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LOW TECHNOLOGY WATER PURIFICATION BY BENTONITE CLAY AND *MORINGA OLEIFERA* SEED FLOCCULATION AS PERFORMED IN SUDANESE VILLAGES: EFFECTS ON *SCHISTOSOMA MANSONI* CERCARIAE

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Abstract—*Schistosoma mansoni* cercariae were removed from clear and turbid water by flocculation with Sudanese bentonite clays, with *Moringa oleifera* seeds as well as with pure bentonite. The flocculation technique employed corresponds to that used to clarify Nile water in Sudanese villages. Moringa seeds, pure bentonite and one type of bentonite clay were able to reduce the number of cercariae by more than 90%.

Key words—water purification, bentonite clay, *Moringa oleifera* seeds, bentonite, flocculation, *Schistosoma mansoni* cercariae

INTRODUCTION

Many people who live on the bank of the River Nile are dependent on the river for their daily water consumption owing to lack of a proper water supply. In the summer months, where heavy rains fall in the highlands of Ethiopia, the tributaries of the Blue Nile and the River Nile carry great amounts of suspended matter which makes the River Nile very turbid. When turbid water is left in containers, it becomes clear by spontaneous sedimentation in 15–20 days (Jahn, 1976). In the Nile valley, women in rural communities are acquainted with different methods to accelerate the clarification process. Flocculation by means of bentonite clay, called rauwāq, and plant material are among these traditional methods of water purification (Jahn, 1981). In the flocculation method, rauwāq or plant material are crushed and added to water in a small bowl, stirred for 10 min and then poured on the turbid water in a jar. Bentonite clay and plant material enhance the sedimentation of small particles in the water by a process of destabilization and particle aggregation (Degrémont, 1979). Water purification is primarily used on a small scale as women bring water from the river and treat it for drinking purposes, cooking and for bathing themselves and their children.

Families who treat the water with rauwāq or plant material seem to have a lower incidence of gastro-intestinal disturbances (Lund and Jahn, 1980) and therefore a project was established to investigate whether rauwāq and plant material, besides their properties as flocculants, were able to reduce the number of pathogens in the water. The project was divided into a bacteriological (reported by Madsen and Schlundt, submitted for publication), a virological (reported by Lund and Nissen, 1986) and a parasitological investigation. Results of the parasitological part is presented in this article and deals with the effect of flocculation on *Schistosoma mansoni* cercariae.

Schistosoma mansoni belongs to the flukes and has a complicated lifecycle involving two different hosts, a definitive vertebrate host and an intermediate molluscan host. The transmission between hosts in the cycle is handled by two free-living larval stages, which are dependent on a fresh water environment. The adult worms are located in venous vessels around the intestine, their eggs pass into the lumen of the bowel and are discharged in faeces. A free-swimming stage, the miracidium, hatches from the egg and is infective to the snail intermediate host. In the appropriate snail, the miracidium goes through several asexual multiplications and finally gives rise to numerous free-swimming cercariae. The cercariae are infective to the vertebrate host as they penetrate the skin. After penetration the cercariae, now called schistosomula, migrate and develop into mature adult worms.

At least 200 million people in the world suffer from schistosomiasis and in some provinces in the Sudan, the prevalence of *Schistosoma mansoni* is between 50 and 80%, depending on age, with high rates of prevalence in children (Cheesmond and Fenwick, 1981).

The aim of the experiments was to evaluate whether addition of rauwāq, pure bentonite or plant material, besides their clarifying properties, have a hygienic effect by removing cercariae from the water phase.

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MATERIALS

Schistosoma strain

A Puerto Rican strain of *Schistosoma mansoni* maintained in *Biomphalaria glabrata* (Puerto Rican origin) and in albino female mice was used in all experiments. The snails were induced to shed cercariae by placing them in dechlorinated tap water under strong illumination for a period of 4 h.

Water types

Experiments were conducted at room temperature in dechlorinated tap water (tap water) and artificial Nile water (Nile water). The Nile water was prepared by Madsen and Schlundt in the following way: To 20 l. of Copenhagen tap water was added 132.9 g of Sudanese clay and 2.0 g of pepton.

Flocculants

Rauwāq from Alti and Kutranj collected in Sudan were employed in experiments. As the main component of rauwāq's with clarifying properties is bentonite (Jahn, 1981), and bentonite is used to adsorb microorganisms in water treatment (Degrémont, 1979), pure bentonite (Sigma B-3378) was tested as flocculant. Flocculants were added in quantities corresponding to concentrations optimal in clarification. As far as rauwāq was concerned, the amount was $10\ g\ l^{-1}$ and for bentonite $2\ g\ l^{-1}$. Before addition, rauwāq were pulverized in a mortar, poured into a small amount of tap water and put into a shaker for 5 min. Bentonite was powdered directly on the water surface in experimental glasses (see below) as bentonite is able to absorb so much water that the procedure for rauwāq was unsuitable. When the powder had settled, the openings of the experimental glasses were sealed, and the glasses turned a couple of times. Seeds from the tree *Moringa oleifera* were also tested as flocculant. The amount of Moringa seeds was $200\ mg\ l^{-1}$ as suggested by Jahn (1981). The seed coat was removed and the pulp crushed in a mortar, put into water and shaken for 5 min. This substance was then poured through a 125 μm sieve.

Turbidity measurements

Turbidity was measured by comparing test water with known standards and expressed in Formazin Turbidity Units (FTU). Temperature and pH were measured in all experiments.

Experimental procedure

Graduated 1 litre cylinders were filled with experimental water, and in experiments where rauwāq or Moringa were used as flocculants, cercariae were added to the water before flocculants. When bentonite was used as flocculant, the addition of cercariae succeeded the addition of bentonite. Cercariae were collected from one batch (> 20 snails) with a Pasteur pipette and 300, 400 or 500 cercariae were added to each cylinder. In imitation of Sudanese earthenware jar, the glass cylinders were covered with plastic in such a way that light could only penetrate from above and the cylinders were left thus for periods of 1–12 h. Cercariae in the supernatant (900 ml) were collected on WH. 541 filters (20–25 μm pore size). Filters were coloured with ninhydrin and dried under light during the night. The total number of cercariae was counted under a dissection microscope. Cylinders with cercariae and tap water or Nile water were controls.

Statistics

Comparisons between average values were made according to "Student's t-test" (Bailey, 1959) using a significance level of 5%.

RESULTS

Temperature and pH

The temperature of experimental water varied from 16 to 26°C but was uniform through each series. One and 6 h after adding the flocculants to Nile water pH was between 7.8 and 7.9 independent of type of flocculant, and pH of Nile water without flocculant was 7.9. Thus, the addition of flocculant did not change the pH of Nile water.

Effect on turbidity

Both rauwāq and Moringa seeds reduced the turbidity of Nile water to < 20% after 1 h of flocculation, while bentonite almost had no effect on water turbidity (Table 1). The time necessary for spontaneous sedimentation of artificial Nile water was short compared to the time for natural Nile water (15–20 days, Jahn, 1976). Therefore, the figures for turbidity can only be used to compare the flocculants and are not to be taken rigorously.

Effect on number of cercariae

The number of cercariae which was removed from the water was dependent on the flocculant employed. The hygienic effect of rauwāq from Alti was poor (Fig. 1). After 1–5 h of flocculation, the number of cercariae removed was only between 10 and 30%, and after 8 and 12 h of flocculation, the number of cercariae was significantly *greater* in glasses with rauwāq from Alti than in controls.

Contrary to this, rauwāq from Kutranj was very effective in removing cercariae (Fig. 2). After 1 and 3 h of flocculation the reduction in tap water was 40 and > 90% respectively. For both 1 and 3 h of

Table 1. Turbidity (expressed in FTU) of Nile water with and without flocculants when left for 1–7 h

Hours	Nile water	Nile water + Alti	Nile water + Kutranj	Nile water + bentonite	Nile water + Moringa
0	> 1000	> 1000	> 1000	> 1000	> 1000
1	500	200	100	400	200
2	250	150	70	200	100
3	150	140	40	140	70
4	100	100	40	100	70
5	100	70	40	100	70
6	100	40	30	100	40
7	100	40	30	70	40

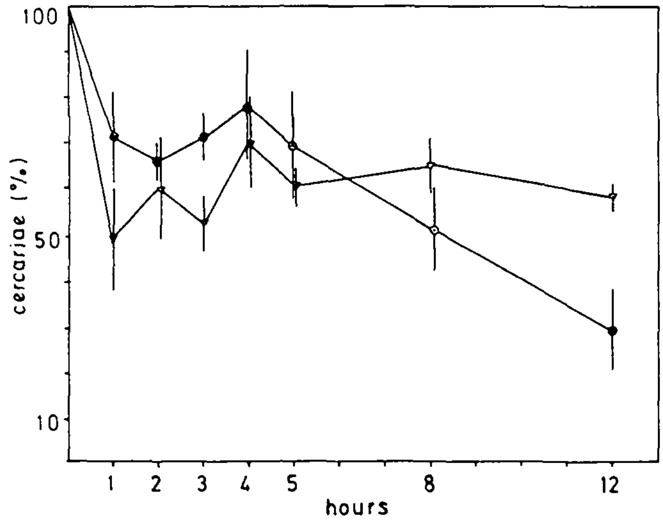

Fig. 1. Changes in occurrence of cercariae (expressed as % of initially added) in the supernatant during flocculation with Alti clay in tap water, ▼——▼ and in controls, ●——●. (n = 6). Vertical lines represent 95% confidence limits.

flocculation the reduction in Nile water was 99%. Thus, the sedimentation of cercariae progressed faster in Nile water than in tap water.

Addition of bentonite also removed cercariae very efficiently (Figs 3 and 4). In tap water, the reduction at all times was more than 90% and in Nile water a 90% reduction occurred after 2 h of sedimentation. In contrast to the experiments with rauwāq from Kutranj, the sedimentation of cercariae with bentonite progressed faster in tap water than in Nile water.

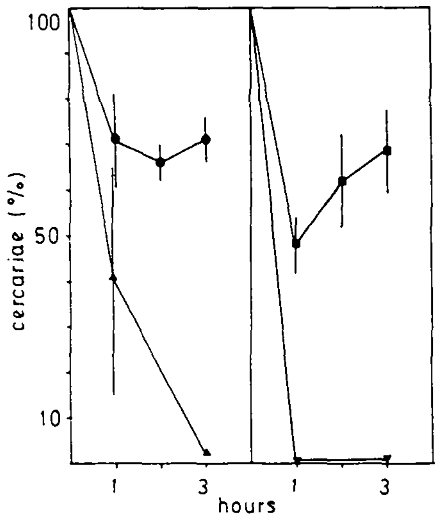

Fig. 2. Changes in occurrence of cercariae (expressed as % of initially added) in the supernatant during flocculation with Kutranj clay. In tap water, ▲——▲ and in controls, ●——●. In Nile water, ▼——▼ and in controls, ■——■. (n = 6). Vertical lines represent 95% confidence limits.

Moringa seeds were able to reduce the number of cercariae as well (Fig. 5). The reduction of cercariae in tap water was 50 and 60% after 1 and 3 h of flocculation respectively. When Moringa seeds were used as flocculant in Nile water, the initial reduction was between 30 and 55%, but after 4 h of flocculation the reduction of cercariae was more than 90%.

Persistence of cercariae in the sediments

When bentonite was used as flocculant, a significant increase in number of cercariae was found after 4 and 5 h of flocculation in both water types (Figs 3 and 4). This increase, most likely, represents cercariae which had been able to free themselves from the sediment. The sediment was about 1–2 cm thick, and, probably, only cercariae in the upper part of the sediment had been able to liberate themselves. Similar to this, a significant increase in number of cercariae was found after 5 h of flocculation when Moringa seeds were employed (Fig. 5). In all these experiments liberated cercariae, however, constituted <4% of the cercariae originally sedimented.

DISCUSSION

Effects on turbidity

Flocculation is routinely a part of the conventional waterworks treatment to remove turbidity. Clarification is obtained through two distinct processes, a destabilization of particles in suspension succeeded by aggregation of particles (Degrémont, 1979). Small particles, <1 μm (called colloids), are characterized by, and derive their stability from, an electrical double layer surrounding each particle. The addition of polyvalent cations such as Al^{3+} and Fe^{3+} to the water represses the double layer and lowers the

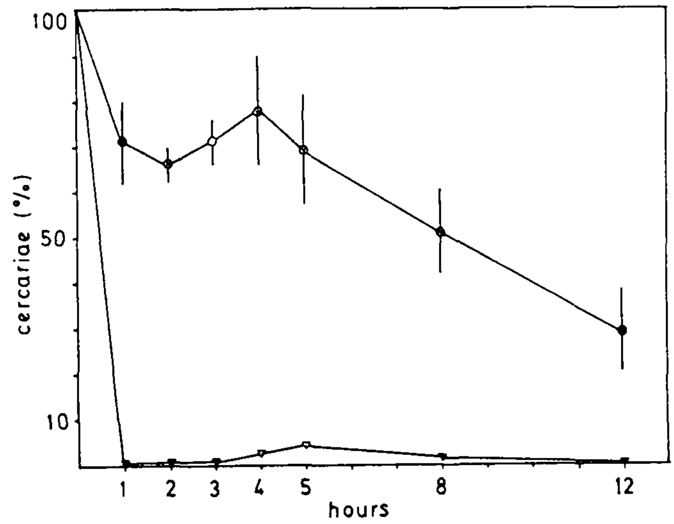

Fig. 3. Changes in occurrence of cercariae (expressed as % of initially added) in the supernatant during flocculation with bentonite in tap water, ▼——▼ and in controls, ●——●. ($n = 6$). Vertical lines represent 95% confidence limits.

stability in the suspension. As a result of the destabilization, the particles aggregate and produce flocs. Flocculants, as activated silica, extracts from algae (alginates) or higher plants (starch) and organic polymers called polyelectrolytes, enhance the sedimentation of particles as they provide nuclei on which flocs can grow, and add weight to the flocs.

The flocculants used in Sudan (rauwāq from Alti and Kutranj and Moringa seeds) proved effective as clarifiers. After 6 h of flocculation, the turbidity was close to the acceptable level in accordance with W.H.O. recommendations (Degrémont, 1979). An acceptable turbidity could have been obtained in a shorter time if the samples had been stirred since stirring enhances the formation of flocs and thereby the sedimentation of particles (Degrémont, 1979). The clarifying properties of bentonite were poor, consistent with the observations of Jahn (1977).

Removal of pathogens

As a consequence of the poor clarifying properties of bentonite, pure bentonite is not used as a proper flocculant in conventional water treatment. On the other hand bentonite is added to strongly polluted

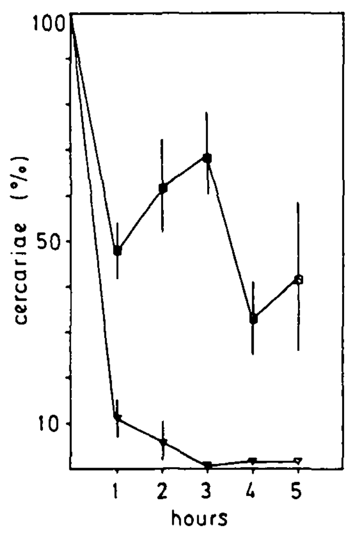

Fig. 4. Changes in occurrence of cercariae (expressed as % of initially added) in the supernatant during flocculation with bentonite in Nile water, ▼——▼ and in controls, ■——■. ($n = 6$). Vertical lines represent 95% confidence limits.

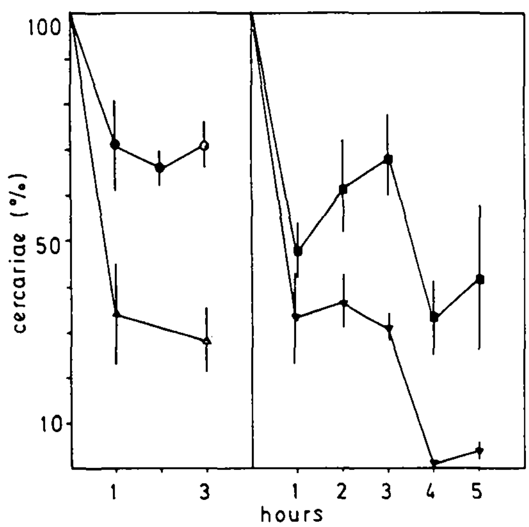

Fig. 5. Changes in occurrence of cercariae (expressed as % of initially added) in the supernatant during flocculation with *Moringa* seeds. In tap water, ▲——▲ and in controls, ●——●. In Nile water, ▼——▼ and in controls, ■——■. ($n = 6$). Vertical lines represent 95% confidence limits.

water to adsorb microorganisms (Degrémont, 1979). It is assumed that addition of bentonite clay initially cause an attachment of bacteria to the surface of the clay particles and that a substantial amount of these attached bacteriae is removed from the water phase by settling of flocs (Madsen and Schlundt, submitted for publication). Enteric viruses have affinity for solids and it is therefore believed that virus is adsorbed to clay particles during flocculation, and that the formation and sedimentation of flocs remove virus from the water phase (Lund and Nissen, 1986). After addition of pure bentonite to a Petri dish with cercariae, particles can be seen to adhere to the cercariae and thereby hamper their movements. However, the reason for this adhesion is not clear.

Removal with bentonite

Bentonite did not show effective as a water clarifier but efficiently removed cercariae from the water phase. Apparently, this removal is not because cercariae are caught within flocs but may be due to the adhesion of bentonite particles to the parasite surface. By adhesion cercarial movability is probably reduced and since bentonite adds weight to the cercariae, the parasite may readily sink out of the water phase. This process progressed faster in tap water than in Nile water, where suspended particles may compete with cercariae for bentonite adhesion.

Removal with clay

Both types of rauwāq effectively clarified Nile water although sedimentation progressed much faster with clay from Kutranj compared to clay from Alti. Thus, both types of clay, presumably, initiate a proper flocculation with a succeeding sedimentation. Differences in clarifying properties are caused by differences in mineral and bentonite content, exposure to open air and in geological history. These factors are unknown for the two types of clay, but the better clarifying properties of rauwāq from Kutranj were also reflected by a much better hygienic effect on cercariae. When added to Nile water, clay from Kutranj removed 95% of the cercariae within 1 h of flocculation. The flocculation was not quite as effective in tap water where the same removal was first obtained after 3 h. The better hygienic effect in Nile water indicates that, although the bentonite component within the clay may still contribute significantly to cercarial removal, an additional effect is obtained by the floc formation.

With rauwāq from Alti, the reduction in the number of cercariae was small ($<30\%$) but significant during the first 5 h of the experiment. However, after 5 h the number of cercariae in samples with rauwāq was higher than in controls indicating a higher viability when clay was added. The decrease in number of cercariae in controls reflects the very limited lifespan of this schistosome stage. The maximum lifespan of a cercariae is ~ 48 h dependent on how quickly their endogeneous glycogen stores are depleted (Lawson and Wilson, 1980). Apparently, the addition of rauwāq from Alti hampers this depletion. Longevity of cercariae can be increased by transtegumental uptake of low molecular organic matter (Asch, 1975). This transtegumental uptake is negligible when cercariae have large endogeneous glycogen stores, but as the cercariae grow older and stores are depleted, the absorption increases (Bruce *et al.*, 1969). However, the organic content in the rauwāq from Alti is $<1\%$ and is, presumably, of only little nutritional value to the cercariae. The increased longevity of the cercariae can, therefore, hardly be due to uptake of organic matter but, more likely, to a less draw on endogeneous pools. If the rauwāq hampers cercarial movements without causing a sedimentation the last hypothesis could be true. Less swimming activity reduces the metabolism of stored glycogen and thereby increases longevity of the cercariae (Lawson and Wilson, 1980).

Removal with Moringa

Removal of cercariae with Moringa seeds occurred with the same success in tap water as in Nile water, and the removal seemed to be independent of the changes in turbidity. After 4 h of "flocculation", a marked decrease in the number of cercariae was observed, but this decrease did not coincide with changes in turbidity (Fig. 5, Table 1). This suggests that the seeds may have cercaricidal properties. Moringa seeds contain a drug, isothiocyanat (Dahlgren, 1975) with bactericidal properties (Kurup and Rao, 1952, 1954; Das *et al.*, 1957; Andersen, 1983; Grabow, 1983) and are also toxic to *Tetrahymena pyriformis*, a ciliated protozoan (Slabbert, 1983). However, the concentration of Moringa seeds and hence the concentration of isothiocyanat used in this investigation is too small to kill bacteriae according to Jahn (1981). It may therefore be doubtful whether it has toxic effects on the cercariae, but the present results suggest that the cercaricidal potential of Moringa seeds needs further investigation.

Persistence of cercariae in the sediments

The chemical flocculants normally applied in water treatment are lime, alum and ferrous and ferric chloride. Lime has been claimed non-effective against cercariae and in experiments with alum, a 100% removal of cercariae could not be obtained (Leiper, 1916; Witenberg and Yofe, 1938; Jones and Brady, 1947). The flocculants from Sudan could not remove 100% of the cercariae either, but more than a 90% reduction was obtained in some experiments. A small proportion, $<4\%$ of the originally sedimented cercariae, was able to free themselves from the sediment after 4–5 h of flocculation. Probably, only cercariae in the upper part of the sediment had been able to liberate from the sediment.

CONCLUSION

The flocculants used by inhabitants in Sudanese villages all showed effective as water clarifiers. The present investigations, furthermore, showed that rauwāq from Kutranj and Moringa seeds had a pronounced hygienic effect by removing more than 90% of cercariae from the water phase. In spite of the clarifying properties of rauwāq from Alti, the hygienic effect was not pronounced. The variable hygienic effect of the different types of rauwāq is probably due to differences in mineral contents, a factor which is not recognizable for the users of rauwāq. This suggests that, in order to obtain an additional hygienic effect, people who use natural flocculants in water purification could, beneficially, choose Moringa seeds as a more reliable flocculant.

Taking into account that application of advanced technology has often been unsuccessful in underdeveloped countries, it appears that traditional techniques are promising alternatives. Thus, flocculation procedures, using bentonite clay or Moringa seeds, are useful alternative methods for obtaining water with less risk of spreading schistosomiasis.

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