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LOW TECHNOLOGY WATER PURIFICATION BY BENTONITE CLAY FLOCCULATION AS PERFORMED IN SUDANESE VILLAGES

VIROLOGICAL EXAMINATIONS*

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Abstract—Viruses were removed from various types of water by flocculation with natural bentonite clays from the banks of the Nile as well as with pure bentonite. The flocculation technique employed corresponds to that used to clarify Nile water in Sudanese villages. If proper flocculation occurred 3 to 4 $\log_{10}$ units of virus could be removed. Some of the conditions for floc formation were examined.

Key words—water purification, bentonite clay, bentonite, flocculation, Sudanese villages

INTRODUCTION

Jahn (1976) and Jahn and El Fadil (1984) have described how the river Nile during the flood season in Sudan, beginning in June and lasting through September, is highly turbid. Suspended solids may be as high as 8000 mg l^{-1}. In the villages, water purification by flocculation has been employed for over 100 hundred years (Jahn, 1977). One of the methods employed in the villages is the addition of a suspension of "rauwaq", clarifier, which is a clay found in varying quality at the river banks. The main flocculating component is montmorillonite (bentonite) (Jahn, 1977). Jahn (1981) describes how pounded rauwaq is prepared as a suspension in a deep plate or calabash and stirred for 10–20 min with a stick or spoon. The suspension is then added to the turbid water in an earthenware jar or other water container. If the woman, who carries out the purification, has chosen the proper concentration for the particular water and rauwaq, flocs will form and settle. Concentrations above and below the proper one will leave the water turbid. Satisfactory clarification will take at least 1 h. Jahn has made a preliminary investigation in 1974 on the removal of coliforms by the rauwaq (clarifier) clay and reports that it is general knowledge in the villages that families who treat their water have lower incidence rates of gastroentestinal disturbances than other families.

Jahn inspired us to look into the hygienic aspects of the purification and provided the clay materials. A study was set up where field work was done in Sudan, and more complicated studies including the virological studies were carried out in Denmark. Bacteriological examinations [preliminary reported by Madsen and Schlundt (1983)], parasitological examinations and virological examinations in continuation of a preliminary work by Lund and Jahn have been carried out. The present report deals with the virological examinations. Preliminary results on the virological work were presented by Lund and Jahn at the 2nd International Conference on the Impact of Viral Diseases on the Development of African and Middle East countries, Nairobi, 1980.

MATERIAL AND METHODS

The rauwaq's (bentonite clays) and bentonite employed

Samples of rauwaq of good quality collected in North Sudan and brought to Copenhagen by special messenger in 1979 were: rauwaq Uqda and rauwaq Gir al. During a visit in 1980 the senior author picked up rauwaq samples as indicated by villagers. These samples were from Wad El Said, Alti and Eseba. The rauwaq samples were estimated for turbidity by appearance in 500 ml bottles after addition to Copenhagen tap water to which had been added 1/10 of cell culture maintenance medium. The pH was around 7.3 and room temperature 20–23°C. The samples were left to stand for 3 h at room temperature and observed for turbidity. In Table 1 the observations are indicated. An optimal concentration is seen for the Uqda type, but for the others only minimal doses were found for flocculation.

Pure bentonite (Sigma B-3378) was a good flocculant, when added to waters of different composition under similar conditions as employed for the rauwaq. The water bottles were inspected with the results indicated in Table 2. As may be seen from Table 2, bentonite could also flocculate in water, if the ionic strength was sufficient.

The water types employed

In all 7 different types of water were employed in the experiments. The purpose was, and also in the experiments carried out in Copenhagen, to simulate as closely as possible the water conditions in Sudan during river flooding periods. Therefore, the artificial Nile water was probably close to the optimal for the flocculation experiments. This water was prepared by Madsen and Schlundt in the following way: to 1.0 l. of Copenhagen tap water was added 6.64 g of mud from the Nile bank and 0.1 g of Bacto Pepton.

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Table 1. Estimation of flocculation ability of rauwaq added to Copenhagen tap water with 10% cell culture maintenance medium, pH 7.4

	Turbidity*					
Type of rauwaq	1 g l^{-1}	3 g l^{-1}	5 g l^{-1}	10 g l^{-1}	20 g l^{-1}	30 g l^{-1}
Uqda	Turbid	Slightly turbid	Clear	Clear	Slightly turbid	Turbid
Gir al			Clear			
Wad El Said	Turbid at 1–30 g l^{-1}					
Alti			Turbid	Turbid	Somewhat turbid	Clear
Eseba			Turbid	Somewhat turbid	Clear	Clear

*Turbidity was observed by visual inspection for the various concentrations added.

Table 2. Flocculation when bentonite was added to water of various compositions*

	Turbidity†						
	In tap water		In demineralized water		With 10% culture medium		
Bentonite (g l^{-1})	With no further addition	With 10% culture medium	With no further addition	With 266 mg l^{-1} of $CaCl_2$	And no further addition	With 133 mg l^{-1} of $CaCl_2$	With 266 mg l^{-1}‡ of $CaCl_2$
0.5	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
1.0	Turbid	Slightly turbid	Turbid	Turbid	Turbid	Slightly turbid	Slightly turbid
2.0	Clear	Clear	Very turbid	Slightly turbid	Turbid	Clear	Clear
3.0	Clear	Clear	Very turbid	Clear	Turbid	Clear	Clear
5.0	Clear	Clear	—	—	—	—	—

*For characteristics of tap water and demineralized water see Table 3.
†*Turbidity was observed by visual inspection.*
‡The same results were obtained with no addition of $CaCl_2$ or employing 133 or 266 mg l^{-1}.

The results obtained with the Nile waters, the artificial Nile water and the Copenhagen tap water were not essentially different in the present experiments. Flocculation was obtained in all cases. The characteristics of the waters employed are given in Table 3, where the pond water is from a highly turbid duck pond of the University park.

Viruses and cell cultures employed

The human enterovirus Coxsackie B3 employed was originally isolated from biologically treated Copenhagen waste water. It is a virus type which has repeatedly been found in waste water all over the world and must be considered a good indicator for faecal virus pollution. The titers obtained were around $10^{8.0}$ $TCID_{50}$ ml^{-1} when titrated in tube cultures of Hela cells, a human cell line which originated from a cervix carcinoma. The cells were grown at 37°C in a medium consisting of Eagle's solution with 10% of new born calf serum with antibiotics. The maintenance medium consisted of the same medium with only 2% calf serum. The titrations were carried out by CPE (cytopathic effect) determinations employing 3 tube cultures for each 10-fold dilution step with 0.1 ml inocula and the titer calculated in $TCID_{50}$ per 0.1 ml.

A strain of human adenovirus type 1 was also isolated from waste water and included in the present work, because *it has a surface and size quite different from the entero-*viruses. It is commonly found in the intestinal tract, but is somewhat less resistant to environmental factors than the enteroviruses. It was grown and employed in the same way as the coxsackievirus. The titers varied somewhat, but was between $10^{6.0}$–$10^{7.5}$ $TCID_{50}$ ml^{-1} during the experients.

The bovine parvovirus (HADEN virus) employed was received from the Danish State Veterinary Virus Research Institute, Lindholm. It was titrated in secondary calf kidney cells received as primary cultures from Lindholm. The media employed were like the ones for the Hela cells except for the use of horse serum instead of calf serum. The titer obtained was around $10^{7.0}$ $TCID_{50}$ ml^{-1}. This virus type was included, although specifically bovine, because parvoviruses are extremely resistant toward environmental factors (Srivastava and Lund, 1980) and a good human type is not available for routine work.

Experimental procedure

The slurries as they were made up from bentonite clays in Sudan involved stirring or shaking by hand. Preliminary experiments showed that floccualtions could be obtained employing a Griffin flask shaker with the same results as with manual procedures, mechanically shaken slurries were employed in the experiments. The desired amount of rau-

Table 3. Characterization of the various water types employed in the experiments*

	Turbidity (FTU)	COD (permanganate) (mg l^{-1})	Total solids (mg l^{-1})	Total hardness		Conductivity (mS m^{-1})	pH
				°dH†	$CaCO_3$		
Tap water		7.0	425	13.5	270	56.2	8.2
Pond water	23	166.0	660	20.2	404	—	7.5
Artificial Nile water	1400	167.0	5385	13.5	270	57.2	7.9
Blue Nile Sept. 82 (flooding season)	1400	167.0	2397	8.6	172	20.3	8.0
Blue Nile April 83 (dry season)	7.8	12.6	340	5.7	114	16.5	7.8
White Nile April 83	48	20.0	525	3.5	70	19.5	8.0
Irrigation Canal, Soba, April 83	170	51	1570	9.5	190	37.0	7.9
Demineralized water						2.5–10	

*The analyses were kindly performed by the Copenhagen Water Works Laboratory. We gratefully acknowledge this assistance.
†One degree of hardness (1 dH) corresponds to 10 mg l^{-1} of CaO.

waq or bentonite was suspended in 100 ml of the water to be tested and mechanically shaken for 30 min. The virus was added after removing a sample for titration to make a final dilution of 1:100 or up to 1:10,000, and the total water sample was made up to 1000 ml. Apparently no difference was found in the removal capacity, whether a high titer of virus or a more diluted material was employed, but at least $10^{3.5}$ $TCID_{50}$/0.1 ml was desired in the flocculation experiments in order to get a good estimation of the removal capacity. The sample stayed overnight at room temperature after careful mixing by hand. Flocculations were in preliminary experiments carried out at 20 and at 37°C. No difference in flocculation efficiency was found between the two temperatures. For each test two flasks were set up. In one the flocs were allowed to sediment spontaneously, and the content of the other one was centrifuged at 5000–7000 *g* for 20–30 min. Both supernatants were titrated for virus. No significant differences in titers were observed between the two supernatants, and the removal efficiency of the flocculation is in the tables indicated as the average of the two values, thus improving the accuracy of the titrations. The flocs from the centrifugation were eluted employing 10% beef extract at pH 9.0 for the elution with a dilution factor of 1:5. After centrifugation at 1000 *g* for 30 min and readjustment to pH 7.4 the eluate was titrated, and the virus titers expressed as amount of virus per g wet wt of centrifuged flocs. Throughout the paper the log units employed are $\log_{10}$ units.

Persistence of infectious virus in the sediments

In connection with corresponding bacteriological examinations suspensions of coxsackievirus were diluted 1:10 with Nile mud to which rauwaq had been added to simulate a floc. The mixture was then distributed in Petri dishes with a layer of around 10 mm in thickness. They were left open to dry exposed to Sudan sunlight. Samples were exposed in the open in such number that three could be taken out each day for 7 days and then less frequently for 30 days in all. The Petri dishes were then closed with tape and kept in the cold until examined in Copenhagen (air transportation by personal messenger). In the laboratory a 1 g sample from each dish was eluated with beef extract, pH 9, to which was added 1000 IU of penicillin and 1000 g of streptomycin, and the eluates were centrifuged at 7000 *g* for 30 min. The pH was adjusted to 7.0 and then titrated in HeLa cell tube cultures employing 0.1 ml inocula. The eluates from the unexposed mud samples had titers between $10^{5.0}$ and $10^{6.0}$ $TCID_{50}$/0.1 ml, the undiluted original virus suspension contained $10^{7.5}$ $TCID_{50}$/0.1 ml. The samples which had been exposed 24 h (13–14 April) contained in one sample $10^{0.5}$ $TCID_{50}$/0.1 ml, and in no other samples virus could be demonstrated. The April temperature was around 32°C, and there was sunshine most of the days. To support this result a laboratory experiment was carried out, where 10 g of soil was mixed with 3 ml of virus suspension and kept in 1 g amounts open at 32°C in an incubator. On each day a sample was removed and eluated like the Sudan weather exposed samples. The zero sample eluate contained $10^{3.8}$ $TCID_{50}$/0.1 ml. After 24 h, the titer was $10^{0.9}$ and later no virus was demonstrated. Thus the drying alone at 32°C was able to inactivate the virus in <48 h.

EXPERIMENTS AND RESULTS

The experiments were carried out during a period of 5 years, and in this period the sensitivity of the cell cultures and the titers of the viruses employed varied somewhat. The actual differences in titer of the virus employed were however little more than experimental error. Therefore, it seems justified to compile the individual results obtained from the various series of experiments. For the titers obtained for virus adsorbed to the flocs the amount adsorbed per g wet wt is indicated. The weight of the flocs varied somewhat between the experiments but was within the interval 15–22 g. The concentration of virus per g of flocs did not give information that could tell much about the adsorbing capacity of the flocs but serve as a control of the experimental procedure, because the difference in titer between the added virus and the one of the treated water could have been the result of virus inactivation rather than removal in the flocculation. In addition the fact that the titer of the eluates was several logs higher than the one of the corresponding supernatant serve to stress the fact that virus did adsorb to the flocs and could be eluted. Therefore the sediments could give potential hygienic problems.

Experiments employing various bentonite clays (rauwaqs) from Sudan on Copenhagen tap water to remove coxsackievirus

Five different rauwaqs were tested as flocculants. The concentrations employed were gauged by the fact that the two of them (from Uqda and Gir al in North Sudan) were known locally to be of good quality, and the indications from the experiments shown in Tables 1–3 were employed. The results from the experiments to remove coxsackievirus are shown in Table 4. It may be seen that rauwaq efficiently removes virus from the water. Reliable removal was obtained when the good flocculants were employed. With too high a concentration of the clay no proper flocculation and little removal of virus was obtained. Even the poorer rauwaq qualities could remove 2 $\log_{10}$ units of virus. A strong adsorption to the flocs was indicated with no virus inactivation, but reversible binding.

Experiments employing a high turbidity duck pond water in an attempt to flocculate coxsackievirus

The pond water employed was turbid and with a high load of organic material. In Table 5 the results are compiled. It may be seen that compared with the results of Table 4 on tap water the results show less efficient or erratic removal of virus. Thus if the water is too heavily loaded the removal efficiency can be apparently very much reduced.

Experiments employing pure bentonite on various waters to remove coxsackievirus

The flocculating, removing factor in a clay definitely depends on the bentonite present, but even with chemical and mineralogical analysis of the bentonite clays employed it would be impossible to predict the removal capacity for viruses. It was therefore considered of interest to work with pure bentonite also. One single batch of bentonite was used throughout the experiments, because even for pure bentonite the activity is not given by the mineral alone. As may be seen in Table 6, excellent removal was obtained with 1 g l^{-1} of bentonite except for the

Table 4. Experiments employing various bentonite clays (rauwaqs) from Sudan on Copenhagen tap water to remove coxsackievirus B3

	Log virus titer			
Bentonite clay	In untreated water	In treated water	Virus removal (%)	Virus adsorbed to flocs (log^{10} TCID$_{50}$ g^{-1} wet wt of flocs)
Rauwaq, Uqda				
1 g l^{-1}	6.7	6.7	0	6.9
	5.1	3.4	98	4.7
	2.7	0.1	99.7	4.8
3 g l^{-1}	2.4	0.7	98	4.8
	6.1	4.1	99	5.3
5 g l^{-1}	2.8	0.1	99.8	5.1
	5.0	2.2	99.8	5.4
	3.8	0.8	99.9	4.8
10 g l^{-1}	4.4	0.8	99.97	4.0
30 g l^{-1}	4.4	3.4	90	4.8
Rauwaq, Gir al				
5 g l^{-1}	4.0	0.7	99.95	4.0
	3.9	0.2	99.98	3.8
Rauwaq, Alti				
30 g l^{-1}	2.6	0.0	99.8	3.3
Rauwaq, Eseba				
20 g l^{-1}	2.6	0.3	99.5	4.0
Rauwaq, Wad El Said				
5 g l^{-1}	5.6	2.4	99.4	5.8

Table 5. Experiments employing bentonite clays on duck pond water with coxsackievirus added

Flocculant	Log virus titer per ml untreated water	Log virus titer per ml treated water	Virus removal (%)	Virus adsorbed to flocs (log$_{10}$ TCID$_{50}$ g^{-1} wet wt of flocs)
Rauwaq, Uqda				
1 g l^{-1}*	6.7	6.7	0	6.8
3 g l^{-1}*	2.4	2.4	0	5.3
	6.1	5.1	90	5.0
5 g l^{-1}	2.8	0.5	99.5	4.8
	3.8	2.9	87.4	4.8
10 g l^{-1}	4.4	2.3	99.2	3.8
30 g l^{-1}*	4.4	4.2	36.9	4.0
Rauwaq, Gir al				
5 g l^{-1}	4.0	2.3	98.0	3.8
	3.9	2.6	95.0	4.5

*Water sample turbid after treatment.

White Nile and the Irrigation Canal water, but even in these cases around 99% removal was obtained.

Experiments with low conductivity water and $CaCl_2$ to remove coxsackievirus

To examine the importance of the ionic strength for flocculation and removal capacity of virus in water some experiments were carried out at pH 7.4 using demineralized water. As may be seen in Table 7 bentonite could not efficiently flocculate under such conditions, and no virus removal was demonstrated. In accordance with the preliminary observations of Table 2 turbidity could be removed if $CaCl_2$ was added. Even when flocculation was obtained the virus removal was less than optimal.

Table 6. Experiments employing pure bentonite to remove coxsackievirus from various waters

Concentration of bentonite	Water type	Log virus titer per ml untreated water	Log virus titer per ml treated water	Virus removal (%)	Virus adsorbed to flocs (log$_{10}$ TCID$_{50}$ g^{-1} wet wt of flocs)
1 g l^{-1}	Tap water	3.8	0.7	99.92	5.0
2 g l^{-1}		3.8	0.8	99.90	4.7
1 g l^{-1}	Artificial Nile water	3.9	0.2	99.98	5.7
	Blue Nile water*	3.8	0.5	99.95	5.0
	White Nile water†	3.8	2.1	98.0	4.4
	Irrigation Canal, Soba*‡	3.8	1.7	99.2	3.4

*Brought to Copenhagen after collection in May 1983.

†Some turbidity left in treated water.

‡Visible particular matter still present in treated water.

Table 7. Experiments employing bentonite on demineralized water (2.5 mS m^{-1}) to which virus suspensions were added in dilution 1/1000

Bentonite concentration	$CaCl_2$ added	Turbidity after treatment	Log virus titer per ml untreated water	Log virus titer per ml treated water	Virus removal (%)	Virus conc. in sediment ($\log_{10}$ units of $TCID_{50}$ g^{-1} wet wt)
1	None	Turbid	4.4	4.7	No removal	4.2
3	None	Very turbid	4.4	4.4	0	4.2
1	133 mg l^{-1}	Clear	4.4	2.7	98.0	6.4
		Clear	4.4	3.4	90.0	6.2
3	266 mg l^{-1}	Clear	4.4	2.3	99.2	6.3

Experiments to remove adenovirus by flocculation

Some of the waters and flocculants employed for the removal of coxsackievirus were examined for the removal of adenovirus. The results seen in Table 8 indicate that the removal of adenovirus functions closely in the same way with around the same efficiency as for coxsackievirus.

Experiments to remove bovine parvovirus

Corresponding to the experiments on adenovirus the ability of rauwaq or pure bentonite to remove parvovirus by flocculation was examined. The results are shown in Table 9. It seems possible that the removal of parvovirus is somewhat less efficient than the removal of coxsackievirus, but the difference demonstrated is small and probably not significant.

DISCUSSION

Flocculation by bentonite

The purpose of the present work was not to look into kinetic or other studies related to flocculation theories, but to study virus removal under conditions simulating what could take place in Sudanese villages. It seems that during winter (Jan.–March) the air temperature in Northern Sudan is around 20–29°C and the water temperature 20–25°C. In summer the air can by 40–44°C and the water temperature 29–31°C. It was found that the flocculation apparently did not change in the region 20–37°C and that the pH around or slightly above 8.0 after addition of bentonite clay was optimal for flocculation. It is important for the removal capacity that the flocs be formed slowly, i.e. during one to several hours and be allowed to settle spontaneously. It seems that it was possible to simulate the flocculation in the laboratory and study some of the factors of importance.

Flocculation in water treatment

Flocculation is routinely a part of the conventional waterworks treatment to remove turbidity. The flocs are often obtained by the addition of alum, and it is recognized that the efficiency of flocculation depends very much on the water quality. Langelier *et al.* (1952) found that a preadjustment of buffer capacity and pH may improve the flocculation. Optimum flocculation requires that an equilibrium be obtained, in which many parameters are involved, such as turbidity, particle size distribution, exchange capacity, pH and alkalinity. The exchange capacity of a water can be increased by the addition of negatively charged colloids, such as activated silica, bentonite

Table 8. Experiments employing bentonite clay and bentonite to remove adenovirus from tap water and artificial Nile water

Flocculant	Water	Log virus titer per ml untreated water	Log virus titer per ml treated water	Virus removal (%)	Virus conc. in flocs ($\log_{10}$ units of $TCID_{50}$ g^{-1} wet wt)
Rauwaq, Gir al 1 g l^{-1}	Copenhagen tap water	6.0	5.2	84.2	6.7
10 g l^{-1}	Tap water	6.3	3.1	99.94	7.0
Rauwaq, Gir al 10 g l^{-1}	Artificial Nile water	5.5	2.7	99.8	6.1
Bentonite					
0.2 g l^{-1}	Tap water	5.8	3.5	99.5	6.7
1 g l^{-1}	Tap water	5.0	1.0	99.99	4.7

Table 9. Experiments to remove bovine parvovirus

Flocculant	Water	Log virus titer per ml untreated water	Log virus titer per ml treated water	Virus removal (%)	Virus adsorbed to flocs ($\log_{10}$ units of $TCID_{50}$ g^{-1} wet wt)
Rauwaq, Uqda	Copenhagen tap water				
1 g l^{-1}		3.7	3.0	80.1	3.2
10 g l^{-1}		2.8	0.0	99.8	4.4
Bentonite					
1 g l^{-1}		3.4	1.0	99.6	5.2
2 g l^{-1}		2.9	0.0	99.9	4.8
Bentonite	Artificial Nile water				
1 g l^{-1}		2.8	0.8	99.0	3.7

and various other materials. Such substances may greatly improve flocculation and clarification. See, for example, Libor *et al.* (1973), Kirch (1974) and Worthington (1978).

Fuller's earth

Bentonite has been used to full woollen cloth and was then called fuller's earth. Fuller's earth is a natural mineral containing bentonite. It consists of volcanic ash and can therefore be found in many geological strata. The quality of fuller's earth not only depends on the mineral contents but also on the exposure of the earth to the open air. Minerals from top layers are more active than from deeper layers. The ion exchange capacity can be for instance 27 m equiv. for a fuller's earth, and pure bentonite can have a capacity of maybe 90 m equiv. The quality of bentonites for fulling has to be empirically determined. The same is the case for their flocculation capacity for turbid wates. In all experiments carried out by the group we used either specified rauwaqs or one single batch of bentonite, and we had to test their flocculation activity and then use a specific batch for comparative studies. This corresponds to the experience in the Sudan villages.

Removal of viruses with clay

In the study by Carlson *et al.* (1968) natural waters, clay suspensions (kaolinite, montmorillonite or illite) were added to water and then phage T_2 or poliovirus 1. The virus content of the supernatant after centrifugation at 1900 *g* was determined in samples with and without clay addition. There was thus no possibility to distinguish between removal by clay and virus inactivation, but it was found that "natural river turbidity" could do the same as the defined clay minerals, i.e. removal of up to 1–2 $\log_{10}$ units of virus. It was also found that resuspension of virus could take place if the ionic strength was lowered; that in fact the process was completely reversible. It was indicated that there would be competition for adsorption sites on the clay between viruses and other proteinaceous materials.

As has been pointed out repeatedly (e.g. Lund *et al.*, 1969; Lund, 1971; Schaub and Sagik, 1975; Wellings *et al.*, 1976) and now has been generally accepted by all who work with environmental samples, the enteric viruses, as far as they have been examined, have affinity for solids, be it clays or particulate matter from faeces. The adsorption is a physical-chemical reversible reaction, and the solid associated viruses are probably more resistant to spontaneous inactivation than free viruses. In a sewage treatment plant about 50% of the virus load follows the primary sludge (Lund, 1971). Clay minerals play an important role in soil microbiology (e.g. Filip, 1979). Among the normally applied chemical flocculants in water and waste water treatments are alum, lime and ferrous and ferric chloride. These are all good virus removing compounds but would probably in many cases require for instance prolonged sedimentation or sand filtration for efficient removal.

The results of the present report indicate that even under primitive conditions such as with bentonite flocculation in a water jar, very good removal of enteric virus may be obtained. The removal is not necessarily followed by a clarification as this would depend on the nature of the turbidity, but a clarified water would have improved hygienic quality from a virological point of view.

Flocculation and sedimentation alone in waterworks cannot be expected to give more than about 99% removal. In the report by van Olphen *et al.* (1984) raw surface water contained from 0 PFU in 100 l. to 5 PFU l^{-1} raw surface water, but 11 of 55 samples of partially purified water were virus positive with up to 3 PFU l^{-1}. The treatment by coagulation and sedimentation followed by rapid sand filtration was found insufficient for complete virus removal.

Chemical disinfections can give a treatment that will remove or inactivate up to 6 $\log_{10}$ units of virus (WHO, 1979). According to South African experience (e.g. Grabow *et al.*, 1980) even 12 $\log_{10}$ units of virus can be expected to be removed by advanced waste water treatment for water reuse. Nonetheless, a 3–4 $\log_{10}$ unit reduction as found in the present report under optimal conditions is a very considerable hygienic improvement.

The choice of viruses for the study

Compared with bacteriological examinations virological ones are complicated and time consuming. There is however a great advantage from the experimental point of view which of course also is reflected in the real life situation that viruses do not multiply in the environment, i.e. outside the proper living cells. In addition the enteric viruses employed are very stable at a wide pH range also at temperatures like here between 20–37°C, so that the time factor is of no importance in the flocculation experiments.

The coxsackievirus employed in the study can almost be considered a viral indicator for faecal pollution, as it is very frequently reported from all over the world. Addy and Otatume (1976) have one of the few African reports. It is a virus which is relatively stable in the environment, but typical for an enterovirus it does not withstand drying very well, as may be seen in the present report. The adenovirus was included, because it is chemically and structurally a very different enteric virus, and so is the parvovirus. All three types were removed with about equal efficiency. It would have been interesting to include a rotavirus in the study, both because it is probably the most important agent for infantile diarrhea, and because it has been reported (Smith and Gerba, 1982), that rotavirus is less efficiently removed in waste water treatments than enteroviruses.

Persistence of virus in the flocs

As can be seen from the results of the present work high titers of infectious virus are contained in the flocs. The native habit of disposing with the sediments from the water by throwing it over the wall of the yard and out in the village street could then be a hazardous procedure. To get an impression of the potential public health problem of the floc disposal the experiments of the present report where virus containing mud was exposed to the Sudan weather were carried out. The virus contents were within 24 h reduced to a not demonstrable level from the added $10^{5.0}$–$10^{6.0}$ $TCID_{50}$/0.1 ml. Laboratory experiments indicated that the drying alone would destroy the virus within 48 h. Consequently, the risk of spreading viral infections with unsanitary disposal of flocs is considerably reduced under dry and sunny conditions as in Sudan. The removal from the water is not connected with any virus inactivation, but with a concentration in the flocs. Therefore the improved water quality depends entirely on a proper separation of the flocs from the water. It could even be imagined, that an amount of water with residual flocs would be of poorer hygienic quality than the untreated water.

What would be a sufficient removal of virus to make a potable water?

The question of acceptable virus standards for drinking water has not been solved but much discussed (e.g. WHO, 1979; IAWPRC Study Group, 1983; Feachem *et al.*, 1981). It is not easy to solve the problem. For piped supplies it might be reasonable to demand a "virus-free' water, but we do not have the methods to guarantee that. In fact we have no good definition for "virus-free". Among other things because we do not even have proper cultivation methods for a number of the relevant human pathogenic viruses. A number of piped supplies have contained demonstrable amounts of virus.

Would a 3–4 $\log_{10}$ unit removal of virus be adequate? As quoted by Feachem *et al.* (1981) polluted surface water has been reported to contain up to 300 infectious units l^{-1}. If this number is realistic then a 3–4 $\log_{10}$ unit removal would probably be an important improvement of water. There is strong epidemiological evidence that the most important spread of enteric virus is from person to person. Consequently a further improvement of drinking water may from a virological point of view not be so important to prevent spread of infections and disease as improvement of sanitary conditions in general. This question is going to be further discussed in a later paper by the whole group.

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