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Effect of water coagulation by seeds of *Moringa oleifera* on bacterial concentrations

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Summary

The effects of a Sudanese water purification method traditionally used in Sudan to treat turbid waters were studied with respect to turbidity reduction and removal of faecal indicator bacteria as well as selected enteric bacterial pathogens. Water treatment was performed at 30°C with *Moringa oleifera* seed material as a coagulant, and the technique employed corresponded closely to that used to clarify turbid water in Sudanese villages. A turbidity reduction of 80.0–99.5% paralleled by a primary bacterial reduction of 1–4 log units (90.00–99.99%) was obtained within the first 1 to 2 h of treatment, the bacteria being concentrated in the coagulated sediment. During the 24 h observation period a secondary bacterial increase due to regrowth in the supernatant water was consistently observed for *Salmonella typhimurium* and *Shigella sonnei*, in some cases for *Escherichia coli*, but not for *Vibrio cholerae*, *Streptococcus faecalis* and *Clostridium perfringens*. The potential of the method when compared with some alternative for the improvement of rural drinking water supplies is discussed.

Introduction

During the flood season, July to September, the Blue Nile and hence also the Nile north of Khartoum become very turbid due to the copious rains in the Ethiopian highlands (Ramadan 1972; Mancy & Hafez 1979). Turbidity maxima of 3000–4000 formazine turbidity units (FTU) have been recorded (Jahn & El Fadil 1984). For more than a century (Pereira 1850) women in the rural communities along the Nile valley have been employing local water purification methods to remove turbidity. Traditionally this was only during the flooding season, but now appears to be more widely used on the turbid water of ponds and hafirs (rain water catchments) as well (Jahn 1977). A number of natural flocculating or coagulating agents of plants or soil origin exist. Their distribution and local use were extensively reviewed by Jahn (1977; 1981). One of the most promising traditional agents for turbidity removal seems to be the crushed seeds of the horse-radish tree, *Moringa oleifera* Lam. (syn. *M. pterygosperma* Gaertn). (Jahn 1979a,b). The coagulating activity of *Moringa* seeds has been ascribed to polypeptides acting as cationic polymers (Barth *et al.* 1982).

The effect of *Moringa oleifera* seed coagulation on the bacteriological counts in turbid water from hafirs in the Sudan has been reported by Jahn and Dirar (1979) and that on *Escherichia coli* and faecal coliforms in river water by Barth *et al.* (1982) and Grabow *et al.* (1985). The present paper deals with the effects of *Moringa oleifera* on bacterial enteric pathogens and indicator bacteria in turbid water as evaluated by field and laboratory experiments.

Materials and methods

MORINGA SEEDS

A single batch of ripe, dried *Moringa oleifera* seeds collected in the Gezira and Blue Nile

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Provinces of Sudan was employed in all experiments. For comparison, ripe, dried seeds of a closely related species, *Moringa stenopetala* (Bak. f.) Cufod, collected from the Malagasy Republic (Madagascar) included in one of the experiments.

WATER TYPES

Four different turbid water types were employed in the study. Field experiments were performed employing freshly collected samples: (1) from the shore-line of the Blue Nile some 20 km south of Khartoum; (2) from the White Nile approximately 20 km south of Khartoum; (3) from an irrigation canal in the green-belt area south of Khartoum. Experiments were conducted within 1–2 h of collection, i.e. while samples still contained their natural bacterial flora.

In the laboratory experiments, carried out in Copenhagen, the purpose was to simulate as closely as possible the water conditions in the Sudan during river flooding periods. To this end, 6.64 g of fresh mud deposits collected from the Nile bank in the flooding season and 0.1 g of Bacto peptone were added to 1.0 litre of unchlorinated, rather hard Copenhagen tap water.

Some of the physicochemical characteristics of the different water types employed are given in Table 1. More suspended solids had to be added to the artificial Nile water to obtain a similar turbidity as in Blue Nile water, suggesting some aggregation of particles in the former.

All experiments were carried out at a water temperature of 30°C, to simulate the temperature of the Nile water in Sudan during the summer months (Jahn & El Fadil 1984).

BACTERIAL STRAINS

In one experiment the effect of *M. oleifera* seed coagulation on the natural bacterial flora of Blue Nile water was studied. In all other cases the waters were seeded with one or more of the following laboratory strains: *Escherichia coli* (serovar 08, resistant to tetracycline), *Streptococcus faecalis, Clostridium perfringens, Salmonella typhimurium* (resistant to streptomycin), *Shigella sonnei* and *Vibrio cholerae* (*NAG*) (ATCC 14374). The test strains were added as a mixture to each sample of water, before addition of coagulant material, to obtain initial concentrations of 10^5 to 10^6 viable bacteria per ml of untreated water.

CULTURE MEDIA AND COUNTING PROCEDURE

For maintenance and short-term storage all strains except *Cl. perfringens* were grown in veal infusion broth (Difco) for 24 h at 37°C and kept as stock cultures at 4°C. *Cl. perfringens* was grown in VL broth (Fievez 1963) and stored similarly.

For isolation and recovery purposes the following media were used:

(1) McConkey agar with an addition of 20 μg ml^{-1} tetracycline chloride (Novo) (*E. coli*).
(2) Mitis salivarius agar (Difco) (*Str. faecalis*).
(3) Iron Sulphite agar (Danish Standard 265.1 for bacteriological examination of drinking water) (*Cl. perfringens*).
(4) BPLS (Merck) plus 15 μg ml^{-1} dihydrostreptomycin sulphate (Novo) (*Salm. typhimurium*).
(5) A *Salmonella/Shigella* differentiation medium developed and routinely used at the State Serum Institute, Copenhagen (Gaarslev 1985) *Salm. typhimurium, Shig. sonnei*).
(6) TCBS agar (Difco) (*V. cholerae* (NAG)).

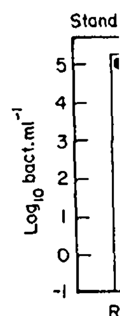
Dilution rows were prepared as 10-fold dilutions in physiological saline (0.9% NaCl) containing 0.1% Bacto peptone (Difco).

All bacterial counts were performed as viable plate counts by surface inoculation of 0.1 ml of the relevant dilutions on the appropriate agar plates. Plates were incubated aerobically at 37°C for 24–48 h, except for *Cl. perfringens* for which plates were incubated anaerobically by the pyrogallol technique (Fievez 1963).

Standard plate, presumptive coliform and faecal coliform counts on the natural flora of Blue Nile water were performed according to the standard methods of the American Public Health Association (1980).

TURBIDITY MEASUREMENTS

In the laboratory turbidity measurements were carried out by the nephelometric method (Danish Standard 290 for water analysis) using a Hach Laboratory Turbiditometer. In the field



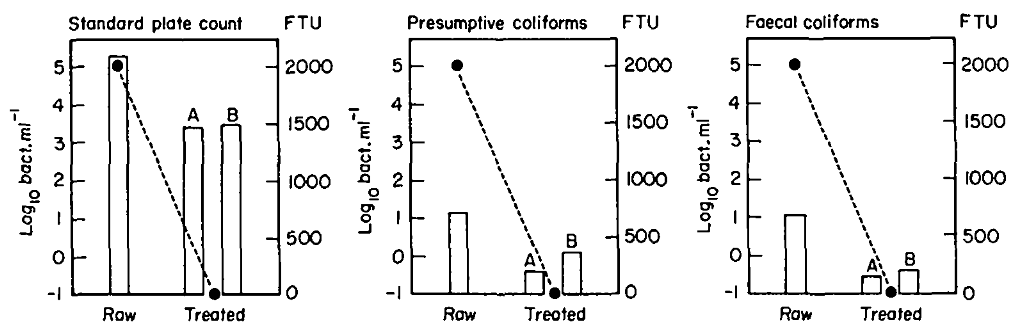


Figure 1. Effect of *M. oleifera* and *M. stenopetala* seed coagulation on turbidity and naturally occurring bacteria in Blue Nile water, flooding season (August 1982). (A) Sample taken after 1 h of coagulation with *M. oleifera* seeds 200 mg l^{-1}; (B) Sample taken after 1 h of coagulation with *M. stenopetala* seeds 200 mg l^{-1}. Turbidity reduction indicated by dashed line. Actual figures: $t=0$; 1875–2250 FTU (estimated); $t=1$ h; 3 FTU (*M. oleifera*), 1 FTU (*M. stenopetala*).

turbidity was measured by visual comparison to formazine standards prepared according to Danish Standard 290.

EXPERIMENTAL PROCEDURE

When *Moringa* seeds are used to remove turbidity by the traditional method, the seeds are crushed in mortars and then mixed with a small amount of water in a deep plate or a calabash. This is sometimes stirred for 10 to 20 min. The suspension is then poured into the turbid water, held in a water container, and left to settle. In most Sudanese villages a fresh supply of drinking water is brought twice daily, i.e. in the morning and before sunset.

In order to simulate Sudanese customs as closely as possible *Moringa* seeds were prepared as follows: the 'wings' but not the coat, of the seeds were removed, 2 g ($\simeq 10$ seeds) weighed out and crushed in a mortar. The seed powder was then mixed with 100 ml of tap water in a screw-capped glass bottle, shaken vigorously for 1 min and allowed to stand for 10 min. This suspension was filtered through a piece of filter gauze (pore size 100 μm), and the filtrate used within 1 h.

Turbidity removal was performed by adding 10 ml of the seed filtrate extract per litre of water (giving a final concentration of 200 mg l^{-1} seed material in the water). This mixture was agitated vigorously with a spoon for 2 min, stirred slowly for an additional 5 min and then left to stand. The water temperature was adjusted to 30°C and bacterial test strains added from stock cultures. In most experiments a series of three one-litre screw-capped glass jars were used. One acted as a control without addition of seed extract and the remaining two as experimental containers to which seed extract was added.

Samples for bacteriological analysis were obtained by pipetting off 1 ml of the supernatant water, the tip of the pipette being placed 5 cm below the water surface. In most cases water samples were taken 1, 3 and 24 h after start of coagulation. After 24 h the container was shaken vigorously and a final sample taken for analysis.

Results

NATURAL WATER FROM THE BLUE NILE COLLECTED DURING FLOODING PERIODS

Figure 1 shows the results of a coagulation experiment on a very turbid freshly collected water from the Blue Nile using *M. oleifera* (A) and *M. stenopetala* (B) seed material as coagulants. There was a very considerable fall in turbidity within 1 h from approximately 2000 FTU in the raw water to 1–2 FTU in the treated water. The turbidity reduction was accompanied by a reduction of the natural bacterial flora—approximately 2 log units (99%) in standard plate counts, and approximately 1–1.5 log units (90–97%) in presumptive coliform and faecal coliform counts. No major

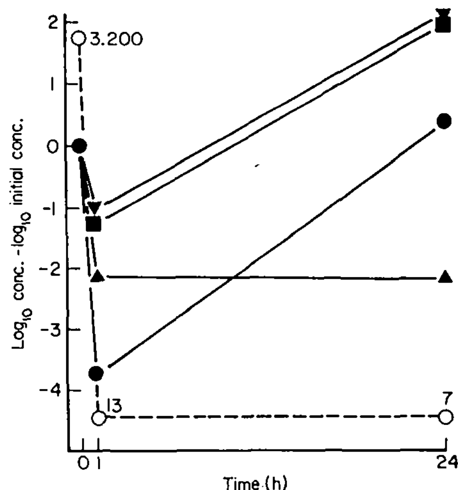


Figure 2. Effect of *M. oleifera* seed coagulation on turbidity and bacterial concentrations in raw Blue Nile water seeded with a mixture of *E. coli, Str. faecalis, Salm. typhimurium* and *Shig. sonnei* (flooding season, August 1982). Actual bacterial concentration at the start of the experiment was approximately 10^5 bacteria per ml. Plot figures are expressed as the logarithmic difference between the initial concentration and the concentration at various times during coagulation. (▼) *Salm. typhimurium*; (■) *Shig. sonnei*; (●) *E. coli*; (▲) *Str. faecalis*; (○) turbidity—FTU.

differences were observed between *M. oleifera* and *M. stenopetala* seed coagulation.

The results of a similar experiment using freshly collected very turbid Blue Nile water seeded with a mixture of *E. coli, Str. faecalis, Salm. typhimurium* and *Shig. sonnei* are shown in Figure 2. As was the case in the previous experiment turbidity was removed very efficiently, dropping from 3200 FTU in the raw water to 13 FTU after 1 h. However, there was almost no further reduction over the rest of the experimental period of 24 h.

Turbidity reduction was paralleled by a bacterial reduction of approximately 1 log unit (90%) for *Salm. typhimurium* and *Shig. sonnei*, 2 log units (99%) for *Str. faecalis* and almost 4 log units (99.99%) for *E. coli* within the first hours. Left to stand for 24 h the reduction of *Str. faecalis* was retained whereas the number of Enterobacteriaceae increased, *E. coli* regaining the jars less initial concentration and *Salm. typhimurium* and *Shig. sonnei* exceeding it by almost 2 log units (99%) after 24 h. There was also bacterial regrowth in an additional experiment in which the supernatant water phase was separated from the sediment. Thus, the secondary bacterial increase may be ascribed to an actual bacterial multiplication and regrowth in the water when left to stand. However, it cannot be completely excluded that there could have been a certain amount of bacterial release from the sediment.

NATURAL WATERS FROM THE WHITE NILE

In general, water from the White Nile is not subjected to the extreme fluctuations in turbidity characteristic of the Blue Nile and turbidity is generally low (Jahn & El Fadil 1984).

Figure 3 shows the results of an experiment using *M. oleifera* seeds on freshly collected White Nile water seeded with a mixture of *E. coli, Str. faecalis, Salm. typhimurium, Shig. sonnei* and *V. cholerae* (NAG). Turbidity fell from 50 FTU to 10 FTU after 1 h and rose again to 15 FTU after 24 h. Almost no reduction was observed in untreated water left to stand, reflecting the low turbidity of untreated White Nile water.

On average there were falls in primary bacterial counts of approximately 1 log unit (90%) after 1 h of coagulation. For the next 23 h *Str. faecalis* remained at almost unaltered concentrations, *E. coli* and *V. cholerae* (NAG) counts declined, while *Salm. typhimurium* and *Shig. sonnei* exhibited regrowth to a level approximately 2 log units (99%) above the initial concentration. Enterobacteriaceae multiplied in the untreated water.

The bacterial reduction obtained for *E. coli* and *Str. faecalis* may most easily be explained as a simple physical removal of bacteria together with the sediment, since resuspension (S in Figure 3) results in initial concentrations being obtained. Although a decrease in numbers with time was observed for *C. cholerae* (NAG), this occurred in both treated water and untreated controls.

WATER FROM AN IRRIGATION CANAL

The results of a similar experiment on freshly collected water from a more turbid irrigation canal are shown in Figure 4. Turbidity is

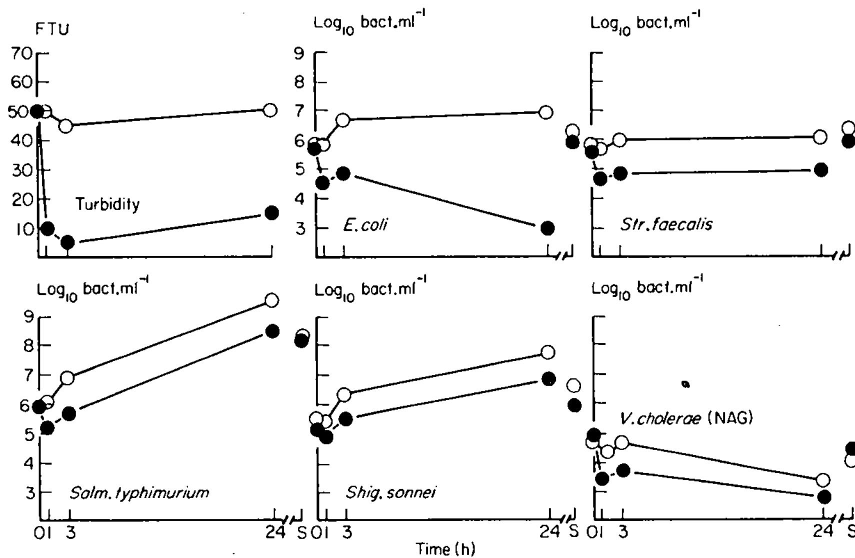


Figure 3. Effect of *M. oleifera* seed coagulation on turbidity and bacterial concentrations in raw White Nile water seeded with a mixture of *E. coli, Str. faecalis, Salm. typhimurium, Shig. sonnei* and *V. cholerae* (*NAG*) (April 1983). Total bacterial concentration in jar water after resuspension of sediment indicated as S. (●) Experimental jar with *M. oleifera* seed material, 200 mg l^{-1} added; (○) control jar, no coagulant added.

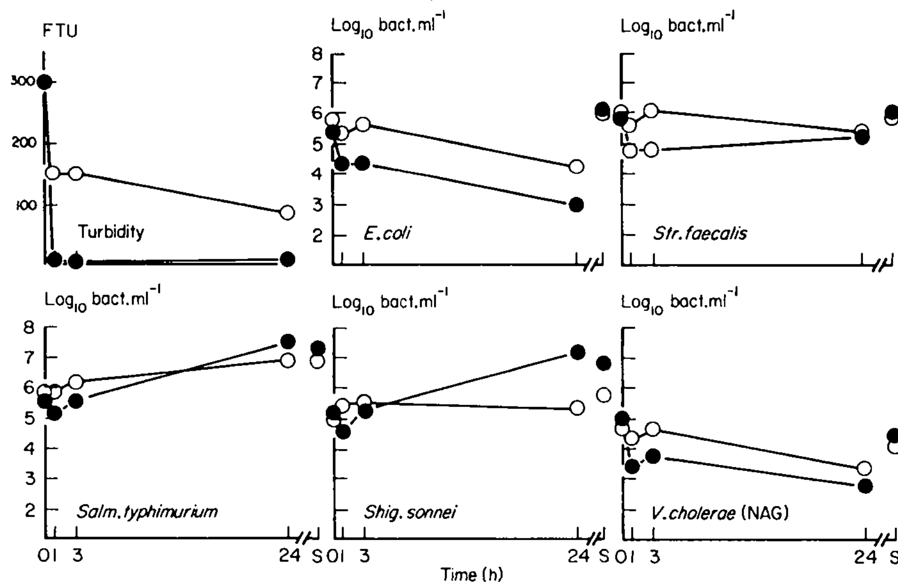


Figure 4. Effect of *M. oleifera* seed coagulation on turbidity and bacterial concentrations in raw water from an irrigation canal seeded with a mixture of *E. coli, Str. faecalis, Salm. typhimurium, Shig. sonnei* and *V. cholerae* (*NAG*) (April 1982). Total bacterial concentration in jar water after resuspension of sediment indicated as S. (●) Experimental jar with *M. oleifera* seed material, 200 mg l^{-1} added; (○) control jar, no coagulant added.

reduced from 300 FTU in the raw water to approximately 10 FTU within the first hour. In contrast to the previous experiment it may be seen from the control curve that part of the turbidity removal may be ascribed to settling of suspended solids by sedimentation, suggesting that the sediments are coarser than those in the Nile water.

An initial bacterial reduction of approximately 1 log unit (90%) was obtained within 1 h. After standing, the *Str. faecalis* count was almost unaltered, *E. coli* and *V. cholerae (NAG)* were further reduced while *Salm. typhimurium* and *Shig. sonnei* exhibited regrowth. By comparing the control curves it seems that this water type is somewhat less suitable than that from the White Nile for bacterial regrowth, *Salm. typhimurium* being the only species showing increasing concentrations in untreated controls.

ARTIFICIALLY PREPARED NILE WATER

The mean results of six separate laboratory experiments employing very turbid, artificially prepared Nile water (Table 1) seeded with a mixture of *E. coli, Str. faecalis, Cl. perfringens, Salm. typhimurium, Shig. sonnei* and *V. cholerae* (NAG) are shown in Figure 5. The general picture is comparable to the results obtained employing natural waters, i.e. a primary bacterial reduction after 1 h of coagulation amounting to 1–2 log units (90–99%) followed by regrowth of *Salm. typhimurium* and *Shig. sonnei* and a further decrease in the remaining four bacterial species in the supernatant water. By resuspension of the sediment (S in Figure 5) it may be seen that some inactivation of *V. cholerae* (NAG) and *Cl. perfringens* had occurred during the standing period, whereas *E. coli, Salm. typhimurium* and *Shig. sonnei* had multiplied. The artificially prepared Nile water was somewhat less suitable for bacterial multiplication than the natural Blue Nile water. Although *Salm. typhimurium* and *Shig. sonnei* actually regrew in treated water, it should be noted that their final concentrations after 24 h standing did not exceed the initial concentration.

Discussion

EFFECT ON THE BACTERIOLOGICAL QUALITY OF WATER

Tropical waters exhibit great variations in bacteriological counts. Untreated drinking water from surface sources such as ponds, irrigation canals and rivers often show heavy faecal pollution due to high water temperatures and a high load of organic material (Evison & James 1973; 1977; Muhammed & Morrison 1975; Egbuniwe 1978; El Attar *et al.* 1982; Wright 1982). The present results on the bacteriological quality of Blue Nile water (Figure 1) are comparable to the data on tropical waters compiled by Feachem (1980) and to the bacteriological data on White Nile water (Jahn & El Fadil 1984).

In terms of bacterial removal, *M. oleifera* is superior to other plant coagulant materials tested and as efficient as alum (Jahn & Dirar 1979; Finch & Smith 1986), bentonite clay (Steinmann & Havemeister 1982; Madsen & Schlundt 1987) and wood ash (Egbuniwe 1978).

Table 1. Characterization of the various water types employed in the coagulation experiments

	Turbidity (FTU)	COD (permanganate) ($mg\,l^{-1}$)	Total solids ($mg\,l^{-1}$)	Total hardness (°dH)*	Conductivity ($mS\,m^{-1}$)	pH
Blue Nile, September, 1982 (flooding season)	1400	167.0	2397	8.6	20.3	8.0
White Nile, April 1983	48	20.0	525	3.5	19.5	8.0
Irrigation canal, Soba, April 1983	170	51.0	1570	9.5	37.0	7.9
Artificial Nile water	1400	167.0	5385	13.5	57.2	7.9

*1°dH defined as the hardness caused by a content of 10 $mg\,l^{-1}$ CaO (Danish Standard DS 250).

We gratefully acknowledge the assistance of the Copenhagen Water Works Laboratory for performing the analysis.

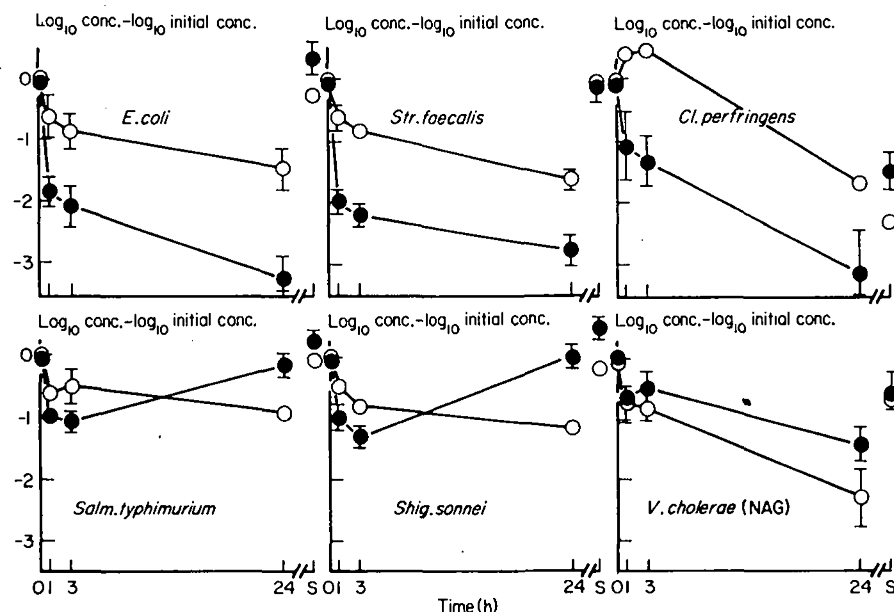


Figure 5. Effect of *M. oleifera* seed coagulation on bacterial concentrations in artificial Nile water seeded with a mixture of *E. coli, Str. faecalis, Cl. perfringens, Salm. typhimurium, Shig. sonnei* and *V. cholerae* (NAG).

Actual bacterial concentration at the start of the experiments was approximately 10^6 bacteria per ml. Plot figures are expressed as the logarithmic difference between the initial concentration and the concentration at various times during coagulation.

Plot figures based on six *M. oleifera* seed coagulation experiments (●) and two controls (○) (*Cl. perfringens, Shig. sonnei* one control). Vertical lines show ± standard error of mean.

(S) Total bacterial concentration in jar water after resuspension of sediment.

The present observations on the removal of enteric bacterial pathogens and indicator bacteria by Moringa seed coagulation are in agreement with the results of Jahn and Dirar (1979), Jahn (1981), Barth *et al.* (1982) and Grabow *et al.* (1985). Thus, a considerable hygienic improvement amounting to a primary bacterial reduction of 1–2 log units (90–99%) or more would seem to be obtained within 1 h by *Moringa* seed coagulation even under primitive conditions. It may be added that the best results were obtained employing very turbid natural waters from the Blue Nile. Reductions in turbidity are therefore associated with improvements in bacteriological quality, a feature which is well-known from flocculation and sedimentation procedures as applied to raw drinking water and sewage (Cox 1964; Steinmann & Havemeister 1982; Finch & Smith 1986).

Although a considerable hygienic improvement may be obtained in terms of primary bacterial reduction by *M. oleifera* seed treatment, it is quite clear from the present results that a treated water of high bacteriological quality when left to stand may deteriorate to be of an even poorer quality than untreated water due to regrowth of bacteria. These results are in agreement with the data reported by Jahn and Dirar (1979), Jahn (1981), Barth *et al.* (1982) and Grabow *et al.* (1985) on similar secondary increases of total bacteria counts and faecal coliforms, and similar bacterial regrowth has been observed following alum treatment (Jahn & Dirar 1979) and treatment employing naturally occurring Sudanese bentonite clays (Madsen & Schlundt 1987). Experimental parameters such as time, water temperature and watery type exert a profound influence on bacterial regrowth. As pointed out by Evison and James (1977) coliform regrowth conditions can be expected to occur regularly in polluted tropical waters where temperatures exceed 20°C. This feature is reflected in the real life situation by multiplication of at least some species of faecal bacteria in the environment under favourable conditions.

Since these results oppose the established concepts of water bacteriology in temperate

climates, and differ from similar experiments performed at lower water temperatures (Finch & Smith 1986) with respect to bacterial regrowth, it should be stressed that the experiments were designed with the purpose of simulating natural tropical water conditions as closely as possible.

It is of particular interest to note then that in the present experiments the indicator bacteria *Str. faecalis* and *Cl. perfringens* in no cases, and *E. coli* only in some cases, exhibited regrowth, whereas regrowth was a consistent feature of *Salm. typhimurium* and *Shig. sonnei*. It appears that in the presence of other actively growing bacterial species, *E. coli* seems unable to compete, an absence of regrowth being the result. It is therefore to be expected that under natural conditions in polluted waters at high temperatures a low level of *E. coli* does not necessarily indicate the absence of enteric pathogens, as previously reported by Gallagher and Spino (1968). Regrowth of *V. cholerae* (NAG) was not observed under the present experimental conditions, which is in contrast to similar experiments performed with another natural Sudanese coagulant—bentonite clay (Madsen & Schlundt 1987).

These conclusions suggest that the validity of the application of the traditional water quality bacterial indicators such as *E. coli*, *Str. faecalis* and *Cl. perfringens* to tropical waters should be questioned. Due to the higher level of pathogenic bacteria present in tropical waters, and to the apparently poor correlation between enteric pathogens and the indicator bacteria relied upon in temperate waters, the detection of the pathogens themselves may be more appropriate.

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