Virological studies of water from the Cape Flats reclamation plant

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Summary

The virological quality of sewage effluent treated by a pilot reclamation plant on the Cape Flats was evaluated. The potable water produced by the treatment was repeatedly shown to be free of virus contamination.

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The Department of Water Affairs recently published its policy on the management of the water resources of the RSA.[1] This provides an outline of the expected population growth as well as the demand for potable water in the Cape Town area. The studies have shown that all known fresh-water supplies will be fully committed by the year 2007 and that thereafter use of water reclaimed from treated sewage effluents and desalinated sea water will have to be implemented. Therefore the City of Cape Town, together with the Water Research Commission, proceeded with investigations into the possibility of reclaiming potable water from treated sewage effluents and with the establishment of the necessary expertise and technology in the Cape Town area. For this purpose a pilot plant, producing 4 500 m^3 of water per day, was constructed at the Cape Flats sewage works and studied over a period of years to determine the efficiency of the plant to produce potable water. In addition to a wide range of chemical and microbiological analyses of the final water produced, a comprehensive study of the virological quality of the water was undertaken.

Viral studies of the water after the various stages of treatment and the surveillance of the final product are reported.

Materials and methods

Water samples were collected in sterile 5 l or 10 l containers before being transferred to an Amicon stainless steel reservoir. The reservoir supplies liquid under pressure to a high-performance ultrafiltration cell with an internal stirrer for rapid concentration utilising a Diaflo XM50 ultrafiltration membrane with a molecular weight cut-off point of 50 000.

After all the water had passed through the membrane — 12-18 hours — the membrane was removed under sterile conditions. Using Eagle's Minimum Essential Medium (MEM) the deposit on the membrane was eluted with the aid of a 'rubber policeman'.

The eluate was shaken with chloroform to remove bacteria and debris and after standing at 4°C for ± 30 minutes, it was centrifuged at 2 000 rpm for 10 minutes. The supernatant fluid was used as the inoculum.

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The greater part of the inoculum was put into large bottles of primary monkey kidney culture. Primary monkey kidney roller tubes containing glass cover slips were also inoculated with the eluate. All bottles and tubes were examined every 2 days and the maintenance medium changed every 3-4 days. After 10 days the cover slips were removed for staining with haematoxylin and eosin for microscopic examination to ascertain whether any viral inclusions were present in the cells. The fluid from the tubes was passaged into fresh tubes and maintained for a further 10 days and the cover slips of these tubes examined. The bottles were maintained for 21 days and if no virus was present passaged into roller tubes for a further 10 days followed by microscopic examination.

Immunofluorescence test for rotavirus

Samples suspected of containing rotavirus were centrifuged at 1 500 g for 1 hour onto cover slips of confluent MA-104 (an embryonic rhesus monkey kidney cell line) or primary monkey kidney cells and incubated overnight at 37°C. The coverslips were fixed with acetone, air dried and then incubated at 37°C for 30 minutes with bovine anti-bovine rotavirus serum (Wellcome), washed and incubated for 30 minutes at 37°C with fluorescein isothiocyanate-conjugated rabbit antibody to bovine IgG (Wellcome).

Rotazyme

The commercial Rotazyme (Abbott) kit was used for detecting rotavirus antigen. The test is based on the 'sandwich-principle'. Beads coated with antibody (guinea-pig anti-rotavirus) are incubated with the sample to be tested. If antigen is present it will bind to the antibody coated on the bead. After washing to remove unreacted sample, the bead is incubated with enzyme-labelled antibody (anti-rotavirus (rabbit): peroxidase (horseradish) conjugate). Excess antibody is removed by washing and the bead is incubated with enzyme-substrate (*0*-phenylene-diamine 2HCl and hydrogen peroxide). The intensity of the colour developed is proportional to the amount of virus bound and locked in the sandwich.

Latex test

A rapid latex agglutination test was also used to detect rotavirus. Rotalex (Orion Diagnostica) and Wellcome Rotavirus Latex Test were the commercial kits used for this test. Latex particles coated with specific antibody react with rotavirus and rotavirus antigen present in the specimen to give agglutination. Control latex particles without antibody are used to ensure that the agglutination is specific. Agglutination of the test but not the control latex indicates the presence of rotavirus in the sample.

Electronmicroscopy

Tissue culture fluids were clarified and concentrated by differential ultracentrifugation and the pellet was negatively stained with phosphotungstic acid and examined in an Elmiskop 1A microscope at 80 kV.

Polyacrylamide gel electrophoresis (PAGE)

Electrophoresis was performed on vertical 7,5% polyacrylamide gels with Laemmli[2] buffer. The gels were run at 4°C for 6 hours at 8 mA. After electrophoresis the gels were stained with silver nitrate as described by Sammons *et al.*[3] with slight modifications. In brief, gels were fixed with methanol 50%, acetic acid 10% for 1 hour, followed by ethanol 10%, acetic acid 0,5% for 1 hour and then stained in 0,19% silver nitrate solution for 1 hour. After two brief washes with tap water and one with distilled water, the gels were covered with a reducing solution consisting of sodium borohydrid 0,01 g/dl, sodium hydroxide 3,0 g/dl and formaldehyde 0,3 g/dl. When the bands were visible, the reducing solution was removed and the gels rinsed in 1% acetic acid and fresh 1% acetic acid added.

RNA extraction

Cells and maintenance medium or stool suspension were frozen and thawed three times, sodium dodecyl sulphate (SDS) was added to 1% and NaCl to 0,2M. After 15 minutes at room temperature 2 volumes of ethanol were added and the mixture was held at −20°C overnight. After centrifugation (1 000 *g*/10 min) the precipitate was suspended in 0,5M sodium acetate 0,01M ethylenediamine tetra-acetic acid (EDTA), pH 5,1. SDS was added to give a 1% solution and sodium perchlorate to 0,5M. The mixture was shaken for 1-2 minutes with an equal volume of chloroform/isoamyl alcohol (24:1) and chilled for 15 minutes. Following centrifugation at 1 000 *g*/10 min the organic phase was discarded and the procedure repeated. NaCl was added to the aqueous phase to a concentration of 0,2M. Two volumes of ethanol were again added and the RNA precipitated overnight at −20°C. After centrifugation at 1 000 *g*/10 min the precipitate was dissolved in 1mM EDTA, pH 8,0.

Haemagglutination

One drop of sample to be tested was mixed with 1 drop of 0,8% human O type red blood cells and 1 drop with 0,55% bovine red blood cells and left at 4°C for 1 hour.

Reovirus types I and II agglutinate human red blood cells but not bovine. Reovirus type III agglutinates bovine red blood cells and human red blood cells only partially.

Results

Purification before carbon columns. Samples taken from stages before carbon column purification were contaminated with viruses on occasions. Of the 32 samples tested 12 contained reoviruses. Haemagglutination and neutralisation showed that the majority of these were reovirus type III. This was confirmed by the electrophoretic patterns on PAGE.

Carbon column effluent without chlorination. Four of the 9 samples studied were infected with viruses. In June 1982 1 sample was contaminated with poliovirus type II, rotavirus and reovirus. A fortnight later a sample showed infection with coxsackie virus B5 and reovirus. The remaining 2 samples were all positive for reovirus. No other viruses were detected.

At this stage the plant was not fully operative.

After sand filtration without carbon treatment. During the later stages of the operation of the pilot plant samples were examined after sand filtration before passing through the carbon columns. The water was consistently uncontaminated in numerous samples taken over a period of 2 years.

Surveillance of the final product CR5. A comprehensive study of the final product of the reclamation plant was conducted over a period of 4 months. All the samples examined during this time were clear and no viruses or coliphages were detected. The results are tabulated in Table I.

Tap-water from the domestic water supply was also tested for viral purity on four occasions. No viruses were detected in these samples, although the water contained more debris than the reclaimed product.

Discussion

The viruses most commonly isolated from water samples in the RSA belong to the Reoviridae family. These viruses seem

TABLE I. SUMMARY OF VIRAL STUDIES OF WATER FROM THE CAPE FLATS RECLAMATION PLANT, JUNE 1982 - JANUARY 1987

Source of sample	No. of samples tested	No. of samples in which virus was detected	Viral identification
Feed water	**6**	**6**	**Reovirus in 6**
Breakpoint chlorination	**2**	**2**	**Reovirus in 2**
Stabilisation tank without chlorination	**3**	**2**	**Reovirus in 2**
Stabilisation tank after chlorination	**1**	**None**	
Sand filter without chlorination	**2**	**2**	**Reovirus in 2**
Sand filter after chlorination	**18**	**None**	
Carbon column without chlorination	**9**	**4**	**Reovirus, poliovirus and rotavirus in 1** **Reovirus and Cox B5 in 1** **Reovirus in 2**
Carbon column intermediate chlorination	**8**	**None**	
Final product CR5	**12**	**None**	

to survive longer than other enteroviruses and are a good indication of the quality of the water.

Although viruses were detected in samples taken from various points during reclamation before the final chlorination stage, it would appear that the final water from the reclamation plant is completely free of viral contamination and is as clear as domestic tap-water. The fact that there was a heavier deposit of discoloured matter on the membrane filter after tap-water had been concentrated would be because of its storage and passage through many pipes.

By virological standards the final water from the Cape Flats reclamation plant is perfectly potable.

The results presented here confirm those reported by the National Institute for Water Research of the South African Council for Scientific and Industrial Research[4] on its studies of a similar pilot plant in Pretoria (Stander Plant) and those reported by Isaäcson *et al.*[5] for the Windhoek reclamation plant.

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