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LOW TECHNOLOGY WATER PURIFICATION BY BENTONITE CLAY FLOCCULATION AS PERFORMED IN SUDANESE VILLAGES: BACTERIOLOGICAL EXAMINATIONS*

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Abstract—The effects of a water purification method traditionally used in Sudan to treat turbid waters were studied with respect to removal of faecal indicator bacteria as well as selected enteric bacterial pathogens. Water treatment was performed with natural bentonite clays (rauwaq) from the banks of the Nile, and the technique employed corresponded closely to that used to clarify Nile water in Sudanese villages. Employing various types of waters a primary bacterial reduction of 1–3 log units (90–99.9%) was obtained within the first 1–2 h of flocculation. During the 24 h observation period bacterial multiplication in the water phase occurred consistently for *Vibrio cholerae* and test organisms belonging to the Enterobacteriaceae group, but not for *Streptococcus faecalis* and *Clostridium perfringens*. Some of the conditions influencing the hygienic effects obtained were examined. The potential and limitations of the method as a local alternative in water improvement are discussed.

Key words—water purification, flocculation, bentonite, montmorillonite, rauwaq, Sudanese villages, *Escherichia coli*, *Streptococcus faecalis*, *Clostridium perfringens*, *Salmonella typhimurium*, *Shigella sonnei*, *Vibrio cholerae*

INTRODUCTION

During the flood season the Blue Nile, and hence also the Main Nile north of Khartoum, become highly turbid due to the copious rains in the Ethiopian highlands (Mancy and Hafez, 1979). At the peak of the flood season the amount of suspended solids may exceed 8000 mg/l (Jahn, 1976). Turbidity maxima of 3000–4000 formazine turbidity units (FTU) have been recorded (Jahn and Omer, 1984).

For some hundred years or more, the women in the rural communities along the Nile valley have been employing local water purification methods to remove turbidity. A number of natural flocculating or coagulating agents of plant and soil origin exist. Their distribution and local use were extensively reviewed by Jahn (1977, 1981).

One of the methods employed in Nile villages is based upon the flocculating properties of a certain clay type called "rauwaq" (clarifier) occurring in certain sites and varying qualities at the river banks. Roentgenographic analyses have revealed the main component of rauwaq to be montmorillonite (bentonite) (Jahn, 1976), a clay mineral also used for flocculation of suspended solids in conventional water works (Cox, 1964). Some skill is required by the women to carry out the clarification satisfactorily since a proper concentration depending on the particular water and rauwaq has to be chosen, whereas concentrations above and below the optimal one will leave the water turbid. In most cases additions of 10–30 g rauwaq/l water is used (Jahn, 1981).

No data are available about the hygienic effect of the method on bacterial pathogens present in untreated water, although it is claimed that villagers treating their water have lower incidence rates of gastro-intestinal diseases. A preliminary investigation by Jahn (1976) indicated some removal of coliforms to take place during the process.

As part of a joint study on the hygienic aspects of the purification method the present paper deals with the results of the bacteriological studies, whereas the results of virological examinations have been reported by Lund and Nissen (1986) and the parasitological results by Olsen (1987). Preliminary results on the bacteriological work were presented by Schlundt and Madsen at the British–Scandinavian Joint Meeting on Tropical Medicine and Parasitology, Copenhagen 1985.

MATERIALS AND METHODS

Flocculants

Samples of dried rauwaq (bentonite clay) of good quality, as indicated by Sudanese villagers (Lund and Nissen, 1986),

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

	Turbidity (FTU)	COD (permanganate) (mg/l)	Total solids (mg/l)	Total hardness (°dH)*	Conductivity (mS/m)	pH
Tap water	0.195	7.0	425	13.5	56.2	8.2
Pond water	23	166.0	660	20.2	—	7.5
Sewage water	35	133.0	1390	31.6	—	7.8
Artificial Nile water	1400	167.0	5385	13.5	57.2	7.9
Blue Nile, Sept. 1982 (flooding season)	1400	167.0	2397	8.6	20.3	8.0
Blue Nile, April 1983 (dry season)	7.8	12.6	340	5.7	16.5	7.8
White Nile, April 1983	48	20.0	52.5	3.5	19.5	8.0
Irrigation canal, Soba, April 1983	170	51.0	1570	9.5	37.0	7.9

*1 °dH defined as the hardness caused by a content of 10 mg/l CaO (Danish Standard DS 250). We gratefully acknowledge the assistance of the Copenhagen Water Works Laboratory for kindly performing the analysis.

were collected from Wad el Said, Alti and Eseba. These samples were used for flocculation experiments during the first months of the study.

As from September 1982 and throughout the rest of the project period until termination July 1983 one single batch of rauwaq from the Nile village Kutranj was employed. The sample was collected during a field visit in the rainy season by the authors and brought still moist and smooth to Copenhagen.

Water types

Seven different 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 approx. 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 water types employed included: (4) unchlorinated Copenhagen tap water; (5) pond water from a highly turbid duck pond of the University park; (6) sewage water collected at the inlet to one of the Copenhagen sewage treatment plants; (7) "artificial" Nile water intended to simulate the water conditions of Sudan during river flooding periods. This water was prepared by adding 6.64 g of fresh mud deposits collected from the Nile bank in the flooding season and 0.1 g of Bacto peptone to 1.0 litre of unchlorinated Copenhagen tap water (Madsen *et al.*, 1987).

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 to the Blue Nile water, suggesting some aggregation of particles in the former.

Experiments designated to elucidate the possible effects of different water temperature levels were conducted at 20, 30 and 37°C (Table 4), whereas experiments simulating natural conditions were carried out at a water temperature of 30°C, the prevalent water temperature of the Nile water during the summer months (Jahn and Omer, 1984).

Bacterial strains

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). A single experiment was carried out using a saprophytic *Mycobacterium* sp. isolated from the soil of the University park as test organism.

In experiments simulating natural conditions the test strains were added as a mixture to each sample of water, before addition of rauwaq, to obtain initial concentrations of approx. 10^6 viable bacteria/ml of untreated water. Experiments directed at the detection of possible effects of different bacterial concentrations in the untreated water (Table 5) were carried out employing concentrations of 10^4 and 10^6 viable bacteria/ml respectively.

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 (Merck) with an addition of 20 μg/ml 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 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)]
(7) 5% blood agar [tryptose blood agar base (Difco), calf blood] (*Mycobacterium* sp.)

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), or the agar medium was incubated by the high agar tube technique described by the Nordic Committee for Food Analysis (NMKL 56).

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

Statistical methods

The data were analyzed using the "Statistical Analysis System" (Barr *et al.*, 1976). The influence of different experimental conditions on the hygienic efficiency of the flocculation was examined in an analysis of variance of log bacteria/ml defined in a three-factor main effects model

using water type, water temperature and the initial bacterial concentration as the independent variables (Tables 3–5). The possibility of interactions was examined in separate analyses, but no significant crossed or nested effects were found.

Experimental procedure

For each type of the rauwaqs tested the optimal concentration for flocculation was estimated in preliminary experiments by appearance after adding increasing amounts of rauwaq to 250 ml of the water type to be investigated. The samples were left to stand at room temperature (20–22°C) and observed for turbidity after 1, 2 and 3 h. No major differences in optimum concentrations were observed when testing one particular rauwaq type in the different water types employed whereas differences in flocculation ability were observed between rauwaq samples of different origins. The optimal concentrations for flocculation were estimated as follows: Wad el Said and Alti samples 30 g/l, Eseba 20 g/l and Kutranj 10 g/l. These concentrations were consequently employed in the rest of the experiments.

In most experiments a series of three one-litre screw-capped glass jars were used. One acted as a control without addition of rauwaq and the remaining two as experimental containers to which rauwaq was added. Prior to flocculation the rauwaq was prepared according to Sudanese custom by making up a slurry consisting of the desired amount of rauwaq and 100 ml of the water to be tested. Bacterial test strains were added from stock cultures to the remaining 900 ml of water to make up final concentrations of 10^4 or 10^6 bacteria/ml water. The rauwaq slurry was then added to the water, preadjusted to the appropriate temperature, and mixing was performed by shaking the container which was left to stand for 24 h. In most cases flocculation was visible within the first 10–15 min after the start of the experiment.

As an alternative to this procedure, in the first experiments the bacteria were added and allowed to adapt to the water by standing overnight at 4°C before flocculation was carried out. No differences in removal capacity as compared to the simultaneous addition of bacteria were observed, though, and for the rest of the experiments bacteria were added to the water immediately before the addition of the rauwaq slurry, as described above.

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. After 24 h the flocculated sediment was resuspended by shaking the container 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 flocculation experiment with Kutranj rauwaq on a very turbid freshly collected water from the Blue Nile seeded with a mixture of *E. coli*, *Str. faecalis*, *Salm. typhimurium* and *Shig. sonnei*. There was a very considerable fall in turbidity within 1 h from approx. 3200 FTU in the raw water to 325 FTU in the treated water. Over the rest of the experimental period of 24 h there was a further reduction down to 60 FTU.

Turbidity reduction was parallelled by a bacterial reduction of approx. 1–1.5 log units (90–96.9%) for *Salm. typhimurium*, *Shig. sonnei* and *Str. faecalis*, and almost 3 log units (99.9%) for *E. coli* within the first hour. Left to stand for 24 h the reduction of *Str. faecalis* was retained whereas the number of Enterobacteriaceae increased, *Salm. typhimurium* and *Shig. sonnei* exceeding the initial concentration by almost 2 log units (99%), *E. coli* remaining approx. 1 log unit (90%) below the initial concentration after 24 h. There was also bacterial regrowth in additional experiments (Table 6) 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.

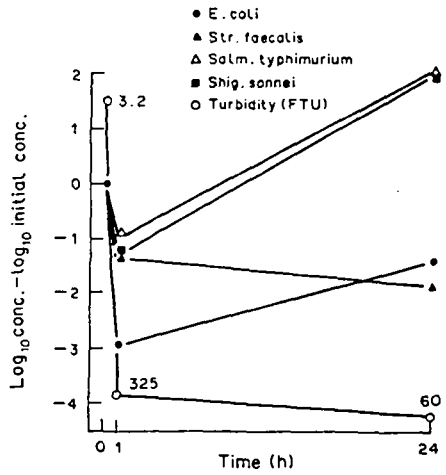

Fig. 1. Effect of rauwaq flocculation on turbidity and bacterial concentrations in raw Blue Nile water seeded with a mixture of *E. coli*, *Str. faecalis*, *Salm. typhimurium* and *Shig. sonnei* (flooding season, September 1982). Actual bacterial concentration at the start of the experiment was approx. 10^5 bacteria/ml. Temperature: 30°C. Plot figures are expressed as the logarithmic difference between the initial concentration and the concentration at various times during flocculation.

Natural waters from the White Nile, and from an irrigation canal

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 and Omer, 1984).

Table 2(a) shows the results of a flocculation experiment with Kutranj rauwaq performed on freshly collected White Nile water seeded with a mixture of *E. coli*, *Str. faecalis*, *Salm. typhimurium* and *Shig. sonnei*. In this experiment addition of rauwaq actually resulted in an increased turbidity of the treated water, whereas no change was observed in untreated water left to stand, reflecting the low turbidity of raw White Nile water.

Despite no turbidity reduction being obtained in the treated water there were falls in primary bacterial counts of 1–3 log units (90–99.9%) after 1 h of treatment. For the next 23 h there was a further decrease

Table 2. Effect of rauwaq flocculation on *E. coli*, *Str. faecalis*, *Salm. typhimurium*, and *Shig. sonnei*: (a) in water from the White Nile and (b) in water from an irrigation canal. Temperature: 30°C. S indicates total bacterial concentration in the jar water after resuspension of flocculated sediment. Experiment performed in Sudan, April 1983

Time (h)	Turbidity (FTU)		*E. coli* (log bact./ml)			*Str. faecalis* (log bact./ml)			*S. typhimurium* (log bact./ml)			*Shig. sonnei* (log bact./ml)		
	Untreated	Treated	Untreated	Treated	% Reduction	Untreated	Treated	% Reduction	Untreated	Treated	% Reduction	Untreated	Treated	% Reduction
(a) White Nile														
0	50	50	5.90	5.90	0	5.91	5.91	0	6.05	6.05	0	5.52	5.52	0
1	50	150	5.91	2.78	99.93	5.69	4.18	96.91	6.13	4.88	94.38	5.38	4.29	91.87
3	45	90	6.76	2.60	99.99	6.03	3.71	99.52	6.96	5.19	98.30	6.28	3.92	99.56
24	50	30	6.96	3.65	99.95	6.11	3.19	99.88	9.56	8.33	94.11	7.70	7.14	72.46
S	—	—	6.33	5.86	—	6.11	5.83	—	8.31	7.99	—	6.60	6.44	—
(b) Irrigation canal														
0	300	300	5.76	5.76	0	5.99	5.99	0	5.85	5.85	0	5.48	5.48	0
1	150	40	5.30	3.38	98.78	5.61	4.10	96.91	5.87	4.51	95.83	5.40	3.90	96.84
3	150	30	5.62	3.04	99.74	6.09	3.71	99.58	6.20	4.81	95.93	5.48	3.30	99.34
24	70	20	4.18	3.14	90.88	5.28	3.19	99.19	6.84	6.58	45.05	5.30	5.00	49.88
S	—	—	6.02	5.90	—	5.79	6.22	—	6.83	6.20	—	5.74	5.49	—

of 1 log unit (90%) for *Str. faecalis*, while concentrations of *E. coli*, *Salm. typhimurium* and *Shig. sonnei* increased by 1–3 log units (90–99.9%). However, Enterobacteriaceae also multiplied in the untreated water and it should be noted that treated water remained of a better bacteriological quality than untreated water left to stand.

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 Table 2) results in initial concentrations being obtained.

The results of a similar experiment on freshly collected water from a more turbid irrigation canal are shown in Table 2(b). Turbidity was reduced from 300 FTU in the raw water to 40 FTU in treated water within the first hour. In contrast to the previous experiment turbidity figures from untreated controls indicate that part of the turbidity removal may be ascribed to settling of suspended solids by sedimentation, suggesting that the sediments are coarser than these in the Nile water.

An initial bacterial reduction of approx. 2 log units (99%) when compared to untreated controls was obtained within 1 h. After standing, *Str. faecalis* was further reduced, *E. coli* was almost unaltered while *Salm. typhimurium* and *Shig. sonnei* exhibited regrowth. By examination of the control figures it seems that this water type was somewhat less suitable than that from the White Nile for bacterial regrowth, *Salm. typhimurium* being the only species showing increasing concentrations in untreated controls.

Tap water, pond water and sewage

The removal efficiency of the purification method was further characterized in laboratory experiments in Copenhagen with *E. coli*, *Str. faecalis* and *Cl. perfringens* as test organisms exposed to different water temperatures (20, 30 and 37°C) and different initial bacterial concentrations (10^4 and 10^6 bacteria/ml).

Figure 2 shows the mean results of a number of experiments, to give an overall expression of the potential of the method when carried out under different experimental conditions, although some of the investigated experimental factors have been found to exert a significant influence on the hygienic effects obtained (see below). In accordance with the previous experiments performed on natural Sudanese waters the main effect was obtained within the first 1–3 h of treatment, 1 log unit (90%) of bacterial reduction in the supernatant water being achieved on an average. Thereafter, *E. coli* exhibited regrowth while concentrations of *Str. faecalis* and *Cl. perfringens* declined. By resuspension of the flocculated sediment after 24 h (S in Fig. 2) an increase in the total bacterial concentration could be demonstrated, indicating that the removed bacteria remain viable and concentrated in the flocculated sediment.

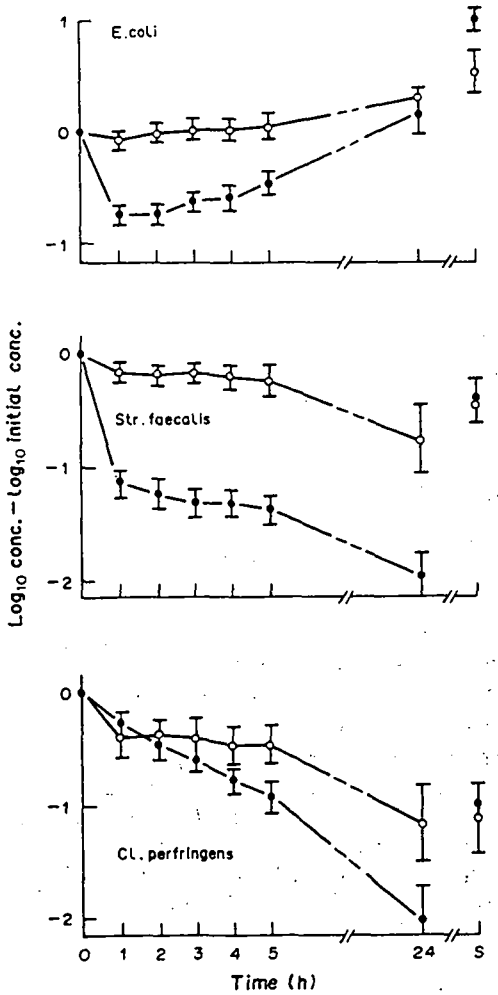

Fig. 2. Effect of rauwaq flocculation on bacterial concentrations in different types of water (tap water, pond water and sewage) seeded with a mixture of *E. coli*, *Str. faecalis* and *Cl. perfringens*. Actual bacterial concentration at the start of the experiments varied from 10^4 to 10^6 bacteria/ml. Plot figures are expressed as the logarithmic difference between the initial concentration and the concentration at various times during flocculation. Plot figures for *E. coli* and *Str. faecalis* based on 44 experiments and 22 controls, for *Cl. perfringens* on 36 experiments and 18 controls. Standard error of mean indicated by vertical lines.

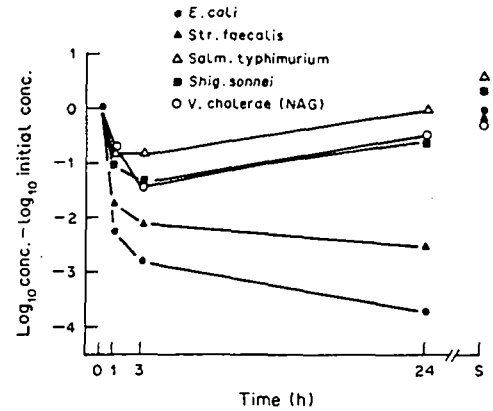

Fig. 3. Effect of rauwaq flocculation on bacterial concentrations in artificial Nile water seeded with a mixture of *E. coli*, *Str. faecalis*, *Salm. typhimurium*, *Shig. sonnei* and *V. cholerae* (NAG). Actual bacterial concentration at the start of the experiments was approx. 10^6 bacteria/ml. Temperature: 30°C. Plot figures are expressed as the logarithmic difference between the initial concentration and the concentration at various times during flocculation. Plot figures based on 5 experiments.

Artificial Nile water

The mean results of five separate laboratory experiments employing very turbid, artificially prepared Nile water (Table 1) seeded with a mixture of *E. coli*, *Str. faecalis*, *Salm. typhimurium*, *Shig. sonnei* and *V. cholerae* (NAG) are shown in Fig. 3. The general picture is comparable to the results obtained employing natural waters, i.e. a primary bacterial reduction after 1–3 h of flocculation amounting to 1–3 log units (90–99.9%) followed by regrowth of *Salm. typhimurium*, *Shig. sonnei* and *V. cholerae* (NAG) and a further decrease in the concentration of *E. coli* and *Str. faecalis* in the supernatant water. By resuspension of the sediment (S in Fig. 3) initial bacterial concentrations were obtained. The artificial Nile water was somewhat less suitable for bacterial multiplication than the natural Blue Nile water. Although *Salm. typhimurium*, *Shig. sonnei* and *V. cholerae* (NAG) actually regrew in treated water it should be noted that their final concentrations after 24 h did not exceed the initial concentration.

A single experiment was carried out with a *Mycobacterium* sp. in artificial Nile water. The effects obtained were closely comparable to the results previously presented for *Str. faecalis* and *Cl. perfringens*, i.e. a primary bacterial reduction of approx. 2 log units (99%) followed by a slow decrease during the 24 h standing period. Resuspension of the sediment demonstrated that only minimal inactivation of bacteria occurred during the process.

Influence of water type, water temperature and the initial bacterial concentration

The data for *E. coli* and *Str. faecalis* from the experiments on tap water, pond water and sewage water (Fig. 2) were examined in an analysis of variance of log (bacterial concentration) using water type, water temperature and the initial bacterial concentration as the independent variables.

The results of the analysis as regards the influence of water type are shown in Table 3. No significant difference in bacterial reduction was detected between the three water types during the first 5 h whereas standing for 24 h resulted in significant differences between water types, both in the supernatant water and in the flocculated sediment. The differences were most pronounced for *E. coli*. In sewage water the concentration of *E. coli* remained approx. 0.5 log units below the initial concentration after 24 h standing; by resuspension of the sediment the initial

Table 3. The influence of water type on the hygienic effect of rauwaq (bentonite clay) flocculation as monitored with *E. coli* and *Str. faecalis*

	E. coli				*Str. faecalis*			
Hours	Significance of difference†	Log (conc.) – Log (initial conc.)			Significance of difference	Log (conc.) – Log (initial conc.)		
		Tap water (n = 14)	Pond water (n = 12)	Sewage water (n = 18)		Tap water (n = 14)	Pond water (n = 12)	Sewage water (n = 18)
0		0	0	0		0	0	0
1		−0.6	−0.9	−0.8		−1.2	−1.2	−1.1
2		−0.8	−0.6	−0.8		−1.3	−1.2	−1.2
3		−0.7	−0.5	−0.7		−1.4	−1.3	−1.3
4		−0.8	−0.4	−0.6		−1.5	−1.2	−1.3
5		−0.7	−0.3	−0.5		−1.5	−1.3	−1.4
24	***	1.0	0.2	−0.5	*	−2.5	−1.9	−1.6
S‡	***	2.0	1.4	0.0	*	−0.8	−0.3	−0.2

†Differences evaluated with an F-test in an analysis of variance; model: bacterial concentration = water type + temperature + initial bacterial concentration; significance levels = $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
‡S indicates resuspension of flocculated sediment after 24 h.

concentration was obtained indicating that no inactivation of bacteria had occurred. As regards tap water and pond water the supernatant water concentration as well as the total concentration of *E. coli* following resuspension after 24 h exceeded the initial concentration, indicating that regrowth had occurred both in the supernatant water and in the sediment. With regard to *Str. faecalis* significant but less marked differences were observed between the water types employed. In all cases the concentration of *Str. faecalis* was well below the initial concentration (1.6–2.5 log units) after 24 h; resuspension of the sediment increased the bacterial concentrations, but not above initial concentrations. From these results it may be concluded that differences between the three investigated water types do not appear to influence the primary removal efficiency on *E. coli* and *Str. faecalis* during the flocculation process. Significant differences between water types after 24 h standing probably reflect different conditions for bacterial regrowth and survival, in accordance with the results obtained with the natural Sudanese waters.

In Table 4 the results of the analysis on the influence of water temperature are shown. No significant differences in bacterial reduction in the supernatant water phase were observed for *Str. faecalis* between the three investigated temperature levels; survival in the sediment was most pronounced at 30°C. As regards *E. coli* significant differences were not noted during the first 2 h, but thereafter the bacterial reductions rapidly diminished in the 30°C containers exceeding the initial concentration by almost 1 log unit after 24 h. Regrowth occurred at 20 and 37°C as well, but at a slower rate, and the bacterial concentration in the supernatant water at 24 h did not exceed the initial concentration.

The results of the analysis on the influence of two different initial bacterial concentrations are shown in Table 5. A significant better removal of *Str. faecalis* was obtained in experiments employing initial bacterial concentrations of 10^4/ml, although the difference was not significant after 24 h. A similar, but less pronounced pattern was observed for *E. coli*. The results imply that the removal capacity of rauwaq flocculation is less efficient if the water is too heavily loaded with bacteria, probably relating to an adhesion of the bacteria to suspended matter prior to and during the flocculation process.

Bacterial regrowth

The results of an experiment investigating the bacterial regrowth are shown in Table 6. Rauwaq flocculation was performed on artificial Nile water seeded with *E. coli*, *Salm. typhimurium* and *V. cholerae* (NAG) either as monocultures or as a mixed culture. After 1 h, a 100 ml sample of the supernatant water was taken and followed separately.

Table 4. The influence of water temperature on the hygienic effect of rauwaq flocculation as monitored with *E. coli* and *Str. faecalis*

	E. coli				*Str. faecalis*			
Hours	Significance of difference†	Log (conc.) – Log (initial conc.)			Significance of difference	Log (conc.) – Log (initial conc.)		
		20°C (n = 14)	30°C (n = 14)	37°C (n = 16)		20°C (n = 14)	30°C (n = 13)	37°C (n = 16)
0		0	0	0		0	0	0
1		−0.8	−0.7	−0.8		−1.2	−1.3	−1.0
2		−0.8	−0.7	−0.8		−1.3	−1.5	−1.0
3	**	−0.8	−0.3	−0.7		−1.5	−1.5	−1.1
4	**	−0.9	−0.2	−0.7		−1.4	−1.5	−1.1
5	**	−0.9	−0.1	−0.5		−1.6	−1.5	−1.2
24	**	−0.1	0.9	−0.3		−2.2	−1.8	−1.9
S‡	***	0.9	1.8	0.4	**	−0.3	−0.1	−0.7

†Differences evaluated with an F-test in an analysis of variance; model: bacterial concentration = water type + temperature + initial bacterial concentration; significance levels = $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
‡S indicates resuspension of flocculated sediment after 24 h.

Table 5. The influence of the initial bacterial concentration on the hygienic effect of rauwaq flocculation as monitored with *E. coli* and *Str. faecalis*

Hours	*E. coli* Significance of difference†	*E. coli* Log (conc.) − Log (initial conc.) Initial conc.: 10^4/ml (n = 24)	*E. coli* Log (conc.) − Log (initial conc.) Initial conc.: 10^6/ml (n = 20)	*Str. faecalis* Significance of difference	*Str. faecalis* Log (conc.) − Log (initial conc.) Initial conc.: 10^4/ml (n = 23)	*Str. faecalis* Log (conc.) − Log (initial conc.) Initial conc.: 10^6/ml (n = 20)
0		0	0		0	0
1		−0.7	−0.8	**	−1.4	−0.8
2		−0.8	−0.7	***	−1.6	−0.9
3	*	−0.8	−0.5	**	−1.7	−0.9
4	*	−0.8	−0.4	**	−1.7	−0.9
5	**	−0.7	−0.2	**	−1.7	−1.0
24		0.2	0.1		−2.1	−1.8
S‡		1.1	0.9		−0.5	−0.3

†Differences evaluated with an F-test in an analysis of variance; model: bacterial concentration = water type + temperature + initial bacterial concentration; significance levels = $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
‡S indicates resuspension of flocculated sediment after 24 h.

After an initial reduction *Salm. typhimurium* and *V. cholerae* (NAG) exhibited the characteristic concentrational increase as seen in the previous experiments. No differences were apparent whether monocultures or mixed cultures were employed. As regards *E. coli* in monoculture the pattern followed the course described above for *Salm. typhimurium* and *V. cholerae* (NAG), whereas the concentration of *E. coli* in mixed culture exhibited a steady decline throughout the experiment. Apart from proving that the observed increase in bacterial concentrations in the previous experiments is actually caused by proper bacterial regrowth the present experiment also indicates that *E. coli* is not always able to compete with actively growing pathogenic bacterial species like *Salm. typhimurium* and *V. cholerae* (NAG).

Survival of bacteria in flocculated sediments

Common practices of water purification by rauwaq flocculation in Sudanese villages includes a disposal of the flocculated sediment by simply throwing it into the yard or out into the street before refilling the jar with fresh Nile water. The results of experiments estimating the survival of *E. coli*, *Str. faecalis*, *Cl. perfringens*, *Salm. typhimurium* and *Shig. sonnei* in mud deposits exposed to Sudanese climatic conditions are shown in Table 7. The bacterial test strains were mixed with Nile mud deposits, water and rauwaq in proportions simulating flocculated sediment, placed in Petri dishes and exposed in the open to sunlight and wind. The Gram-negative bacteria *E. coli*, *Salm. typhimurium* and *Shig. sonnei* died out very

Table 6. Effect of rauwaq flocculation on *E. coli*, *Salm. typhimurium*, and *V. cholerae* (NAG) as monocultures or mixed cultures in artificial Nile water. Temperature: 30°C. The regrowth was monitored in 100 ml samples of supernatant water separated from the flocculation jar after 1 h. Bacterial concentrations expressed as $\log_{10}$ bact./ml

Time (h)	*E. coli* Separate	*E. coli* Mixture	*Salm. typhimurium* Separate	*Salm. typhimurium* Mixture	*V. cholerae* (NAG) Separate	*V. cholerae* (NAG) Mixture
0	3.65	4.04	4.23	4.15	2.90	2.82
1	1.00	2.11	3.90	3.72	1.78	2.11
3	1.30	1.60	3.78	3.79	1.30	1.00
24	1.48	<1	4.73	4.92	1.90	1.78

Table 7. The survival of *E. coli*, *Str. faecalis*, *Cl. perfringens*, *Salm. typhimurium*, and *Shig. sonnei* in mud deposits (simulated flocculated sediment; see text) exposed to Sudanese climate conditions during April 1983. Sunshine, day temperatures 35–45°C, night temperatures 25–35°C, no precipitation. (Figures average of 4 experiments, bacterial concentrations expressed as $\log_{10}$ bact./g dry matter)

Days	*E. coli*	*Str. faecalis*	*Cl. perfringens*	*Salm. typhimurium*	*Shig. sonnei*
0	8.72	8.58	8.51	8.23	8.53
1	2.66	7.33	6.32	2.20	ND
2	ND*	7.16	5.80	ND	—
3	—†	6.85	5.56	—	—
4	—	6.98	5.49	—	—
5	—	7.07	5.77	—	—
7	—	6.47	5.15	—	—
9	—	7.13	4.80	—	—
12	—	5.91	4.03	—	—
33	—	3.26	2.00	—	—

*ND = not detectable, i.e. <100 bact./g dry matter (detection limit).
†— no test performed.

quickly, i.e. down to negligible concentrations below the detection limit of 100 bacteria/g dry matter in 1–2 days. It should be added, though, that *Salm. typhimurium* could be isolated by selective enrichment procedures from the terminal samples after 33 days of exposure. As regards *Str. faecalis* and *Cl. perfringens* the elimination was slow, both species were isolated from all samples taken during the experimental period of 33 days.

Toxicity and mutagenicity

Water samples originating from the White Nile and an irrigation canal (Table 1) were tested for endotoxin (LPS) contents in the *Limulus* Amoebocyte Lysate (LAL) assay immediately before, respectively after 3 h of rauwaq flocculation. Different endotoxin levels were recorded in the two types of water, i.e. White Nile 2.5–5 ng endotoxin/ml water, irrigation canal 50–100 ng/ml water, but no significant differences were observed between the untreated water samples and samples taken after 3 h of flocculation.

Ames *Salmonella* mutagenicity assays were carried out testing rauwaq clay samples originating from Kutranj. The assays were performed as duplicate plate incorporation tests using tester strains TA98 and TA100 in the presence or absence of liver preparations, done in 5-fold including positive, negative and sterility controls. No toxic effects of the test material on tester strains were observed. Concentrations of up to 100 g/l of rauwaq had no detectable mutagenic effect on the tester strain TA100, whereas a weak mutagenic response was detected in the tester strain TA98 when rauwaq concentrations of 20 and 100 g/l were tested. However, no mutagenic effects were observed at these levels either if the rauwaq sample was autoclaved before testing and the tester strain TA98 was pre-incubated with the test solution for ten minutes before plate inoculation. It is thus possible that the observed response may be attributed to organic impurities in the rauwaq sample tested.

DISCUSSION

Removal of bacteria with bentonite clay

Bentonite either in pure form or as naturally occurring bentonite-containing clay minerals has for some considerable time been used to assist flocculation in the treatment of municipal and industrial wastewater as well as raw surface water prior to consumption (Louis, 1956; Rebhun *et al.*, 1974; Brecht *et al.*, 1974; Delaine, 1978). As pointed out by Lund and Nissen (1986) the role played by bentonite clays in the flocculation process may largely be ascribed to the increase of the water's exchange capacity by addition of bentonite.

Reports on bentonite flocculation of turbid waters have indicated that very good removal of enteric viruses (Lund and Nissen, 1986) and *Schistosoma* cercariae (Olsen, 1987) may be obtained even under primitive conditions. As regards the specific hygienic effect of bentonite flocculation on bacterial pathogens and indicator bacteria no reports seem available. In general, the estimated removal capacity of flocculation as performed in surface water treatment for drinking water purposes seems to constitute around 0.7–1 log unit (80–90%) reduction with respect to coliforms and faecal coliforms (Kool, 1979). In field and laboratory experiments employing treated sewage and various flocculants a bacterial reduction of 0.3–3 log units (47.9–99.9%) was obtained (Steinmann and Havemeister, 1982; Finch and Smith, 1986).

The results of the present report indicate that even under primitive conditions such as with bentonite flocculation in a water jar a primary bacterial reduction of 1–3 log units (90–99.9%) or more may be obtained, which is a considerable hygienic improvement. In contrast to removal of viruses and metazoic parasites by flocculation, however, experimental parameters like time, water temperature and water type exert a profound influence on the further changes in bacterial contents of the treated waters due to bacterial multiplication and regrowth. 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, a fact which must be considered when recommendations with respect to application and extension of the present method are elaborated.

Regrowth of faecal bacteria

As regards coliform bacteria it has been documented that multiplication in aquatic environments frequently occurs (Deaner and Kerri, 1969; Dutka, 1973), whereas *E. coli* in temperature climates usually is regarded as a reliable indicator of faecal pollution in water sources, although isolated cases of regrowth of *E. coli* associated with rotting vegetation and elevated temperatures have been reported (Robertson *et al.*, 1966; Taylor, 1972). Regrowth of faecal streptococci in aquatic environments has not been demonstrated.

The most important determinants of regrowth or die-off of faecal bacteria in water include water temperature, organic matter, effective surface and the presence of indigenous bacterial populations. Water temperatures around 10°C regularly result in a steady die-off of coliforms as well as faecal streptococci (McFeters *et al.*, 1974) whereas temperatures exceeding 20°C lead to growth of both coliforms and *E. coli* (Evision and James, 1973, 1977). In contrast to the very high nutritional requirements of faecal streptococci, regrowth of *E. coli* has been observed in water containing as little as 0.28 ppm organic matter in solution (Allen *et al.*, 1952). The effect of a very often complex, indigenous bacterial flora is difficult to assess, but it may be assumed that complete or partial removal of the indigenous flora favours a rapid regrowth of remaining bacteria with short generation

intervals (Lonsane *et al.*, 1967). The observations of a separate study carried out by the authors in Sudan in which a secondary regrowth of coliforms amounting to 2 log units in a water reservoir following an initial coliform reduction of 1 log unit by sand filtration seem to support this view.

The regrowth of *E. coli*, *Salm. typhimurium*, *Shig. sonnei* and *V. cholerae* (NAG) following an initial removal by rauwaq flocculation as observed in the present study conforms to the results cited above. The consistent regrowth of the enteric pathogens may then be explained by the fact that the experiments were performed at 30°C employing waters rich in organic material in order to simulate natural conditions. As regards *E. coli* it may be concluded that although regrowth was observed in most experiments it was recorded also under certain conditions, i.e. in the presence of other actively growing species, *E. coli* seemed unable to compete, an absence of regrowth being the result. It is thus 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).

Potential of water purification by rauwaq flocculation

From the presented results it may be concluded that water treatment by rauwaq flocculation even under primitive conditions may result in a considerable hygienic improvement amounting to 1–3 log units (90–99.9%) of bacterial reduction within 1–2 h of flocculation. The type and composition of the raw water may be expected to exert some influence on the efficiency of the flocculation, so it is of particular interest to note that the most efficient bacterial reduction was obtained employing very turbid water collected from the Blue Nile in the flooding season.

It is quite clear, though, that a treated water of high bacteriological quality may deteriorate to a poor quality by standing due to the demonstrated bacterial regrowth. However, it should be noted that in no cases the regrowth resulted in bacterial concentrations of treated water exceeding the concentrations of untreated controls within the experimental period of 24 h.

Parallel to the problems encountered in sewage treatment processes the removal of bacteria from the water phase by rauwaq flocculation is accompanied by the formation of a flocculated sediment containing viable bacteria in, at times, high concentrations. The potential public health risk of spreading bacterial infections by the disposal of infected sediments seems negligible under the dry and sunny climatic conditions of the Sudan, though, as reflected in the present results on survival of bacteria in infected sediments.

Possible adverse effects on human health by drinking rauwaq-treated water have not yet been thoroughly investigated. However, on account of the present although limited investigations on possible endotoxin formation and mutagenicity no reasons seem to justify the arrest of the employment of the method so far.

In conclusion, the described method of water purification by rauwaq flocculation may be a simple and valuable tool improving the hygienic quality of the water and thus reducing the risk of waterborne infections. The method may in particular prove to be useful in rural developing areas where the alternative for the population, in a long time to come, is to drink the water untreated. In recommending the method, though, the importance of the time factor must be stressed, and treated water should if possible be consumed within the first hours of purification.

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