RECYCLING OF WATER FOR IRRIGATION: PERSISTENCE OF ENTEROVIRUSES IN SEWAGE EFFLUENT AND NATURAL WATERS RECEIVING THE EFFLUENT

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Abstract. A virological analyses of a sewage treatment plant which provided chlorinated, activated sludge treated sewage effluent to irrigate a complete two-year crop of sugarcane was made. The raw, the activated sludge treated and the chlorinated sewage effluent, as well as streams and a harbor receiving sewage effluents were concentrated by either the polymer two-phase, PE-60, $Al(OH)_3$, protamine sulfate, or cellulose membrane method and assayed for human enteric viruses. Viruses were recovered from 100% (11/11) of the raw sewages tested at concentrations ranging from 27 to 19 000 PFU l⁻¹ while 76% (13/17) of the activated sludge treated effluent was positive at concentrations ranging from 7 to 5222 PFU l⁻¹. After chlorination, 58% (31/53) of the samples was positive for virus at concentrations ranging from 2 to 750 PFU l⁻¹. Human enteroviruses were also isolated from shallow flowing streams at distances up to 3 mi (5 km) from the closest known sewage effluent discharge point and from a harbor approximately 0.5 mi (0.8 km) from the point of sewage discharge entering the harbor. The viruses most often isolated were echovirus 7, coxsackievirus B-4, B-5 and poliovirus 1, 2, and 3. These results indicate that although activated sludge treatment plus chlorination remove approximately 90% of the virus from the raw sewage, the final treated sewage effluent, which is normally discharged into a stream and in this experimental study to irrigate sugarcane, still contains a significant concentration of infectious viruses. Furthermore, the recovery of enteroviruses from waterways at points distant from the sewage treatment plants indicates that sewage-borne viruses persist in natural water environment. The significance of enteric viruses in waters accessible to the public and used for irrigation purposes remains to be determined.

1. Introduction

Approximately 90% of the fresh water needs of Oahu, the major island in the State of Hawaii, is obtained from deep underground water which results from the natural filtration of rainwater through the unique soil and rock formations of the island (2020 Plan, 1971). The quality of this fresh underground water is of such purity that no chlorination is required for human consumption. However, since the quantity of water which can be stored underground cannot be increased and since the demand for more fresh water in the near future will exceed the available supply (2020 Plan, 1971), plans to conserve water must be implemented. One such alternative is the recycling of sewage effluent for irrigation. If implemented, this will make available for domestic consumption, the large volume of fresh water currently being used for agricultural purposes. However, before such a plan can be put into practice, the presence of undesirable sewage components such as toxic chemicals, heavy metals, pesticides, pathogenic microorganisms and viruses and their possible effects on the soil, the crops, and the health of people must be assessed. These parameters were

examined in a multi-disciplinary pilot field study to determine the feasibility of using treated sewage effluent to irrigate the high water requiring sugarcane, the major agricultural crop of the state.

This report addresses itself mainly to assessing the concentration of enteroviruses in the raw, the activated sludge treated and the final chlorinated sewage effluent used for the irrigation studies over a two-year period. In addition, natural bodies of water receiving the sewage effluents were monitored for sewage-borne viruses. From these analyses, it was possible to: (a) evaluate the practicality and efficiency of the various methods used to concentrate viruses from sewage and sewage contaminated waters, (b) characterize the virological content of sewage from a given community, (c) determine the efficiency of a secondary (activated-sludge) sewage treatment plant to reduce the concentration of viruses in the sewage, and (d) demonstrate that sewage-borne viruses persist in the receiving waters.

2. Materials and Methods

2.1. CELLS AND MEDIA

African green monkey kidney cells either primary, secondary or tertiary passaged (AGMK) or an established line (BGM), as well as the human diploid cells, WI-38, were used for virus isolation and growth. The BGM (Dahling *et al.,* 1974) cell line was kindly supplied by G. Berg (Environmental Protection Agency). The mouse L-cells were used to plaque and identify reovirus while the established rhesus monkey kidney cell line (LLC-MK$_2$) was used in many of the enterovirus identification tests. All cells were grown in Eagle's basal medium (EBM) using either Hank's or Earle's salt and supplemented with 5 to 10% fetal calf serum (FCS).

2.2. VIRUS AND VIRUS ASSAYS

Stock pools of poliovirus type 1, originally isolated from a case in Hawaii, were prepared in BGM cells and purified by isopycnic banding in CsCl. This virus preparation was used to evaluate the various virus concentration methods used. The Sabin type 1 attenuated poliovirus strain (LSc2ab) was used as a control in the characterization of the RCT$_{40}$ marker of the isolated polioviruses. Virus growth was quantitated using either the 50% tissue culture infectious dose (TCID$_{50}$) method or the plaque method. The isolated enteroviruses were identified by the neutralization test (Lim and Benyesh-Melnick, 1960) using pooled antisera supplied by the National Institutes of Health. Reovirus was identified but not typed using fluorescein isothiocyanate conjugated rabbit anti-reovirus type 2 gamma globulin to specifically stain reovirus infected L cells (Oie *et al.,* 1966).

2.3. WATER SAMPLES AND SAMPLING SITES

The sewage effluent used to irrigate the sugarcane was obtained from Mililani sewage treatment plant (STP) located in central Oahu (Figure 1). This STP which employs
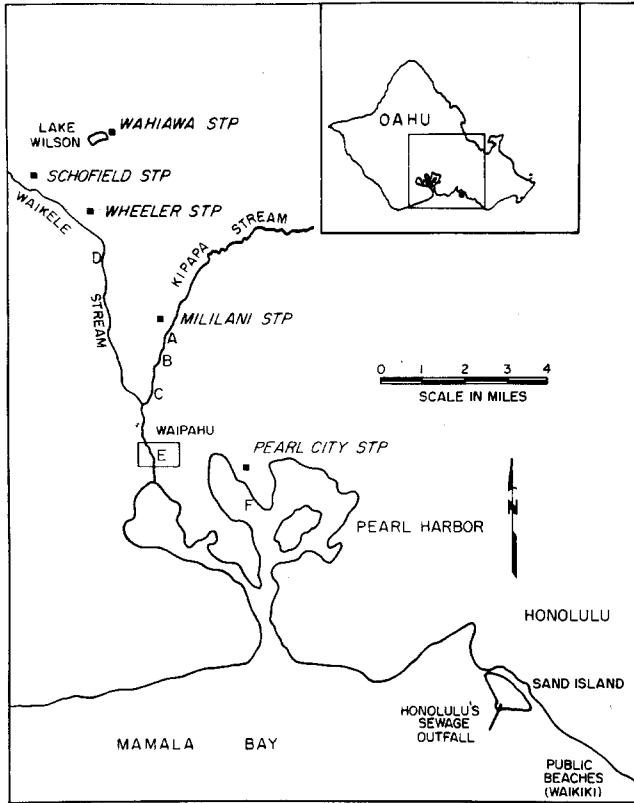
Fig. 1.   Virus sampling sites on Oahu. Inset of island of Oahu is enlarged to show sampling area. Virus
sampling points are designated as A, B, C, D, E, F and further described in Table V.

settling, activated sludge and chlorination, processes 3.2×10^6 l of sewage day^{-1}
serving an exclusively residential community (Mililani) of approximately 8000
people. Comparative sewage samples were obtained from Wahiawa STP (Figure 1)
which also employs settling, activated sludge and chlorination to process 6.8×10^6 l
of raw sewage day^{-1} and from Pearl City STP (Figure 1) which employs only settling
and chlorination to process 2.2×10^7 l of raw sewage day^{-1}. Water samples from
natural bodies of water which received sewage effluent from these sewage treatment
plants were also assayed for virus. These include water samples from Kipapa and
Waikele stream which receive treated sewage effluent from Mililani as well as other
sewage treatment plants and also from Pearl Harbor which receives the sewage
effluent from Pearl City sewage treatment plant (Figure 1).

   All water samples were obtained by the grab method and transported back to the
laboratory where it was immediately processed or stored at 4°C for not more than 15
to 20 h. The water samples were initially clarified by filtration through an AP-20 or
AP-25 fiberglass pad (Millipore Corp., Bedford, Mass.) before they were concen-

trated. Initially, the clarifying filters were prewashed with 1% Tween 80 or 10% calf serum. However, this procedure did not improve virus recovery from sewage samples presumably due to the high concentrations of membrane coating components present in sewage. Consequently, the clarifying filters were prewashed for clarifying stream and harbor waters but not for undiluted sewage effluents. The final sample concentrates were freed of bacteria by filtration through either 10% calf serum or 3% beef extract washed 0.45 μm membranes (Millipore Corp.) before inoculation into the appropriate cell cultures.

2.4. METHODS OF SAMPLE CONCENTRATION

2.4.1. *Polyelectrolyte 60 (PE-60)*

The batch technique (Wallis *et al.*, 1969) of using the insoluble PE-60 (Monsanto Co., St. Louis, Mo.) was employed. Four different lots of PE-60 were tested and were found to vary in their efficiency to adsorb poliovirus. Two of the better lots of PE-60 were water washed and 0.8 to 1.0 g was added to each 4 l of sewage water which had been clarified and adjusted to pH 5.0 to 5.5. The sample was then mixed for 2 h at room temperature, after which the PE-60 was recovered by filtering the entire sample through an AP-25 pad. Virus adsorbed to the PE-60 was then eluted by the addition of 10 ml of 0.05 M borate buffer plus 10% calf serum to each 1 to 2 g of PE-60, followed by vigorous mixing for 10 min at a final pH of 9.0. This was followed by centrifugation for 10 min at 13 000 $\times$ g to pellet the PE-60 and the supernatant (the concentrate) was immediately adjusted to pH 7.0 to 7.5 with 0.6 N HCl.

2.4.2. *Polymer two-phase*

A modification of the polymer two-phase separation method as described by Shuval *et al.* (1969) was used. Briefly, 2 g of sodium dextran sulfate 500 (Pharmacia Fine Chem., Piscataway, N.J.), 64.5 g of polyethylene glycol 6000 (Union Carbide, New York, N.Y.) and 17.5 g of NaCl were dissolved into each liter of clarified and pH 7.0 to 7.5 adjusted sewage sample. The samples were then transferred into a separatory funnel and kept at 4°C for 15 to 24 h to allow the polymers to separate into two phases. The lower dextran sulfate phase plus the interphase were collected and mixed well with 50 mg ml^{-1} of NaCl. After an additional overnight separation at 4°C, the resulting top aqueous phase was diluted 1:7 with sterile water or dialyzed against phosphate buffered saline, pH 7.2, to render the concentrate isotonic.

2.4.3. *Aluminium hydroxide*

The stock Al(OH)$_3$ suspension at twice the concentration as described by Wallis and Melnick (1967) was prepared and 40 ml of this reagent was added to each 4 l of sewage which had been clarified and adjusted to pH 7.2 to 7.4. The sample was then

mixed for 2 h at room temperature and filtered through an AP-25 pad. The Al(OH)$_3$ precipitate was recovered from the filter pad and vigorously mixed with 10 ml of 0.05 M borate buffer plus 10% calf serum (final pH 9.0) for 10 min to elute the adsorbed viruses. This was followed by centrifugation for 10 min at 13 000 × g to pellet the precipitate and the supernatant (the concentrate) immediately adjusted to pH 7.0 to 7.5 with 0.6 N HCl.

2.4.4. *Protamine sulfate*

A modification of the method as described by England (1972) was used. To each 4 l of clarified and pH 7.2 to 7.5 adjusted sewage sample, 6.25 ml of calf serum was added followed by protamine sulfate (Calbiochem., La Jolla, Calif.) to a final concentration of 0.025%. The sample was mixed for 2 h at room temperature and the resulting precipitate was recovered by filtering the entire sample through an AP-20 filter pad. The virus-precipitate complex was trapped on the filter pad and was subsequently dissolved by slowly filtering through the filter pad, 10 ml of 1 N NaCl for each 4 to 8 l of sewage processed. The eluate was then diluted 1:7 with sterile distilled water or dialyzed against phosphate buffered saline pH 7.2, to render the concentrate isotonic.

2.4.5. *Cellulose membrane*

The method as described by Wallis *et al.* (1972) was used to concentrate viruses from natural waters receiving the sewage effluent. The natural water samples were initially clarified, the pH adjusted to 4.0 and 0.5 g of AlCl$_3 \cdot$ 6H$_2$O added to each 4 l of water. The water samples were then filtered through a 90 mm cellulose nitrate membrane of 0.45 μm porosity (Millipore Corp.) at the rate of 4 l per 10 to 15 min. The membrane adsorbed virus was eluted by placing the membrane into a beaker along with 20 ml of 0.05 M borate buffer plus 10% calf serum (final pH 9.0) and mixed well for 15 min using a magnetic bar. The resulting eluate (virus concentrate) was recovered and the pH immediately adjusted to 7.0 to 7.5 with 0.6 N HCl.

3. Results

3.1. EFFICIENCY OF THE VIRUS CONCENTRATION AND RECOVERY METHODS

The efficiency and facility of the various virus concentrations methods are known to depend on the volume and turibidty of the water sample (Hill *et al.,* 1971). Sewage samples were relatively turbid and volumes of less than 20 l were concentrated using primarily the PE-60 and polymer two-phase methods. Samples from the natural waterways were relatively clean and volumes up to 40 l were processed using the PE-60 or cellulose nitrate membrane adsorption methods. To determine the expected efficiency of these methods to concentrate and to recover virus from field samples, poliovirus type 1 at a final concentration of 10^3 to 10^5 PFU ml^{-1} was added as marker virus to clarified samples of either dechlorinated Mililani treated sewage

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effluents or Waikele Stream water and the samples concentrated using the appropriate procedures. The results (Table I) indicate that the efficiency of the various methods to remove the added type 1 poliovirus from the samples and to recover the virus in the final concentrate varied. The best lot of PE-60 removed 42% of the total virus added to the sewage effluent and 36% was recovered in the final concentrate. Other lots of PE-60 were markedly less efficient, with recoveries ranging from 10 to 30%. The limitation of the different lots of PE-60 is their inefficiency to adsorb virus, for with all lots of PE-60 most of the adsorbed virus could be recovered in the eluate. The polymer two-phase method removed 79% of the poliovirus added to the sewage effluent and 68% was recovered in the concentrate. The efficiency of poliovirus recovery by the polymer two-phase method appears to be superior to that of the PE-60. However, the polymer two-phase method is for practical reasons and convenience limited to processing up to 6 l of sample while the batch PE-60 method can process up to 20 l of sample with ease.

Two additional methods [protamine sulfate and Al(OH)$_3$] were subsequently employed and their virus concentrating efficiency evaluated. The Al(OH)$_3$ method removed 97% of the poliovirus added to the sewage effluent but only 44% was recovered in the final concentrate indicating that the limitation of this method is the recovery of the virus from the precipitate. The method yielding the poorest efficiency of poliovirus recovery was the protamine sulfate method which removed less than 10% of the added virus from the sewage effluent and only 3% was recovered in the final concentrate. This poor adsorption and recovery of poliovirus by protamine sulfate supports England's (1972) data that protamine sulfate is a poor method for concentrating the small enteroviruses. However, the method when applied to field samples readily recovered a number of enteroviruses (poliovirus 1 and 3, coxsackie-

TABLE I

The relative efficiencies of the various virus concentration methods used

Method	Suspending medium	Total volume	Strain of Type 1 poliovirus added	% of virus removed	% of virus recovered
PE-60 (Batch)	Chlorinated effluent	1 l	Laboratory strain	42	36
Two-Phase	Chlorinated effluent	1 l	Laboratory strain	79	68
Prot. Sulfate	Chlorinated effluent	1 l	Laboratory strain	10	3
Prof. Sulfate	Chlorinated effluent	1 l	Field isolate	66	60
Al(OH)$_3$	Chlorinated effluent	1 l	Laboratory strain	97	44
Membrane adsorption	Waikele stream	4 l	Laboratory strain	85	38

virus B-4, B-5, and echovirus 1). In an attempt to explain this apparent contradiction, a sample of sewage effluent was seeded with poliovirus type 1 previously isolated by the protamine sulfate method and the sample concentrated by the same method. Under these conditions, 66% of the added field isolated poliovirus was removed from the sewage effluent and 60% was recovered in the final concentrate (Table I) indicating that the inconsistency of this method in concentrating the various enteroviruses may be related to the past history of the virus rather than its specific type. However, the exact nature for this difference remains to be defined.

Table I also shows that the cellulose nitrate membrane method removed 85% of the poliovirus added to 4 l of Waikele stream water. However, only 38% of the added virus was recovered in the final concentrate. These results indicate that the cellulose nitrate membrane method efficiently adsorbs virus although the adsorbed virus could not be efficiently eluted off the membrane.

3.2. VIROLOGICAL ASSESSMENT OF MILILANI STP

To determine the concentration of human enteric viruses in raw sewage and the effectiveness of the sewage treatment processes in removing viruses, 2 to 20 l of either the raw, activated sludge treated or the final chlorinated effluent from Mililani STP were concentrated and assayed for enteric viruses. It was found (Table II) that

TABLE II

Virus isolations from Mililani sewage treatment plant

Source of sample	No. of samples tested	Sample assayed (l)	No. and percent of samples positive for virus	Range of virus concentration (PFU l⁻¹)
Raw sewage	11	1–6	11 (100%)	27–19 000
Activated-Sludge treated effluent	17	2–16	13 (76%)	7–5222
Chlorinated effluent	53	5–20	31 (58%)	2–750

11/11 (100%) of the raw sewage tested were positive for virus at concentrations ranging from 27 to 19 000 PFU l⁻¹ while 13/17 (76%) of the activated sludge treated effluent tested were positive at concentrations ranging from 7 to 5222 PFU l⁻¹. In the final chlorinated effluent, 31/53 (58%) of the samples tested were positive for viruses at concentrations ranging from 2 to 750 PFU l⁻¹. These results demonstrate that the raw domestic sewage contains a relatively high concentration of infectious human viruses. Furthermore, although sewage treatment at the STP reduces this concentration of viruses, the final treated sewage effluent still contains a significant amount of infectious viruses.

3.3. IDENTIFICATION OF THE VIRUS ISOLATES

The viruses isolated from the sewage samples obtained from Mililani STP were identified (see Materials and Methods) and correlated with the method of concentration, the date of isolation and the source of sewage (Table III). The results show that over the two-year period of study, the viruses most often isolated were poliovirus types 1, 2 and 3, coxsackievirus B-4 and B-5 and echovirus 7. Furthermore, the various methods of virus concentration did not appear to be selective for a given type of virus as evidenced by the isolation of poliovirus types 1, 2 and 3 as well as coxsackievirus B-4 and B-5 using either the PE-60, polymer two-phase, Al(OH)$_4$ or protamine sulfate methods. Similarly, echovirus 7 was isolated using either the PE-60 or polymer two-phase methods while reovirus was isolated using either the PE-60 or Al(OH)$_3$ methods. The results also indicated that the predominating virus in the sample was being isolated as evidenced by the identical virus isolates when either the PE-60 and polymer two-phase methods were used to concentrate virus from the same samples (12-12-72, 12-18-72, 1-3-73, 2-27-74). However, in the other cases when identical samples (2-20-73, 12-11-73, 2-6-74) were concentrated by two different methods, two different viruses were isolated indicating that the chance of isolating more kinds of viruses increases when more than one method is used to concentrate a given sample. Nevertheless, as seen in samples 8-14-73 and 4-29-74, even one method of concentration can result in multiple virus isolations.

3.4. VIRUS ISOLATION FROM SEWAGES OTHER THAN MILILANI STP

Sewage samples from two other STP which serve a much larger and heterogenous population including industrial wastes were obtained and examined for viruses. The Wahiawa STP employed settling, activated sludge and chlorination while the Pearl City STP employed only settling and chlorination. The results (Table IV) again demonstrate that 100% of the raw sewage tested was positive for virus and that the sewage treatment process did not adequately remove or destroy the viruses in the raw sewage. Furthermore, the kinds and concentrations of viruses were similar to that found at the Mililani STP. These results indicate that all sewages in the state, whether treated or untreated are sources of infectious human viruses.

3.5. CHARACTERIZATION OF THE POLIOVIRUS ISOLATES

Twenty-six of the enteroviruses isolated were identified as poliovirus and it is of public health significance to determine whether these isolates belong to either the attenuated or virulent strains. One of the most stable genetic markers (Melnick, 1960) for poliovirus virulence is the ability of the virulent poliovirus to grow effectively at both 37°C and 40°C (T+), whereas the attenuated strain grows well at 37°C but is inhibited at 40°C (T–). The T marker property of all the poliovirus isolates was determined and compared with the attenuated Sabin type 1 poliovirus. The isolates were considered T– when growth at 40°C was at least 2 log$_{10}$ TCID$_{50}$ less

TABLE III

Viruses isolated and identified from Mililani sewage treatment plant

Date	Method	Raw sewage	Activated-Sludge treated effluent	Chlorinated effluent
07-05-72	PE-60	ECHO-1		
07-18-72	PE-60	Coxsackie B-4	Reo	
08-02-72	Two Phase	Polio-1		
08-29-72	PE-60		Coxsackie B-5	
09-05-72	Two Phase		Coxsackie B-5	
09-12-72	PE-60		Polio-3	
10-26-72	PE-60			Coxsackie B-5
11-21-72	Two Phase			Coxsackie B-5
12-05-72	PE-60			Coxsackie B-5
12-07-72	PE-60			ECHO-7
12-12-72	Two Phase			ECHO-7
	PE-60			ECHO-7
12-18-72	Two Phase			ECHO-7
	PE-60			ECHO-7
01-03-73	Two Phase			ECHO-7
	PE-60			ECHO-7
01-09-73	PE-60			ECHO-7
01-16-73	PE-60			ECHO-7
02-13-73	PE-60			ECHO-1
02-20-73	Two Phase			ECHO-7
	PE-60			ECHO-27
03-27-73	Two Phase	Coxsackie A-16		
04-19-73	Two Phase	Polio-1		
05-10-73	Two Phase	Coxsackie B-5		
05-15-73	Two Phase		Polio-3	
05-22-73	PE-60		Polio-2	Reo
05-23-73	Two Phase		Coxsackie B-5	
06-01-73	Prot. Sulfate		ECHO-1	
07-17-73	Al(OH)$_3$			Reo
08-14-73	Al(OH)$_3$			Polio-2, 3
09-11-73	PE-60			Polio-2
09-27-73	Al(OH)$_3$			Coxsackie B-4
10-02-73	Two Phase		.	ECHO-15
12-11-73	Two Phase	Coxsackie B-4		
	Prot. Sulfate	Coxsackie B-5		
12-18-73	Two Phase	Coxsackie B-4		
	Prot. Sulfate			Polio-1
02-06-74	Prot. Sulfate		Coxsackie B-4	
	Two Phase		Coxsackie B-5	
	PE-60			Coxsackie B-4
02-13-74	Prot. Sulfate		Coxsackie B-5	
	PE-60			Polio-1
02-20-74	Prot. Sulfate		.	Polio-3
	PE-60			Polio-3
02-27-74	Two Phase			Polio-3
	PE-60			Polio-3
03-20-74	PE-60			Polio-2
04-03-74	PE-60			Polio-1
04-29-74	Two Phase	Polio-1, -3		
07-13-74	Two Phase			Polio-1
08-06-74	PE-60			Polio-1
08-29-74	Two Phase			Polio-1

TABLE IV

Virus isolations from Wahiawa and Pearl City sewage treatment plants

Source of sample	No. of samples tested	No. of positive samples[a]	Types of virus identified	Range of virus concentration (PFU l⁻¹)
A. Wahiawa STP				
1. Raw sewage	2	2	ECHO-15, Polio-1	50-118
2. Chlorinated effluent	9	2	Polio-2, 3	Not done
B. Pearl City STP				
1. Raw sewage	2	2	Coxsackie B-2, Polio-3	5-268
2. Chlorinated effluent	2	2	ECHO-15 Coxsackie B-4 Coxsackie B-5 Polio-3	25-34

[a]The PE-60, polymer two-phase or protamine sulfate methods were used to concentrate the samples.

than at 37°C and were considered T+ when the difference between the growth at 37°C and 40°C was not greater than 1 $\log_{10}$ $TCID_{50}$. Of the 26 poliovirus isolates tested, three isolates of poliovirus type 1 obtained from Mililani chlorinated effluent (4-3-74, 8-6-74, 8-29-74) were considered T+ or indicative of a virulent strain. It is apparent that most of the poliovirus isolates are of the attenuated strain.

3.6. PERSISTENCE OF SEWAGE-BORNE VIRUSES IN THE NATURAL WATERWAYS

It is a common world-wide practice, as it is in Hawaii, to discharge the sewage effluent into some body of natural water. Since infectious human viruses were shown to survive the sewage treatment processes including chlorination (Table III), it follows that sewage-borne viruses are entering the natural waterways. Thus, the sewage effluent from Wahiawa STP is discharged into Lake Wilson which is used for boating, fishing and irrigation while the effluent from Pearl City STP is discharged into Pearl Harbor, a major naval harbor (Figure 1). The effluent from Mililani STP is discharged into Kipapa stream 2.4 km before it feeds into Waikele stream, which continues southward for another 2.4 km, passing through a major community (Waipahu) and finally emptying into Pearl Harbor (Figure 1). To determine whether these sewage-borne viruses persisted in the streams and harbor into which they were discharged, several attempts were made to isolate viruses from these waters. Sampling sites from which grab samples were obtained are shown in Figure 1. Table V lists the sampling points, their distances downstream from the nearest STP, the volumes of water assayed, the method of concentration used and the virus isolated. Viruses were isolated at least once at all the sampling sites tested, indicating that infectious viruses persist in the natural waterways. The sources of these viruses are presumed to be the sewage treatment plants.

TABLE V

Virus isolations from natural bodies of water receiving sewage effluents

Sampling site	Distance downstream from nearest STP	# positive / # attempts	Date	Method used	Volume assayed (l)	Virus isolated
A. Upper Kipapa Stream	Entrance of Mililani STP effluent into Kipapa Stream	1/3	8/6/74	PE-60	16	Polio-1
B. Middle Kipapa Stream	1.6 km from Mililani STP effluent discharge into Kipapa Stream	2/3	9/30/74 10/3/74	PE-60 Two-phase	16 4	Polio-3 Polio-3
C. Lower Kipapa Stream	2.4 km from Mililani STP effluent discharge into Kipapa Stream	1/3	9/10/74	Two-phase	4	Polio-1
D. Upper Waikele Stream	1.5 to 3 km from (Wheeler and Schofield) STP	1/1	3/5/75	PE-60	8	Polio-1
E. Lower Waikele Stream	5 km from Mililani STP and 6 to 8 km from Wheeler and Schofield STP	2/3	3/25/75 4/1/75	PE-60 Membrane	20 40	Polio-1 Polio-1
F. Pearl Harbor (Middle Loch)	0.8 km from Pearl City STP discharge into Pearl Harbor	1/5	6/9/75	Membrane	40	Polio-3

4. Discussion

A virological assessment of three sewage treatment plants on Oahu demonstrated that infectious human enteric viruses were present in high concentrations in the raw sewages and that while the conventional sewage treatment processes, such as settling, activated sludge and chlorination markedly reduced the concentration of viruses, they could not be relied upon to completely eliminate viruses consistent with findings in an experimental sewage treatment plant (McLean, 1973). Thus, 58% of the final chlorinated sewage effluent obtained from the Mililani STP was positive for virus at concentrations ranging from 2 to 750 PFU l^{-1}. Since each PFU is capable of causing an infection in humans (Katz and Plotkin, 1967) the concentrations of viruses in the chlorinated sewage effluent represents a potential source for the transmission of virus infection and disease. In a corollary study (Leung, 1973) the Mililani sewage treatment process was found to be very effective in reducing the total coliform concentration in the raw sewage from 5.6 to 22.4×10^7 (100 ml)$^{-1}$ to an acceptable level of 2 to 1600 (100 ml)$^{-1}$ in the chlorinated sewage effluent. These results confirm other reports (Liu et al., 1971; Scarpino et al., 1972) that human enteroviruses are more resistant to chlorination than bacteria and as a consequence the coliform index is an inadequate indicator for the absence of viruses. It should be noted that the actual concentration of viruses in the field samples must be far greater than that determined since (a) the samples were preclarified of debris larger than 1 μm to which viruses are known to be adsorbed (Moore et al., 1975), (b) the methods of virus concentration are inefficient (Table I), and (c) some kinds of viruses will be excluded on the basis of the method of concentration as well as the cell culture system used.

Significantly, the present data (Table III) indicated that the concentration methods employed were not selective for specific enteroviruses nor did the sewage treatment processes appear to be selective in their removal or inactivation of the various viruses isolated. The viruses most often isolated from Mililani sewages were poliovirus types 1, 2, 3, coxsackievirus B-4, B-5 and echovirus 7. The growing concern that the immunization level against poliovirus in the U.S. has been declining to a dangerous level raises the question as to whether the virulent strains of poliovirus is still endemic in our communities. In the current study, 23/26 poliovirus isolates were determined to have the T– marker indicating that most of the isolated poliovirus were of the attenuated vaccine strain. However, the three poliovirus isolates with T+ marker suggest that the virulent strain is still endemic in the population and may represent a source of transmission to the non-immunized. Significantly, the State of Hawaii passed a state law in 1974 requiring all school children to be immunized against poliovirus and initiated a massive state-wide vaccination program to achieve this goal. The exclusive recovery of poliovirus from the sewages during 1974 probably reflect the activities of the vaccination program. Since poliovirus is the only enterovirus to which the population is vaccinated, the

other enteroviruses isolated from the sewage must represent natural infections. Thus, the exclusive recovery of echovirus 7 within a three month period (Table III) indicates that the community underwent a 'silent epidemic' of this virus infection during that period. Presumably, echovirus 7 was a new virus to which the population at Mililani lacked herd immunity and consequently this virus was easily established. A similar 'silent epidemic' of echovirus 7 involving 115 families in Louisiana has been previously reported (Henigst *et al.,* 1961). The accumulated data suggest that continued monitoring of the sewage for human enteric viruses would be useful in determining the endemic viruses in the community as well as recording and perhaps predicting the next epidemic of virus infection in that community.

The isolation of poliovirus from the natural bodies of water receiving sewage effluents demonstrates that enteric viruses persist in the natural water environment km aways from their presumed point of entry. These findings are essentially similar to those reported by Lamb *et al.* (1964) and Shuval *et al.* (1969). The exclusive isolation of poliovirus (Table V) from these waters may indicate either the unusual stability of this virus or may merely reflect the predominant virus present, since poliovirus was also exclusively isolated from the Mililani STP during this same period. The isolation of virus from upper Waikele stream (Site D, Figure 1) before its mergence with Kipapa stream is not surprising since two other STP discharged their effluents within 1.6 to 3.6 km north of this sampling site. Consequently, the source of the virus isolated from lower Waikele stream (Site E, Figure 1) could have originated from either upper Waikele stream or from Kipapa stream. The isolation of virus from lower Waikele stream is significant because this part of the stream passes through a populated area which is used by children for swimming and wading. The isolation of virus from Pearl Harbor (Site F, Figure 1) is also significant because this harbor is a natural habitat for oysters and clams which are known to concentrate and transmit viruses. Although the oyster and clam beds are currently closed to the public, future plans are to raise these shellfishes for human consumption.

The current study has clearly shown that the highest concentration of viruses is found in raw sewage. This makes the health hazard potential associated with discharging raw sewage into any body of water considerably greater than that of treated sewage. Until recently, the city of Honolulu's sewage which comprises approximately 50% of Oahu's sewage and represents about 2.3×10^9 1 day^{-1} was discharged untreated into the ocean only 1 km offshore at a depth of 12 m and within 8 km from the popular swimming beaches (Figure 1). The sewage is currently being discharged from a newer discharge pipe extending 3 km offshore and at a depth of 73 m. The survival, movement and fate of these sewage-borne viruses in these marine waters are currently being investigated and will be the basis of another report. The results of the present study support the earlier conclusions by Gerba *et al.* (1975) that the public health consequences of infectious viruses in the disposal and reuse of sewage must be properly evaluated. In one exceptional study enteroviruses were eliminated from the effluent of a Santee, California sewage treatment

plant after holding in an oxidation pond and filtration through a natural sand bed, in addition to final chlorination, and this water has been used successfully for recreational purposes (England *et al.,* 1967). Significantly, in all current water pollution regulations, the problem of viruses as a specific pollutant has been omitted.

Acknowledgments

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