibrios, *Aeromonas* spp., *Plesiomonas shigelloides*, *Shigella* spp., *Salmonella* spp., and *Campylobacter* sp. using conventional culture technique following the procedures described by Stoll *et al.*, 1982.

Results

Fig. 2 shows the faecal coliform concentrations in water samples collected from the wastewater and non-wastewater ponds. Raw wastewater (L-5) showed the highest counts of faecal coliform. Other spots showed similar counts irrespective of wastewater and non-wastewater ponds.

Fig. 3 shows the faecal coliform counts in duckweeds in non-wastewater and wastewater ponds. The duckweeds collected from ponds situated both in non-wastewater and wastewater areas showed almost similar counts of faecal coliforms. In case of fish ponds, a slightly higher counts of faecal coliform in wastewater pond were observed than non-wastewater pond.

Fig. 4 shows the concentration of faecal coliform in fish samples collected from wastewater and non-wastewater poonds. The faecal coliform concentration were found almost similar in gills of fish which were caught from the ponds located both in wastewater and non-wastewater areas. In case of fish intestines, the faecal coliform count was slightly higher in fish caught from non-wastewater pond than wastewater pond. Fish intestine contained more bacteria than the gills. In fish samples, faecal coliforms concentrations were highest in comparison with water and duckweed (except raw wastewater).

The stool samples from the handlers worked in non-wastewater and wastewater areas yielded no enteric bacterial pathogens.

Discussion

There is no significant difference in faecal coliform concentrations between wastewater and non-wastewater grown duckweed and fish. The faecal coliform concentrations were similar in water collected from both wastewater and non-wastewater ponds (except in raw wastewater). These results indicated that the pollution level and the potential for transmission of diarrhoeal diseases are similar both in wastewater and non-wastewater ponds.

The fish farm workers had no complain regarding any infection from these sources. Fish were normal, no symptoms of disease was noticed. Fish growing pond waters were of desired quality for fish culture (fecal coliforms concentrations were <10^2/ml). Strauss (1985) reviewed the literature on the survival of pathogens in and on fish and concluded that invasion of fish tissues by pathogens increased with the duration of exposure to the contaminant. In Bangladesh, various studies showed that faecal coliforms concentration in natural water are quite higher (counts 10^2-10^3/ml of water) compared to fish growing pond waters of this study (Islam *et al.*, 1994a, Morshed *et al.*, 1985). Previous studies demonstrated that pathogenic bacteria are also very common in pond waters in Bangladesh (Islam *et al.*, 1991, 1992, 1994b, 1995, 1996) and elsewhere (Nair *et al.*, 1985; 1988). Pathogenic bacteria like *V. cholerae*, *Shigella* spp., *Campylobacter* spp. and others can also go to viable but non-culturable (VBNC) state (Colwell *et al.*, 1985; Colwell & Huq, 1994; Colwell *et al.*, 1996). Non-culturable pathogenic bacteria may also exist in these study ponds (Colwell *et al.*, 1985; Huq *et al.*, 1990; 1992). Further study should therefore be designed with the aim of detecting viable but non-culturable (VBNC) enteric pathogens from duckweeds, wastewater and fish from both non-wastewater and wastewater areas.

This study demonstrated that duckweeds can efficiently reduced the faecal coliform counts. The results also showed that following treatment with duckweeds, there was no significant difference between wastewater and nonwastewater interms of FC counts. The faecal coliform counts in duckweed and fish were semilar in both wastewater and nonwastewater areas. There was no isolation of enteric bacterial pathogens from the stool of handlers of duckweeds and fish.

Therefore, this study clearly demonstrated that duckweed can be grown in wastewater lagoons and can safely used as fish feed without having any potential risk for transmission of diarrhoeal diseases from fish. The workers who handle both the contaminated duckweed grown in wastewater and the fish fed by wastewater grown duckweed were also free from diarrhoeal pathogens. Therefore, the wastewater grown duckweek and fish fed by these duckweed do not pose any health hazard to the handlers.

The present study also showed that duckweed can be grown in wastewater lagoons which will help to treat the wastewater at the same time the wastewater grown duckweed could be used safely as fish feed for pisciculture. This study provided some vital information about microbiological safety of using wastewater for growing duckweed which can be used for pisciculture.

We can conclude from this study that in developing countries, duckweeds can be used as a good means for wastewater treatment. The duckweeds can also be used as fish feed. This study therefore important from microbiological safety point of view of wastewater grown duckweed which is used as fish feed as well as the handlers who are involved in duckweed based fish culture project.

Acknowledgements

This research was supported by Swiss Development Cooperation (SDC) and the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The ICDDR,B is supported by countries and agencies which share its concern for the health problems of developing countries. Current donors include: the aid agencies of the Governments of Australia, Bangladesh, Belgium, Canada, China, Denmark, Germany, Japan, The Netherlands, Norway, Republic of Korea, Saudi Arabia, Sweden, Switzerland, the United Kingdom and the United Stated;

international organizations including the Arab Gulf Fund, Asian Development Bank, European Union, International Atomic Energy Centre, the United Nations Children's Fund (UNICEF), the United Nations Development Program (UNDP), the United Nations Population Fund (UNFPA) and the World Health Organization (WHO); private foundations including Child Health Foundation, Ford Foundation, Population Council, Rockfeller Foundation and the Sasakawa Foundation; and private organizations including American Express Bank, Bayer A.G., CARE, Family Health International, Helen Keller International, the Johns Hopkins University, Procter Gamble, RAND Corporation, SANDOZ, Swiss Red Cross and the University of California Davis and others. We thank Mr. Md. Anisur Rahman for typing the manuscript.

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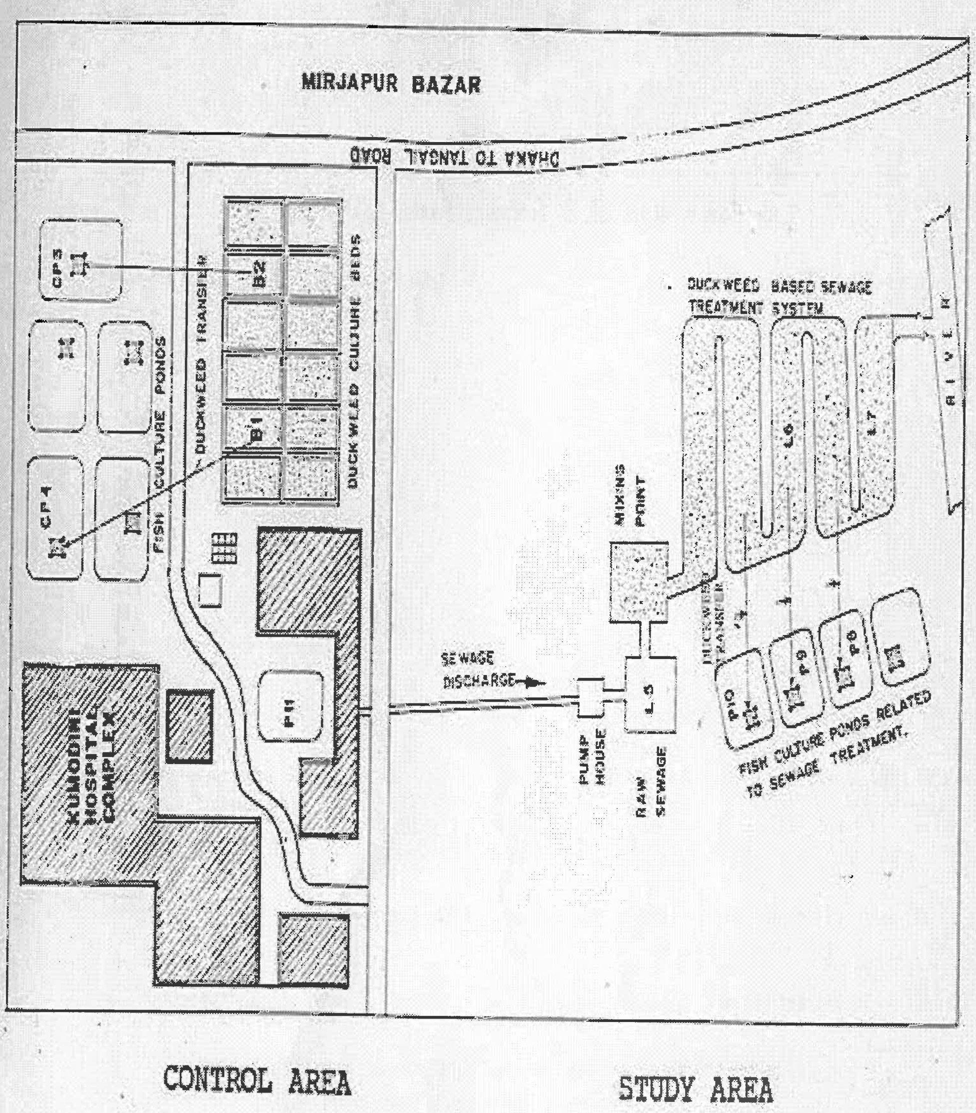

Fig-1: Schematic diagram of the sampling site at Mirzapur, Tangail with specific sampling points.

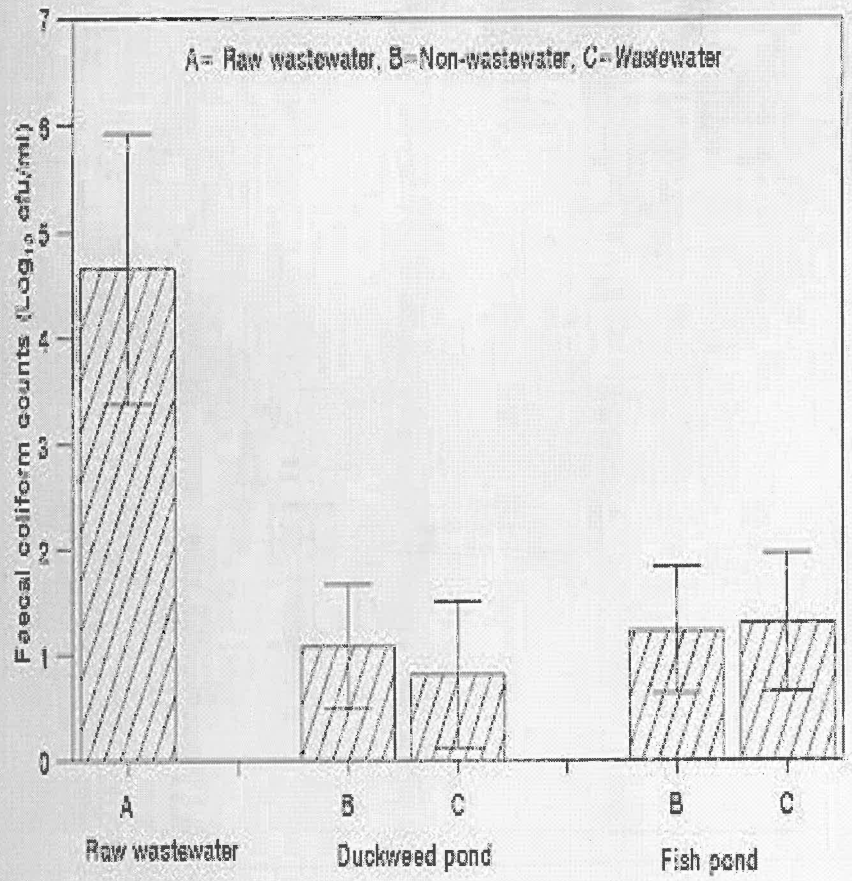

Fig-2: Faecal coliform counts of water collected from raw wastewater, non-wastewater and wastewater ponds.

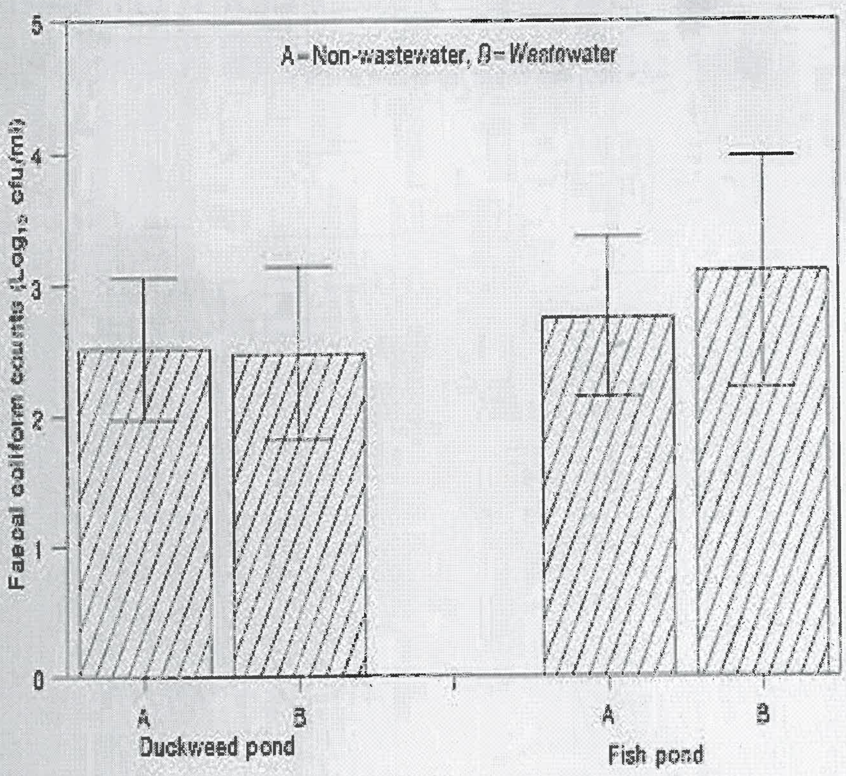

Fig-3: Faecal coliform counts in duckweed collected from non-wastewater and wastewater ponds.

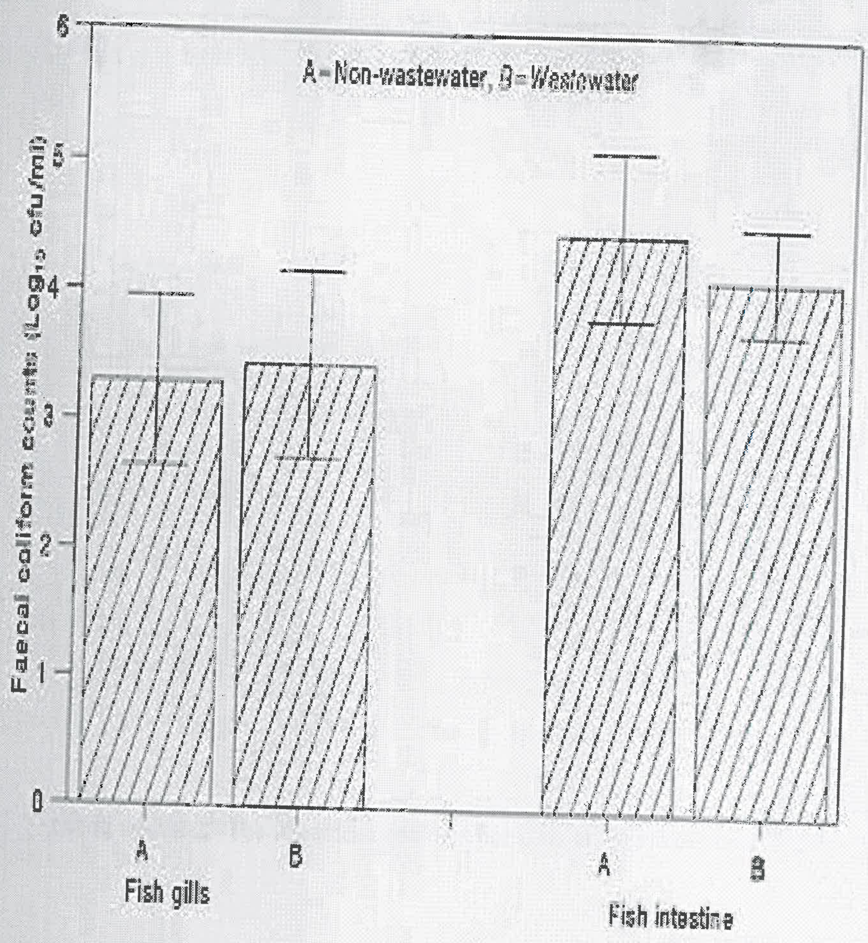

Fig-4: Faecal coliform counts in fish-gills & intestine of non-wastewater and wastewater ponds.