

MIGRATION OF POLIOVIRUS TYPE 2  
IN PERCOLATING WATER THROUGH SELECTED OAHU SOILS

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Technical Report No. 36

January 1970

Project Completion Report

of

POLLUTION EFFECTS ON GROUND WATER RECHARGE IN HAWAII

OWRR Project No. A-000-HI, Grant Agreement No. 14-01-0001-1630

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Project Period: July 1, 1968 to June 30, 1969

The programs and activities described herein were supported in part by funds provided by the United States Department of the Interior as authorized under the Water Resources Act of 1964, Public Law 88-379.



## ABSTRACT

*A laboratory study was made to determine the ability of three Oahu soils, Wahiawa, Lahaina, (both Low Humic Latosols), and Tantalus cinder to prevent possible contamination of the basal-water lens by the passage of virus.*

*The study was performed utilizing columns containing the selected soils and subject to intermittent percolating water containing a known concentration of Poliovirus Type 2, simulating the action of a cesspool leaching into the ground. The effluent from the soil column was collected and analyzed for viral content by the plaque forming techniques.*

*Breakthrough of the virus occurred in both Wahiawa and Lahaina soils at soil thicknesses of 6-inch, 2 1/2-inch, and 1 1/2-inch at applied concentration of  $1.5 \times 10^5$  pfu/ml of feed solution. The virus removal was over 97 percent of the applied titer.*

*The Tantalus cinder proved ineffective in withholding the virus at the applied concentration of  $1.5 \times 10^5$  pfu/ml of feed solution. The virus breakthrough varies between 39 percent to 78 percent of the applied titer.*



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## INTRODUCTION

Accompanying the growth of the population in the United States, has been a movement of people from rural areas to metropolitan centers. This has brought about increasingly complex problems of water consumption and wastewater disposal. Within the last five years, there have been several epidemics and numerous lesser outbreaks of viral diseases which have been attributed to water-borne viruses (1, 2, 3, 4, 5, 6, 7, 8, 9). Involved in the majority of cases were small well-water supplies contaminated by subsurface seaage disposal systems. To accumulate knowledge concerning the movement of viruses through subsurface aquifers was the objective of this study.

Hawaii has had no reported contamination of the ground-water supply attributed to enteroviruses which include the polio group, Coxsackie group, and ECHO group (enteric cytopathogenic human origin) In a 1960 survey by the American Water Works Association, Hawaii was one of only three of the 50 states that could report no health incidents due to water contamination (10). With the continued increase of cesspools and septic tanks, there is no assurance that this condition will continue. The dangers of contamination of the basal lens still exist. Thus, in order to safeguard and prevent outbreaks of viral diseases attributed to water-borne viruses, there is a great need for information regarding virus movement through the soils.

The Hawaiian Islands are in a tropical area. Some of the world's highest annual rainfall have been recorded on the thickly forested mountains. These orographic rains are brought mainly by the moisture-laden tradewinds that sweep in from the sea and up the mountain slopes. The rain can trickle or percolate downward through the soil blanket and the underlying partly weathered lava. About one-third of the rain filters down through the ground into the island mass where it becomes part of basal-water lenses (11). These basal water lenses are formed as the fresh water reaches the porous rock underlying most of the area, filling the void spaces within the rock.

The ground water used for the domestic water supply is purified naturally through the slow filtering action of intervening soil and rock (12). An area of 31 square miles makes up the Honolulu watershed

in the Koolau Mountains above the City. Entry to this area is closely restricted so that the water does not become polluted by human access (13).

The primary source of Honolulu's water and the water supplying other areas on the island of Oahu are pumped from the basal-water supply beneath the island. Contrary to practices from other localities where water is pumped from lakes or rivers and where filtration, chlorination, and other treatment are the preferred methods of treatment in water-works operation, over 90 percent of the Honolulu water supply receives no chlorination or treatment and the remaining 10 percent receives only nominal chlorination to maintain a chlorine residual of 0.1 ppm (14). The nominal dosage is primarily a safety measure rather than a required treatment.

In many areas, small housing developments mushroomed in suburban sections of Oahu where the construction of sewer systems was uneconomical. The only available method of sewage disposal was by means of a cesspool. Problems associated with cesspools and septic tanks on Oahu were mainly due to poor soil conditions, high water table, and the concentration of numerous homes on small lots. As a result, the overflowing of cesspools and septic tanks is inevitable. (These cesspools and septic tanks located directly above Oahu's basal-water supply can be a potential source of ground water pollution.)

Findings in a recent study of cesspool problems on Oahu conducted by a consulting engineering firm were as follows (15):

- 1) Some 38,000 cesspools exist on Oahu. A drastic reduction does not appear imminent.
- 2) Some 8,700 defective cesspool (1965) requiring City's service may increase to 12,000 within the next five years, a 33% increase.
- 3) In spite of the accelerated sewer construction program (some 7 million dollars per year) the number of cesspools on Oahu has been increasing. The cesspool problem will remain critical for many years.
- 4) During the period 1960-1963, 5,380 new residences were constructed with cesspools, or 35% of a total of 15,286 new residences.

5) In recent years, an average of 1,000 new cesspools has been constructed each year for about 15-30% of all new residences.

Previous studies (16) concerning the passage of virus through selected soils indicated that virus of the type typified by coliphage T4 Br<sup>II</sup> mutant, a plant type virus, could breakthrough selected Oahu soils when dosed upon the surface and permitted to percolate through the soil.

Certain clay-type soils, prevalent on Oahu, retained the virus when depths of greater than 2.5 inches of soil existed. Tantalus cinder, a volcanic ash, proved to be ineffective in preventing the passage of the virus.

### PURPOSE AND SCOPE

This laboratory investigation and study was designed to determine the ability of three types of Oahu soils to prevent possible contamination of the basal-water lens by the passage of virus through them.

The poliovirus was selected for this study because 1) it is one of the smallest enteroviruses that can contaminate soils and the basal-water lens, 2) poliovirus can be cultivated in vitro using continuous cell lines, 3) a research worker can be highly immunized against poliovirus, and 4) accurate quantitative viral assay can be made by the production of plaques with agar overlay techniques. The Poliovirus Type 2 (Lansing) H8 was the only virus utilized in this study.

The Wahiawa and Lahaina soil series which were selected for this study, are most commonly found on the island of Oahu (Fig. 1). They occur throughout the central Wahiawa plain where the predominant agricultural use of the land is the cultivation of pineapple and sugarcane. Yet, the soil thickness overlying the basal-water table varies from near bare to considerable depth.

Tantalus soil was selected because it is strategically located above the basal-water lens found in the Tantalus mountain area where large portions of watershed areas occur. This soil is in areas where it is believed rainfall percolates directly into the basal-water lens without any hindrance from impermeable strata.

The study was performed utilizing columns containing the selected soils, which were subjected to intermittent percolating water with a

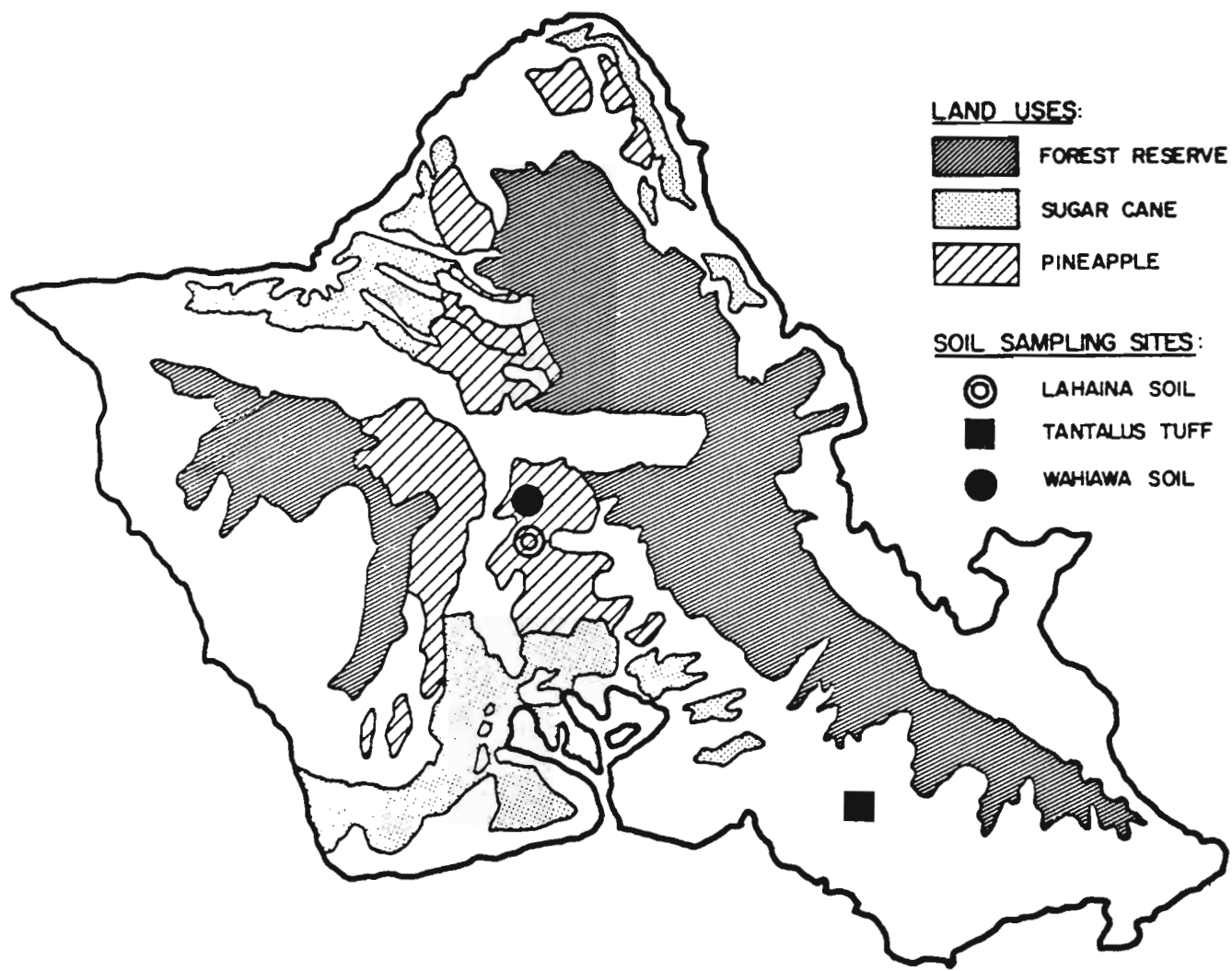


FIGURE 1. MAP OF OAHU SHOWING LAND USES AND SOIL SAMPLING SITES.

known concentration of virus, and simulating the action of a cesspool leaching into the ground. The effluent from the soil column was collected and analyzed for viral content by the plaque-forming techniques.

## LITERATURE STUDY

### Polioviruses

Polioviruses are known to cause poliomyelitis which is an acute, febrile, communicable disease. The Greek roots from which the name is derived point to an infection of the gray matter in the spinal cord.

The poliovirus is a spherical particle (Fig. 2), with a diameter of about 28 mu, as determined by electron microscopy, gradual membrane filtration, or other means (17). Chemical analysis reveals that the particles are nucleoproteins of the RNA (ribonucleic acid) type and that the nucleic acid core comprises 20 to 25 percent of the total mass. There is evidence that RNA core of the particle is surrounded by a series of 60 protein sub-units (18, 19). The resistance of the virus to ether, because of its lack of essential lipids, is demonstrated by retention of full infectivity after treatment with 20-percent ethyl ether for 18 hours at 4°C (13).

The International Subcommittee on Virus Nomenclature has recommended that viruses be classified into major groups on the basis of common biochemical and biophysical properties. Therefore, the polioviruses are classified as enteroviruses of picornaviruses of human origin.

The antigenic composition of polioviruses studied earlier had indicated the existence of more than one poliovirus serotype, but it was not until 1949 that the classification of three types, namely, Type 1, Type 2, and Type 3, were established (20).

Polioviruses are resistant to all known antibiotics and chemotherapeutic agents. Laboratory disinfectants, such as 70-percent alcohol, 5-percent lysol, or 1-percent quaternary ammonium compounds are ineffective. They are insensitive to ether, deoxycholate, and various detergents, which destroy other viruses (arboviruses, myxoviruses, etc.). Polioviruses are also stable at pH 3.0, but are inactivated rapidly by heat, desiccation, ultraviolet light (21), and

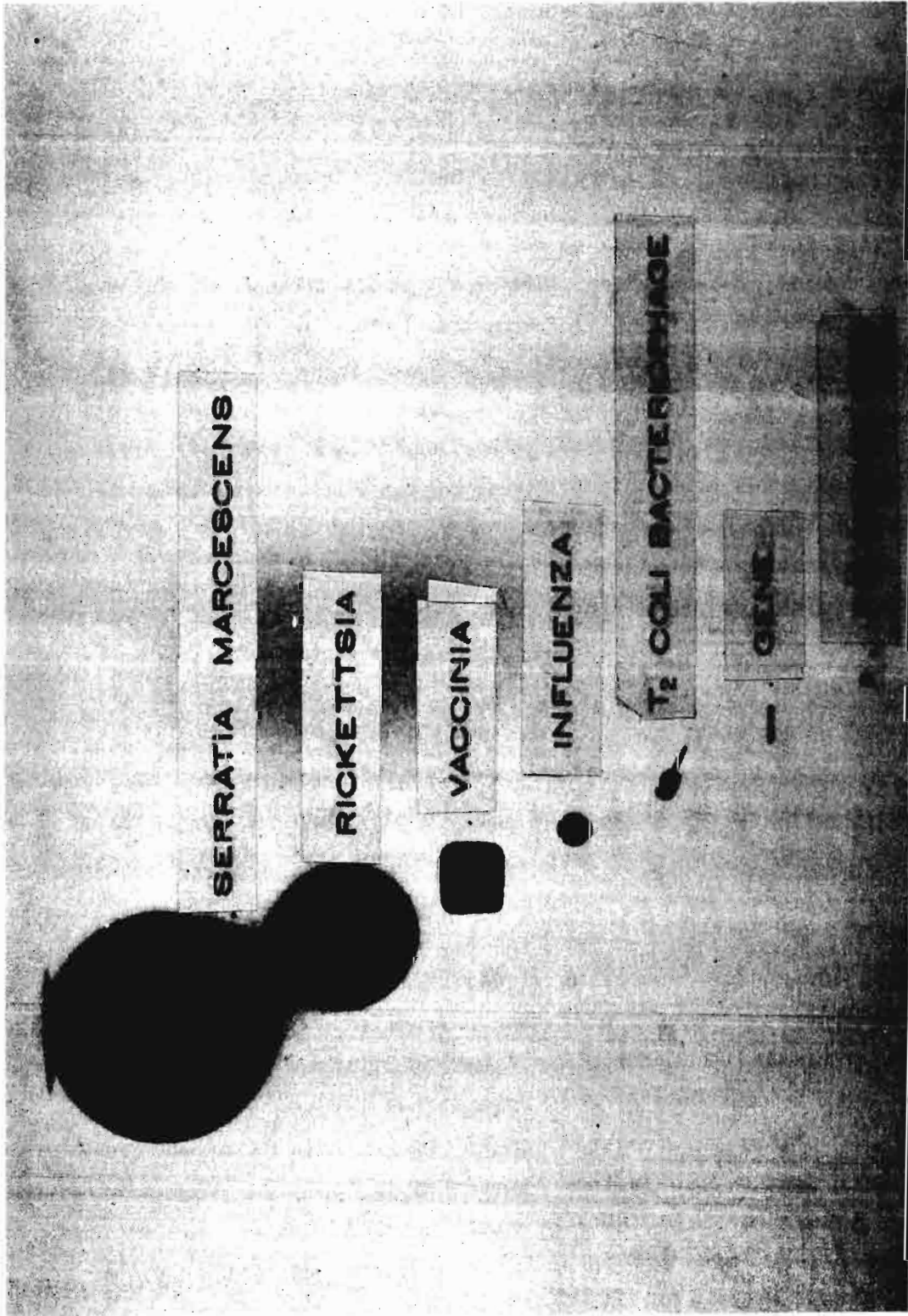


FIGURE 2. A COMPARISON OF SIZES AND SHAPES.

treatment with 0.3-percent formaldehyde, 0.1 N HCl, or free residual chlorine at a level of 0.3-0.5 ppm, but the presence of extraneous organic matter protects the virus from inactivation (22).

The discovery that polioviruses can be cultivated in vitro, especially in non-neural tissues, opened up a new phase of investigation of polioviruses and poliovirus infections (23). It was found that both primary and continuous cell-line tissue cultures derived from a wide range of primate organs and tumors are highly susceptible to polioviruses (24).

The development of the plaque method for viral growth and assay on epithelial monolayers in tissue culture, especially cultures derived from trypsinized monkey kidney cells, represented a further important advance in the cultivation of polioviruses (25).

At present, cells from monkey kidney and those from a human carcinoma of the cervix (He La) are most widely used for poliovirus cultivation and assaying.

#### Virus Movement through Soils in Ground Water

A literature survey revealed very few investigations of virus movement through soils. Most of the limited amount of literature is concerned with the removal of virus by treatment processes.

Rapid development of the science of microbiology and virology together with improved epidemiological methods of investigation attributed known outbreaks of water-borne disease, 555 cases in the United States alone, to contamination of public and private water supplies during the period from 1938 to 1960 (1, 2).

Epidemiological studies have documented several outbreaks of water-borne infectious hepatitis virus which passed through soils. Mosley's (23) study shows nine outbreaks, involving 1,015 cases of hepatitis, infected by virus-contaminated underground water supplies. Clarke and Chang (24) list 5 outbreaks, involving 538 cases of hepatitis, also traced to virus-contaminated underground water supplies. Other cases of infectious hepatitis caused by contamination of ground water through soils have been reported (5, 6, 7, 8, 9). In all of these cases, the underground waters were contaminated by cesspools or septic tank seepage. The virus-contaminated water traveled anywhere

from a few to several hundred feet through soil to enter water supplies.

In most cases, virus appeared to have traveled through the soil into the water by means of seepage channeled through fissures, or cracks. Laboratory data obtained in investigations of virus movement in ground water or survival of viruses in ground water environments is nearly non-existent. Data which are available are based on studies with bacteriophages and chemical contaminants.

Gilcreas and Kelly (26) conducted an experiment on comparative behavior of enteric viruses and bacteria during seepage of water through soil by suspensions of Coxsackie virus and *E. coli* in spring water. The suspensions were percolated through garden soil of 4-percent moisture content with 6, 18, and 36-inch columns of soil in 36-mm glass tubes and the percolate was collected and assayed. Gilcreas and Kelly concluded that:

percolation through at least 36 inches of soil was necessary for the reduction of either virus or bacteria, and even distance of travel was insufficient to remove them completely.

The results given by Gilcreas and Kelly show approximately 50-percent recovery of Coxsackie virus, 30-percent recovery of *E. coli*, and 75-percent recovery of bacteriophage following percolation through a 36-inch column of soil. However, this investigation did not reveal the effective soil column distance for virus removal, the effect of rate of flow, or different types of soil.

Drewry and Eliassen (27) conducted an experiment with bacteriophage T1, T2, and f2, which are effective against *E. coli*. Nine different soils were used in this study. Five soils were obtained from various sites in San Mateo County, California, and were used in the studies relating to T1 and T2 bacteriophage. Four soils obtained from sites in Washington County, Arkansas, were used in the study on f2 bacteriophage.

Two test procedures, batch tests and column studies, were utilized. All batch tests were performed by mixing an equal weight of soil and water containing the desired amount of bacteriophage by volume. The results show typical adsorption isotherms obtained with the bacteriophage and the various soils. Thus, Drewry and Eliassen indicated that removal of various bacteriophage from liquid solutions by solid media was an adsorption process.

Nine columns were utilized with different soils of various depths in a 1 1/8-inch I.D. lucite cylinder for the column experiments. The column feed solutions contained phage from  $7.88 \times 10^6$  to  $4.30 \times 10^7$  pfu/ml. The feed solution was pumped through the soil column at rates varying from 0.078 to 0.313 ml/min. Soil depths ranging from 1 to 8 cm were examined by centrimeter increments and it was found that all columns removed over 99 percent of the viruses.

Tanimoto (16) conducted a laboratory study of the ability of three Oahu soils, Wahiawa, Lahaina, and Tantalus, to remove or absorb bacteriophage T4. The Wahiawa and Lahaina soils were 100 percent effective in the removal or adsorption of bacteriophage T4 from percolating water at the applied concentration of  $2.5 \times 10^6$  per ml of feed solution at depths of 6 and 2.5 inches. Columns were operated for 5 consecutive days with intermittent irrigation totalling 30 inches of water.

Breakthrough of the bacteriophage occurred in both Wahiawa and Lahaina soil columns of 1.5-inch depth at an applied concentration of  $1.5 \times 10^6$  per ml of feed solution. The breakthrough occurred immediately and the numbers which passed through the soil increased rapidly with time. A greater number of viruses passed through the Lahaina soil than the Wahiawa soil. Unfortunately, no precise enumeration was obtained for the last three days of sampling except that abundant breakthrough occurred.

The Tantalus cinder subsoil proved ineffective in holding the bacteriophage at the recorded thicknesses of 15, 12, and 6 inches at the applied concentration of  $1.5 \times 10^6$  per ml of feed solution. The breakthrough concentration was  $5 \times 10^5$  per ml, thus representing approximately 67 percent or 33 percent passage through the column.

#### Problems in Research Concerned with Viruses in Ground Water

Through research efforts within recent years, a great deal of information has been obtained regarding the presence of viruses in water systems, and transmission by, and isolation of viruses from recreational water (28, 29, 30). Present monitoring systems determine exact quantitative viral contamination of waste-water effluents and potable waters.

Kelly and Sanderson (31) found that wastewater treatment processes do not completely remove or inactivate viruses, therefore the viruses are discharged in the effluents to receiving waters. It is known, however, that treated wastewater contains a fairly low concentration of viruses.

Clarke, *et al.*, (32), determined the survival times of enteric viruses and bacteria in river water and wastewater. The data obtained substantiated earlier observations (33, 34) that viruses were able to survive longer in clean river water than in moderately polluted river water. The evidence seems to indicate that there is the probability of the presence of many different types of viruses in natural waters.

Studies of the isolation and identification of viruses in ground water or any water supplies indicate that the detection of low number of viruses in large volumes of water present enormous technical problems. Viruses can survive in a water which is relatively free from nutritive material (32, 33, 34) but do not multiply outside living cells. Thus, living cells are required for their detection, and the techniques involved require great technical skill and expense. It has been calculated (32) that the average density of enterovirus in sewage is 500 virus particles per 100 ml and in polluted waters only 1 per 100 ml. Therefore, for satisfactory detection, sample concentration of at least one hundredfold is required, even in the case of highly polluted water.

Presently, there is no accepted quantitative method of examining waters for the presence of viruses which is simple and inexpensive enough to be used as a routine procedure.

## METHODS AND PROCEDURE

### The Poliovirus Type 2 (Lansing)

Poliovirus Type 2 (Lansing) H8 was utilized in this laboratory study. Stock virus and He La cells were obtained from Dr. H. K. Oie, of the Microbiology Department, University of Hawaii.

The virus pool was prepared in He La cell cultures throughout the entire study. Titration of virus in cell culture was done using

the plaque technique as described by Dulbecco and Vogt (25). Titers of prepared virus pool varied between  $5.0 \times 10^7$  to  $8.7 \times 10^8$  per ml.

### Preparation of Glassware for Cell Culture

The importance of employing clean glass surfaces for the preparation of cell cultures has been recognized since the introduction of tissue culture techniques.

It has been shown that soft and hard glass surfaces require different treatment to permit attachment and growth of cells, and that the medium employed plays a large part in the suitability of the glass for cell cultures (35, 36, 37).

All glassware was boiled in a 0.5-percent solution of Micro-Solv tissue culture detergent (Microbiological Associates, Inc.) for five minutes. After being cooled to room temperature, the glassware was scrubbed with a bottle brush using the boiled detergent solution. After brushing, the glassware was rinsed ten times with tap water and twice with distilled water. Prior to use, the glassware was either autoclaved at  $121^\circ\text{C}$  (15 pounds per square inch pressure) for 30 minutes or sterilized with dry heat at  $160^\circ\text{C}$  for one hour.

### The Media

The following media were used for the growth and maintenance of cell cultures: Eagle's Growth Medium (EGM), Eagle's Maintenance Medium (EMM), Glucose Potassium Nitrogen Medium (GKN), and Overlay Medium.

Composition and preparation of the media used throughout the duration of this experiment are described in Appendix A.

### Preparation of He La Cell Culture

Cultivation of He La cells was done with a fluid medium (Basal Medium Eagle with Hank's Balance Salt Solution) in stationary cell culture bottles. The He La cell cultures consisted of confluent monolayers of cells in original state. They were utilized for the preparation of subcultures. The medium was removed and the cell sheets were washed twice with 10.0 ml of Glucose Potassium Nitrogen (GKN)

medium (see Appendix A). The cells were dispersed by the addition of 0.1 ml of 2.5-percent trypsin in 10.0-ml GKN medium directly to the cell sheet and incubated at 35°C for 5 to 10 minutes and examined for rounding off. When almost all the cells were rounded, the trypsin was removed and replaced with Eagle Growth Media (EGM). Any remaining clumps of cells were dispersed by pipetting the suspension back and forth. The dispersed cells were then counted in a hemocytometer. The cell suspension was diluted with EGM to desired concentration and dispersed into culture bottles. The cultures were incubated at 35°C for two to three days and the EGM was replaced with 10.0 ml of fresh EGM. Complete monolayers were formed in 7 to 10 days. Prior to infection of the cultures with the virus, the EGM was removed and replaced with 10.0 ml of the Eagle Maintenance Media (EMM).

To prepare the stock cell culture, trypsinized He La cells were diluted in EGM to give a suspension containing  $2 \times 10^6$  cells per ml and 1.0 ml of cell suspension was transferred into a 200-ml milk dilution bottle. The suspension was then fed with 10.0 ml of EGM as before.

#### Preparation of Virus Pool Stock

Confluent monolayer He La cells which were seven to ten days old were used for the preparation of virus pool stock (Fig. 3). The He La cell culture medium was removed and washed twice with 10.0 ml of GKN medium. The cultures were inoculated with one milliliter of Poliovirus Type 2 and incubated at 35°C to adsorb for 1 to 2 hours with redistribution of inoculum every 15 to 20 minutes. The inoculum was then washed with three rinses of the GKN medium and infected cells were fed with 10.0 ml of EMM. After incubation of 35°C for 72 hours, they showed complete degeneration of cells. The infected fluids were kept frozen at -20°C until ready to process for harvesting.

The infected cells were disrupted by three cycles of freezing at -20°C and thawing in running tap water. A tissue-free supernatant fluid containing the poliovirus was obtained by agitating the virus suspension in a centrifuge at 500-600 rpm for 15 minutes. The fluids were pooled and dispersed in 100-ml aliquots and stored at -20°C. Prior to the use of the fluid for experiment, the stock poliovirus was assayed, using the plaque method.

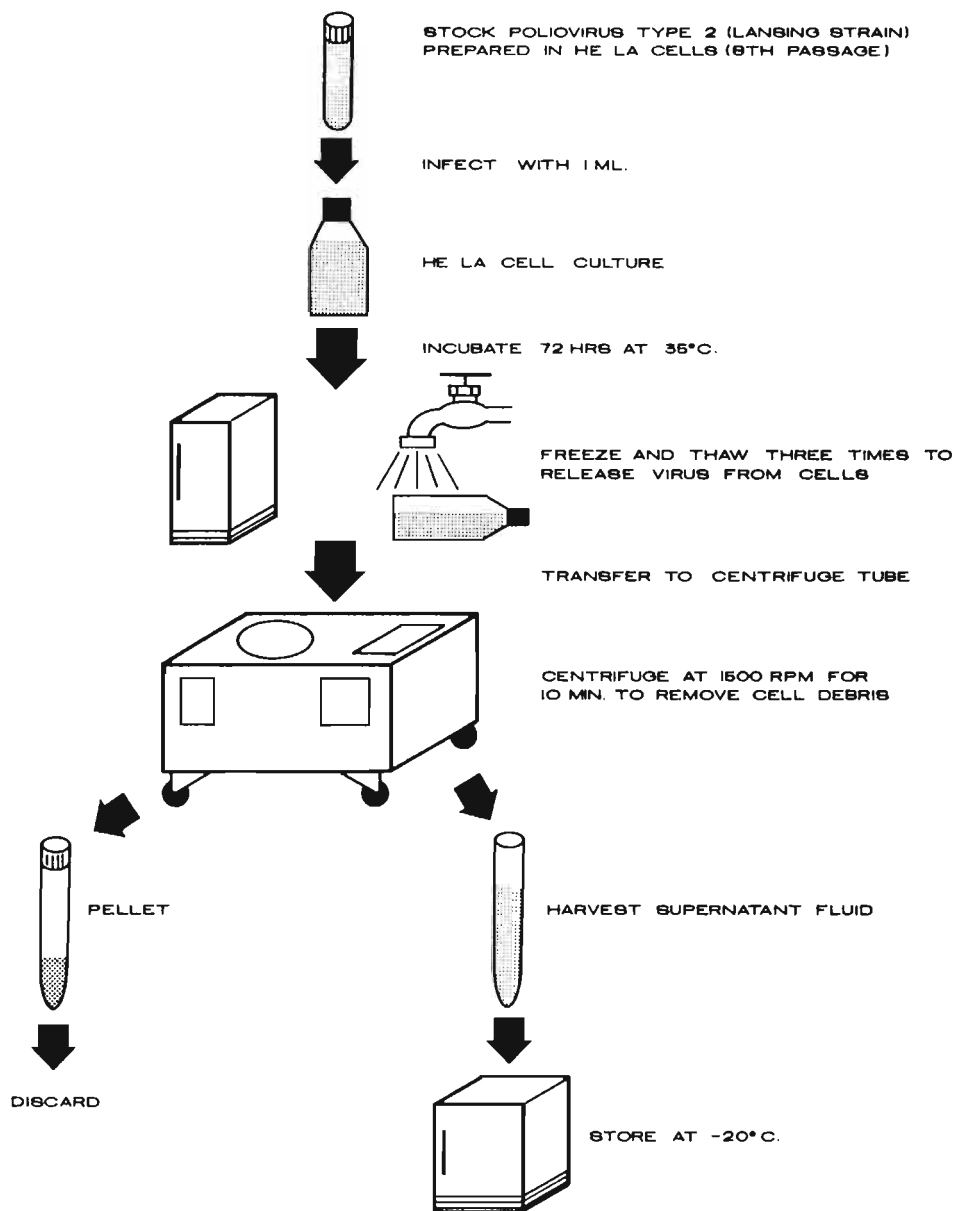


FIGURE 3. SCHEMATIC DIAGRAM SHOWING THE PREPARATION OF STOCK VIRUS POOL.

## Virus Assay

The tissue cultures were prepared in 2-ounce prescription bottles from trypsinized cells as described previously. When a confluent monolayer of cells was produced in seven to ten days, the EGM was removed from the monolayers of cell cultures and the cell sheets were washed twice with 2.0 ml of GKN medium (Fig. 4). Serially diluted viral suspensions were inoculated into the bottle cultures in volumes of 0.1 ml. The inoculum was distributed evenly over the monolayer and the cultures were incubated one hour at 35°C to permit viral adsorption. After the adsorption of virus on the cell sheet was completed, the residual virus was washed off with two rinses of the GKN medium (2.0 ml/rinse). Overlay medium was prepared (see appendix) and held at 45°C in a water bath during the addition to the cell culture. Approximately 5.0 ml of overlay medium was added into the bottle cultures of virus and carefully distributed over the cell monolayer.

The bottles were placed on a flat surface, cell sheet down, for one hour to permit hardening of the agar, then were incubated in darkness at 35°C for 24 hours. Poliovirus plaques appeared after 24 hours of incubation.

### Staining and Counting of Plaques

The overlay medium from each incubated culture were decanted and the cell sheet rinsed twice with 2.0 ml of GKN medium. Each culture was stained with 0.5 ml of 0.01-percent crystal violet solution for two minutes. Virus spreads from the initially infected cell to adjacent cells and produces circular areas of infected cells called, *plaques*, which do not take the crystal violet stain and appear as clear unstained areas against a background of stained cells. The plaques were counted under reflected light. Virus titers were obtained by multiplying the number of plaques counted by the dilution factors and reported as plaque-forming units per milliliter (pfu/ml).

### Preparation of Soils and Soil Columns

Soil samples were collected from sites described by Sakuichi Nakamura, soil scientist, United States Department of Agriculture,

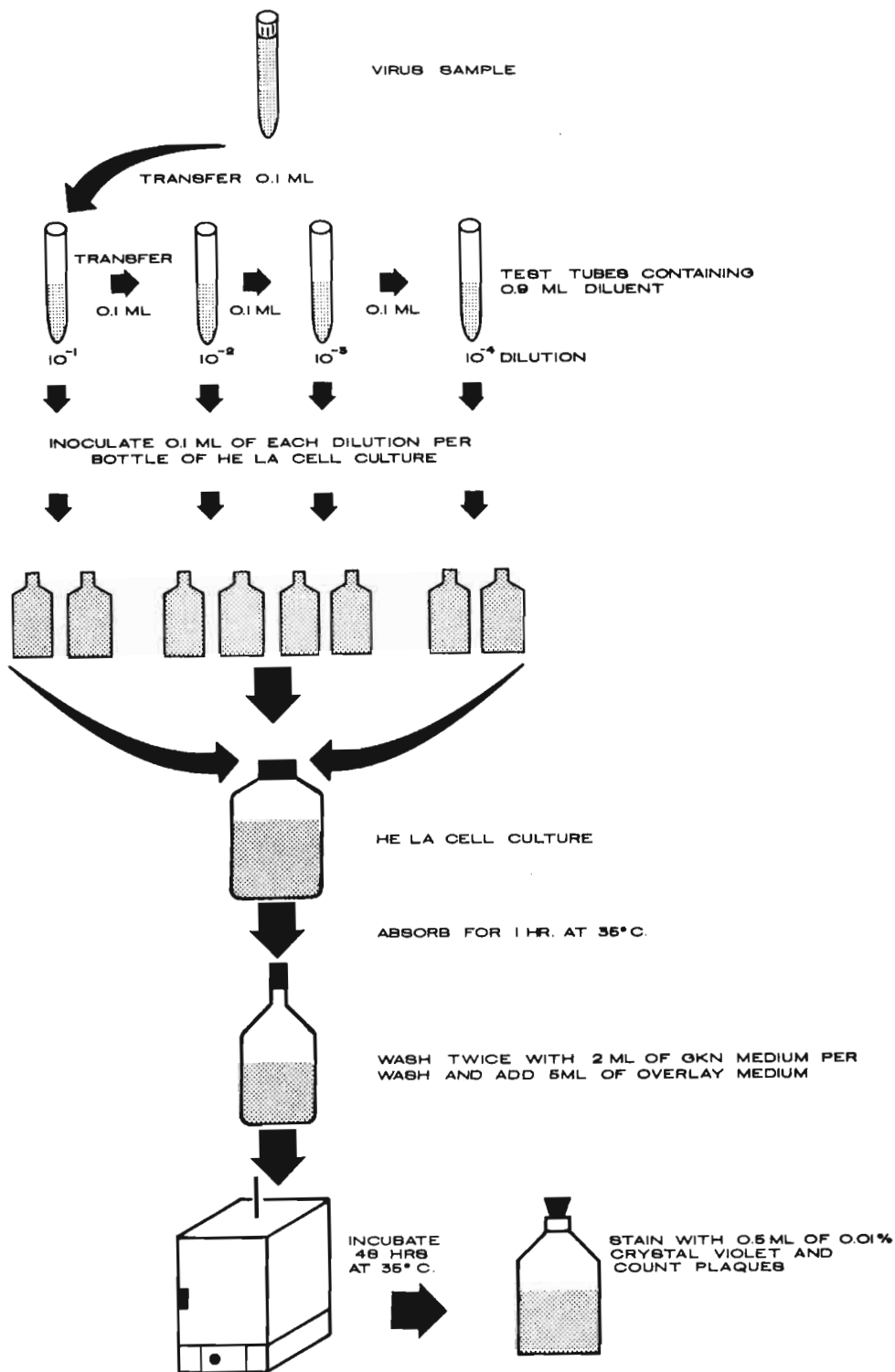


FIGURE 4. SCHEMATIC DIAGRAM OF THE PROCEDURE USED IN ASSAYING THE VIRUS.

Soil Conservation Service, from depths of about 6 to 8 inches below the ground surface (Table 1).

TABLE 1. A SUMMARY OF SOIL pH.

SOIL SERIES	CLASSIFICATION <sup>**</sup>	HORI	S.C.S. <sup>***</sup>	YOUNG	ETO	TANIMOTO
WAHIAWA	TROPEPTIC EUTHRORTHOXS	5.0	5.6	5.5	5.7	6.2
LAHAINA	TROPEPTIC HAPLUSTOX	5.3	5.2	6.3	5.9	4.9
TANTALUS	TYPIC DYSTRANDETS	7.2	-	-	-	7.7

<sup>\*\*</sup> DENOTES CLASSIFICATION AS OF JULY 1966.

<sup>\*\*\*</sup> U. S. SOIL CONSERVATION SERVICE.

The Wahiawa soil sample was collected from a site 0.7 miles east then 400 feet north from Kamehameha Highway near the entrance road to Mililani Cemetery in the Dole Corporation field number 4101, Block 30.

The Lahaina soil sample was obtained from a site approximately 2,500 feet SSE of the junction of the entrance road to Mililani Cemetery and the Dole loading station.

The Tantalus soil sample was obtained from Round Top Drive located along the roadside in the Sugar Loaf area near Mt. Tantalus.

The Wahiawa and Lahaina soil samples were brought to the laboratory and screened by hand to remove rocks and large organic matter. Portions of screened soils were then passed through a #10 sieve. The screened soils were sterilized in 8.25 quart stainless steel beakers that were sealed and autoclaved at 121°C with applied pressure of 15 pounds per square inch for one hour. The Tantalus soil was sterilized in the same manner, but the screening procedure was omitted.

The plastic columns used for the percolation study were 18" x 1.75" I.D. cylindrical columns covered at the base with three layers of #16 mesh nylon screen. The columns were washed with detergents, tap water, sterilized with 95-percent ethanol, and allowed to dry. Each column was packed with approximately 2.5 inches of sterile glass wool (Owens-Corning Fiberglass Corporation) then filled to specified thickness and were immersed in sterile distilled water. They were then conditioned by passing 10 inches/day of sterile distilled water through the columns for two days prior to the experiment.

Eight columns were utilized for the study on the Wahiawa and Lahaina soil samples. Six columns were paired and were carefully filled with 1.5, 2.5, and 6 inches of Wahiawa and Lahaina soils. The remaining two columns were packed with 6 inches of Wahiawa and Lahaina soil and used as controls (Fig. 5).

Precautionary measures were taken throughout the experiment against virus contamination for the research worker as well as the environment. Two 21" x 13" stainless steel pans, which contained virucidal agent (Aloetox), were placed beneath the column to collect spillage, if any.

The columns were dosed by carefully pouring the poliovirus in a water solution on the soil surface until the water ponded to a depth of one-half inch followed. Additional one-half inch doses were added as each dose passed into the soil column.

During the course of the experiment, 6 one-inch applications were made each day at intervals of 1 1/2 hours for five consecutive days for a total dosage of 30 inches of virus suspension in water to each test and control column, respectively. The intermittent application attempted to simulate the loading of a cesspool waste leaching into the ground.

When experimentation on the Wahiawa and Lahaina soils was completed, all the columns were washed, sterilized, and dried as described previously. Then the columns were packed with Tantalus cinder subsoil to a thickness of 15 inches, 12 inches, and 6 inches in pairs. Two columns with a soil thickness of 15 inches were utilized as controls.

Since Tantalus cinder is characterized by rapid drainage, the ponding application was not utilized. A 250-ml dosage of the liquid (equivalent to a six-inch depth of liquid in the column) was applied to the cindery substratum prior to each sampling run per column. Six samples were obtained, one hour apart, for the one-day test.

The percolants collected from Wahiawa, Lahaina, and Tantalus soils were placed in 18 x 150-mm screw cap sterile test tubes and stored by freezing until ready to be assayed.

### Soil Permeability Test

Five sets of soil permeability tests were conducted to determine



FIGURE 5. PHOTOGRAPH OF COLUMNS FOR PERCOLATION STUDY OF WAHIAWA AND LAHAINA SOILS.

the average permeability of Wahaiawa and Lahaina soils. Soil permeability tests were performed on columns filled with the soils to specified thicknesses. The solution was applied to maintain a constant hydraulic head two inches above the soil surface. The percolant was collected in 400-ml beakers for a period of 10 minutes.

The computations of the permeability of the Wahaiawa and Lahaina soils are shown in Appendix B.

## DISCUSSION OF THE RESULTS

### Soil pH

Determinations of soil pH confirmed the results reported by others (38, 39, 40, 16). The Wahaiawa and Lahaina soils are acid with pH values of 5.0 and 5.3, respectively, and Tantalus cinder has a pH of 7.2.

### Permeability

Permeability of the soils was determined by using a constant head of 2 inches above the soil surface. Average flow rates for the soils confirmed the work of others. The Lahaina soil was more permeable than the Wahaiawa soil and the coefficients of permeability were determined as 140 gallons/day/square foot (23.8 cm/hr) and 100 gallons/day/square foot (23.3 cm/hr), respectively (Table 2). It is

TABLE 2. OBSERVED PERMEABILITY OF WAHIAWA AND LAHAINA SOILS AT DIFFERENT THICKNESSES.

SOIL SERIES	SOIL THICKNESS IN COLUMN (IN INCHES)	VOLUME COLLECTED (ML/600 SEC)	K (GAL/DAY/FT <sup>2</sup> )	K (CM/HR)
WAHIAWA	6	103.2	177	30.1
	2 1/2	120.2	152	25.9
	1 1/2	92.2	90	15.3
	AVERAGE	105.2	140	23.8
LAHAINA	6	71.0	121	20.6
	2 1/2	59.8	76	12.9
	1 1/2	105.0	103	17.5
	AVERAGE	78.6	100	17.0

recognized that the permeability of the soil varies with packing and the properties of the test fluid.

The permeability of Tantalus cinder was not determined in this study. The soil has a characteristic granular structure, drains rapidly, and does not retain liquids. Ishizaki and Young reported a permeability rate of 35,200 gallons/day/square foot or 6,000 cm/hr (41).

#### Percolation Test Results of Wahiawa and Lahaina Soils

Percolation tests with Wahiawa and Lahaina soils and Tantalus cinder demonstrated that short soil columns (6 inches, 2 1/2 inches and 1 1/2 inches) did not completely remove Poliovirus Type 2 from percolating water when the virus was applied at a dosage of  $1.5 \times 10^5$  pfu/ml. Breakthrough occurred immediately for the 1 1/2 and 2 1/2-inch soil columns (Figs. 6, 7, 8, and 9) and the 6-inch columns (Figs. 10, 11, and 12) demonstrated a breakthrough of virus after the passage of less than bed-volume of percolant (Tables 3-6).

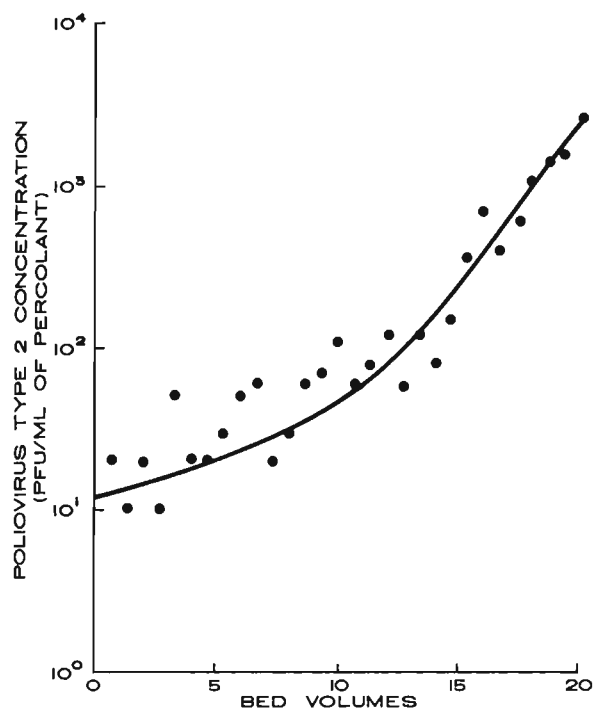


FIGURE 6. BREAKTHROUGH CURVE OF POLIOVIRUS TYPE 2 THROUGH 1 1/2-INCH TEST SOIL COLUMNS OF WAHIAWA SOIL.

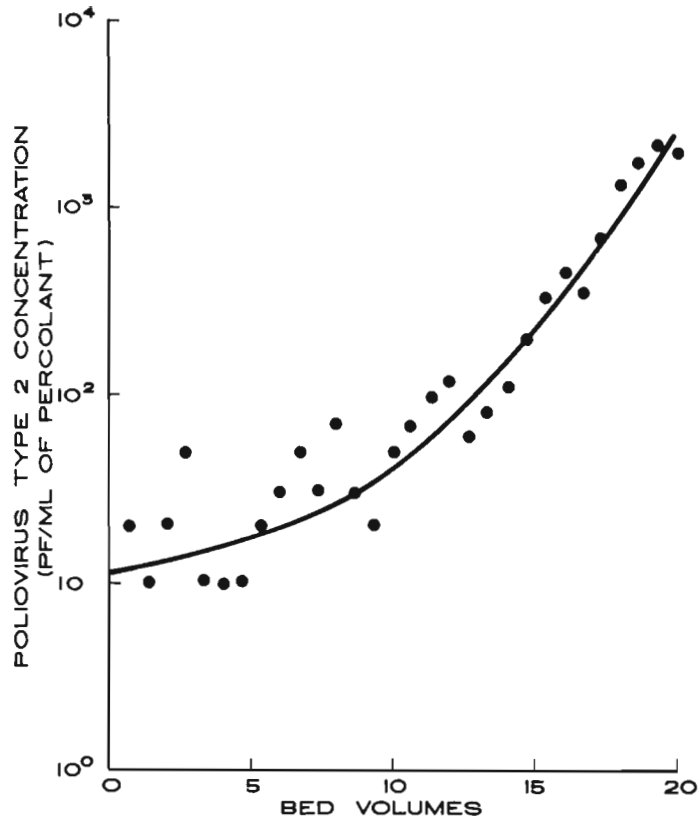


FIGURE 7. BREAKTHROUGH CURVE OF POLIOVIRUS TYPE 2 THROUGH 1 1/2-INCH TEST SOIL COLUMNS OF LAHAINA SOIL.

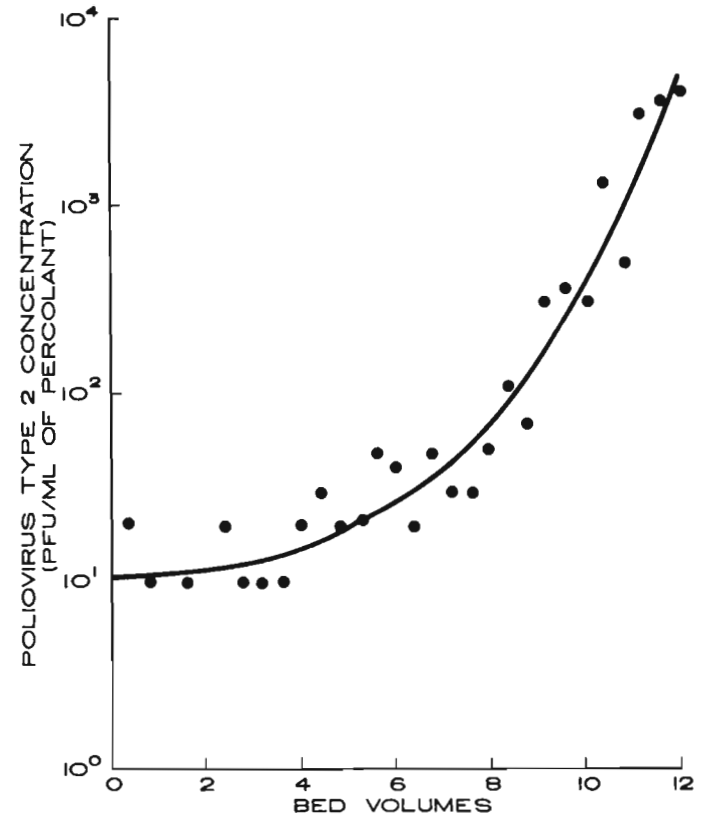


FIGURE 8. BREAKTHROUGH CURVE OF POLIOVIRUS TYPE 2 THROUGH 2 1/2-INCH TEST SOIL COLUMNS OF WAHIAWA SOIL.

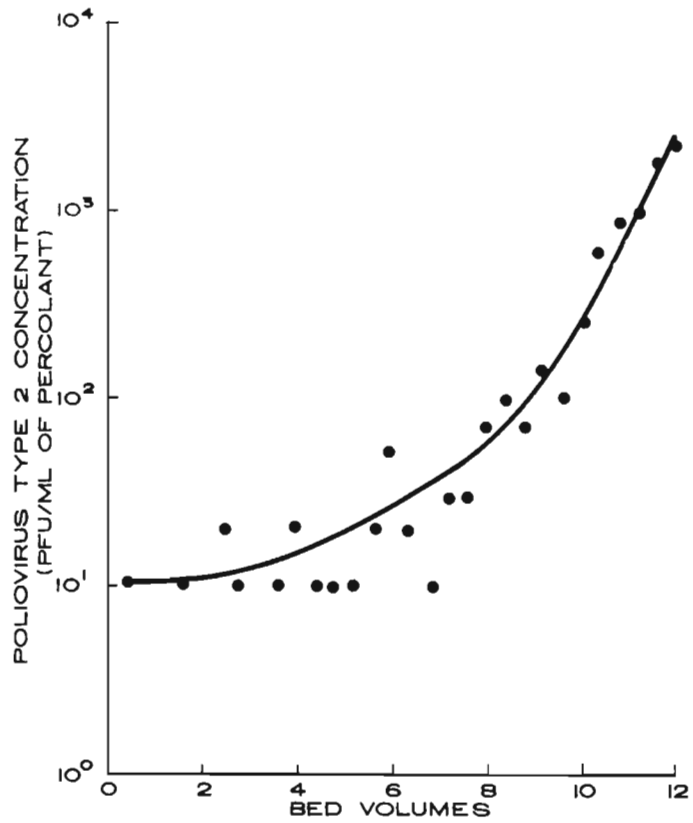


FIGURE 9. BREAKTHROUGH CURVE OF POLIOVIRUS TYPE 2 THROUGH 2 1/2-INCH TEST SOIL COLUMNS OF LAHAINA SOIL.

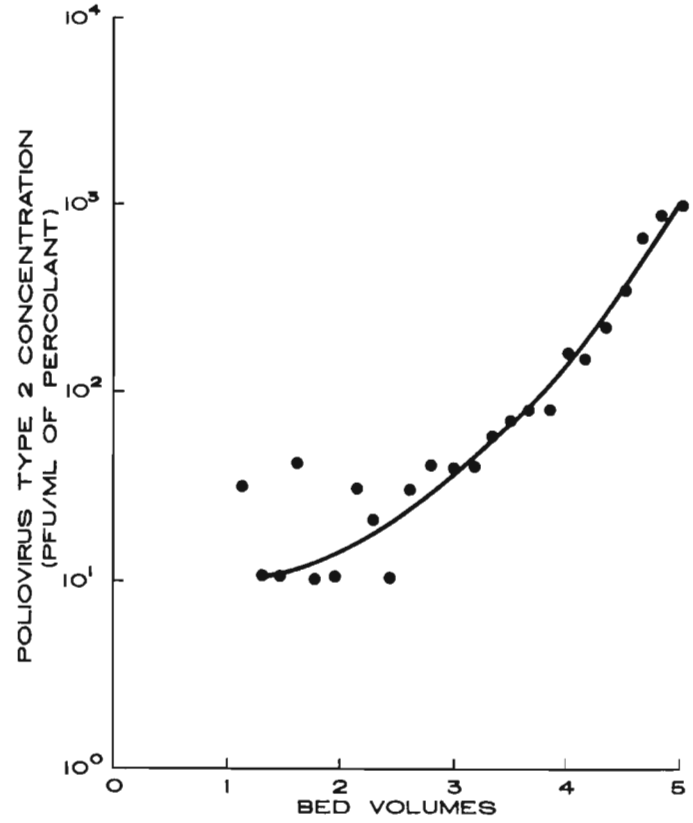


FIGURE 10. BREAKTHROUGH CURVE OF POLIOVIRUS TYPE 2 THROUGH 6-INCH TEST SOIL COLUMNS OF WAHIAWA SOIL.

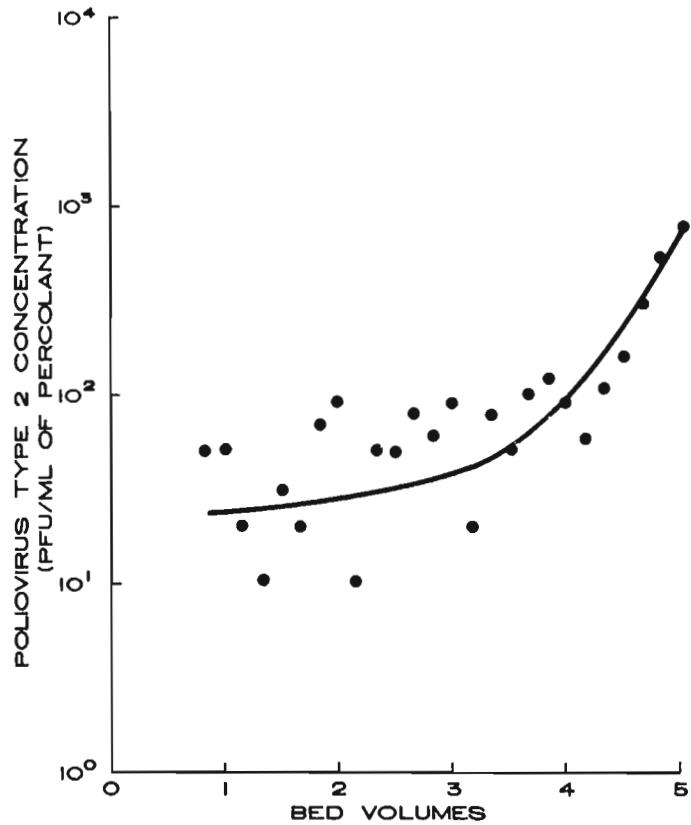


FIGURE 11. BREAKTHROUGH CURVE OF POLIOVIRUS TYPE 2 THROUGH 6-INCH TEST SOIL COLUMNS OF LAHAINA SOIL.

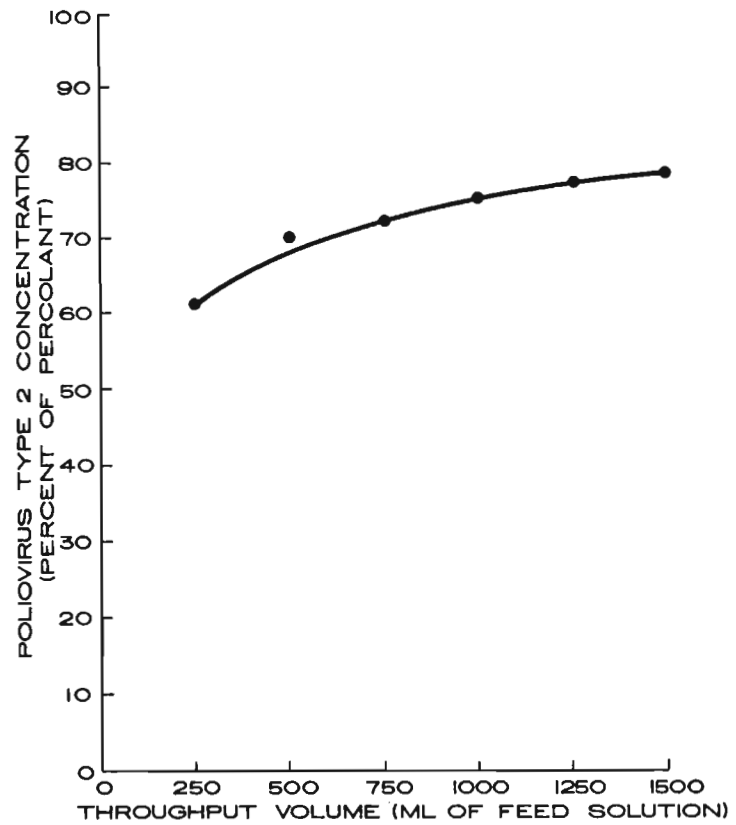


FIGURE 12. BREAKTHROUGH CURVE OF POLIOVIRUS TYPE 2 THROUGH 6-INCH TEST SOIL COLUMNS OF TANTALUS CINDER.

TABLE 3. AVERAGE BREAKTHROUGH NUMBERS (PFU/ML) OF POLIOVIRUS TYPE 2 FROM 1 1/2-INCH TEST SOIL COLUMNS OF WAHIAWA AND LAHAINA SOILS ( $1.5 \times 10^5$  PFU/ML APPLIED).

SAMPLING DAY	BED VOLUME	WAHIAWA		LAHAINA	
		EXP.	CONTROL	EXP.	CONTROL
FIRST DAY	0.668	20	0	20	0
	1.336	10	0	10	0
	2.004	20	0	20	0
	2.672	10	0	50	0
	3.340	50	0	10	0
	4.008	20	0	10	0
SECOND DAY	4.676	20	0	10	0
	5.344	30	0	20	0
	6.012	50	0	30	0
	6.680	60	0	50	0
	7.348	20	0	30	0
	8.016	30	0	70	0
THIRD DAY	8.684	60	0	30	0
	9.352	70	0	20	0
	10.002	110	0	50	0
	10.688	60	0	70	0
	11.356	80	0	100	0
	12.024	120	0	120	0
FOURTH DAY	12.698	60	0	60	0
	13.360	120	0	80	0
	14.028	80	0	110	0
	14.696	150	0	200	0
	15.364	360	0	340	0
	16.032	700	0	460	0
FIFTH DAY	16.700	400	0	360	0
	17.368	600	0	700	0
	18.036	1100	0	1300	0
	18.704	1500	0	1800	0
	19.372	1600	0	2200	0
	20.040	2800	0	2000	0

TABLE 4. AVERAGE BREAKTHROUGH NUMBERS (PFU/ML) OF POLIOVIRUS TYPE 2 FROM 2 1/2-INCH TEST SOIL COLUMNS OF WAHIAWA AND LAHAINA SOILS ( $1.5 \times 10^5$  PFU/ML APPLIED).

SAMPLING DAY	BED VOLUME	WAHIAWA		LAHAINA	
		EXP.	CONTROL	EXP.	CONTROL
FIRST DAY	0.401	20	0	10	0
	0.802	10	0	0	0
	1.203	0	0	0	0
	1.604	10	0	10	0
	2.005	0	0	0	0
	2.406	20	0	20	0
SECOND DAY	2.807	10	0	10	0
	3.208	10	0	0	0
	3.609	10	0	10	0
	4.010	20	0	20	0
	4.411	30	0	10	0
	4.812	20	0	10	0
THIRD DAY	5.213	20	0	10	0
	5.814	50	0	20	0
	6.215	40	0	50	0
	6.616	20	0	20	0
	7.017	50	0	10	0
	7.418	30	0	30	0
FOURTH DAY	7.819	30	0	30	0
	8.220	50	0	70	0
	8.621	110	0	100	0
	9.022	70	0	70	0
	9.423	300	0	140	0
	9.824	360	0	100	0
FIFTH DAY	10.225	300	0	250	0
	10.626	1300	0	600	0
	11.027	480	0	770	0
	11.428	3100	0	960	0
	11.829	3600	0	1800	0
	12.230	4000	0	2200	0

TABLE 5. AVERAGE BREAKTHROUGH NUMBERS (PFU/ML) OF POLIOVIRUS TYPE 2 FROM 6-INCH TEST SOIL COLUMNS OF WAHIAWA AND LAHAINA SOILS ( $1.5 \times 10^5$  PFU/ML APPLIED).

SAMPLING DAY	BED VOLUME	WAHIAWA		LAHAINA	
		EXP.	CONTROL	EXP.	CONTROL
FIRST DAY	0.167	0	0	0	0
	0.334	0	0	0	0
	0.501	0	0	0	0
	0.668	0	0	0	0
	0.835	0	0	50	0
	1.002	0	0	50	0
SECOND DAY	1.169	30	0	20	0
	1.336	10	0	10	0
	1.503	10	0	30	0
	1.670	40	0	20	0
	1.837	10	0	70	0
	2.004	10	0	90	0
THIRD DAY	2.127	30	0	10	0
	2.338	20	0	50	0
	2.505	10	0	50	0
	2.672	30	0	80	0
	2.839	40	0	60	0
	3.006	40	0	90	0
FOURTH DAY	3.173	40	0	20	0
	3.340	60	0	80	0
	3.507	70	0	50	0
	3.674	80	0	100	0
	3.841	80	0	120	0
	4.008	160	0	90	0
FIFTH DAY	4.175	150	0	60	0
	4.342	220	0	110	0
	4.509	350	0	160	0
	4.676	660	0	300	0
	4.843	880	0	520	0
	5.010	1000	0	800	0

TABLE 6. AVERAGE BREAKTHROUGH NUMBERS (PFU/ML) OF POLIOVIRUS TYPE 2 THROUGH VARIOUS SOIL THICKNESS OF TANTALUS CINDER ( $1.5 \times 10^5$  PFU/ML APPLIED).

THROUGHPUT VOLUME (ML)	15" TEST COLUMNS	12" TEST COLUMNS	6" TEST COLUMNS	15" CONTROL COLUMNS
250	$5.8 \times 10^4$	$6.6 \times 10^4$	$9.2 \times 10^4$	0
500	$7.6 \times 10^4$	$7.9 \times 10^4$	$10.5 \times 10^4$	0
750	$8.0 \times 10^4$	$8.6 \times 10^4$	$10.8 \times 10^4$	0
1000	$9.0 \times 10^4$	$9.2 \times 10^4$	$11.2 \times 10^4$	0
1250	$9.5 \times 10^4$	$10.4 \times 10^4$	$11.5 \times 10^4$	0
1500	$9.8 \times 10^4$	$10.7 \times 10^4$	$11.7 \times 10^4$	0

Despite breakthrough it was established that both types of Low Humic Latosols, Wahaiawa and Lahaina, in short columns remove over 96 percent of this extremely small virus. In the 6-inch column retention of virus was up to 99.3 percent for both types of soil; however, these high removal rates did not prevent the passage of these pathogenic animal viruses through the soil columns. These results are shown in Tables 3, 4, and 5 and Figures 10, 11, and 12.

Percolation test results with Tantalus cinder dosed at  $1.5 \times 10^5$  pfu/ml of Poliovirus Type 2 demonstrated very low virus retention. With a 6-inch column, only 22 percent of the virus was retained. The 12-inch column increased virus removal to 30 percent and the 15-inch column removed 35 percent of the virus (Figs. 13 and 14). Table 6 indicates that the magnitude of virus breakthrough varies with soil thickness and that the experimental results fit a linear adsorption isotherm.

Significant differences were observed between the bacteriophage studies of Tanimoto (16) and this work on animal virus. The results obtained with bacteriophage showed that 2 1/2-inch soil columns of Wahaiawa and Lahaina soil removed 100 percent of the applied Coliphage T4 at a feed concentration of  $1.5 \times 10^6$  pfu/ml. The poliovirus experiment showed that even the 6-inch columns of Wahaiawa and Lahaina soils were unable to effect 100-percent retention of Poliovirus Type 2 with an initial feed concentration of  $1.5 \times 10^5$  pfu/ml. The amount of removal obtained with Tantalus cinder was much smaller. Thus, if bacteriophage is used as a model in percolation studies similar to that conducted for animal viruses, the limitations and basic differences must be recognized and evaluated.

Adsorption of viruses by soils is influenced by many factors. Physical adsorption of virus is only slightly affected by temperature variations, but pH plays a major role. Although viruses exist in other forms, the only unit of viral structure which has been isolated and subjected to direct examination is the virion (42). Since the virion is composed of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), it possesses the amphoteric behavior of proteins. Therefore, as the pH decreases, there is also a decrease in the ionization of the hydroxyl groups occurs and the virions behave as anions. Soil is known to have a negative charge, hence adsorption takes place.

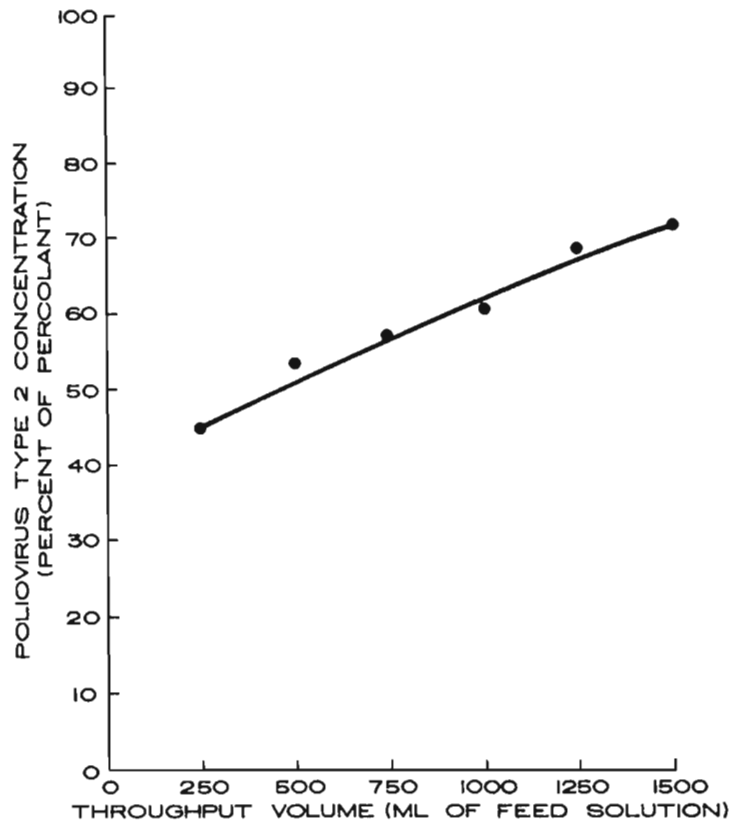


FIGURE 13. BREAKTHROUGH CURVE OF POLIOVIRUS TYPE 2 THROUGH 12-INCH TEST SOIL COLUMNS OF TANTALUS CINDER.

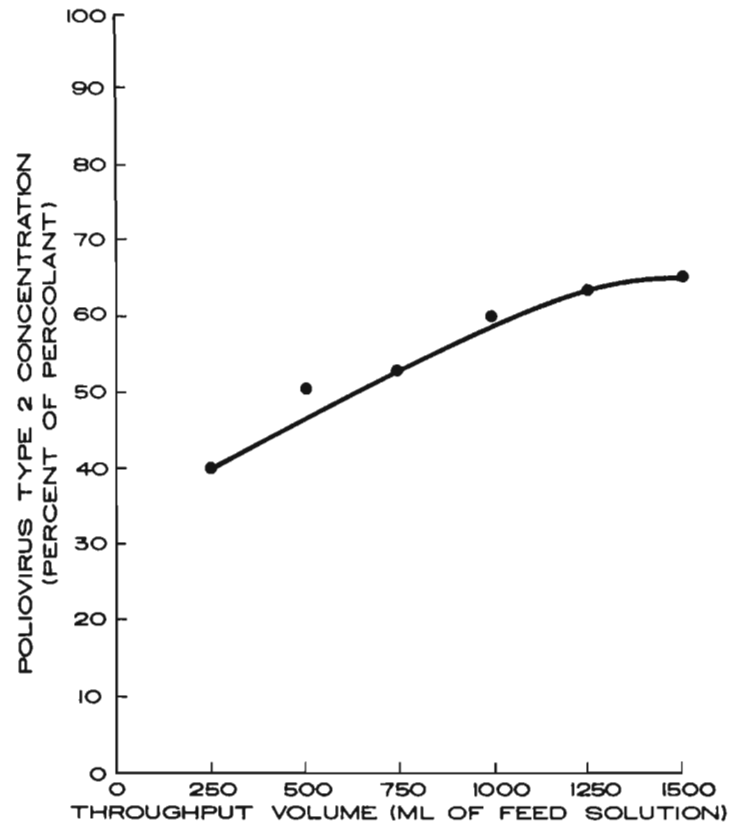


FIGURE 14. BREAKTHROUGH CURVE OF POLIOVIRUS TYPE 2 THROUGH 15-INCH TEST SOIL COLUMNS OF TANTALUS CINDER.

The use of ion exchange to remove or adsorb virus is well known. It has been shown that virus adsorption by soils increases with increasing ion-exchange capacity, clay and organic content, and presence of other minerals in the soils (27). High concentrations of iron, manganese, and clay in Wahiawa and Lahaina soils increase their ion-exchange capacity and hence, tend to support previous findings.

The results of this study indicate that the possibility of viral pollution of Hawaiian ground water does exist. However, due to the high adsorptive capacities of Hawaiian soils, the probability of the passage of viruses through the soil layer is fairly small. Furthermore, individuals who consumed very small quantities of virus may have undetectable infections in spite of the highly infectious nature of some of the viruses. To date there is no documented instance of movement of viruses through soils on Oahu, but the possibility of such movements exists through agricultural soil, especially where fissured or fractured substrata could provide for direct channeling of contaminants to ground water. The problems associated with the detection of low numbers of viruses in a large quantity of water is well known. Viruses can survive, but they are unable to multiply outside of living cells. Tissue cultures or direct inoculation into animals are required to grow and cultivate them and this requires much skill on the part of the technician and expensive laboratory equipment.

#### LIMITATIONS OF THE STUDY

The methods utilized for this study made no attempt to investigate the mechanisms of removal or inactivation of the viruses, the increasing or decreasing of the feed solution titer, flow rate, feed volume, or other physical and chemical factors. Only one animal virus, Poliovirus Type 2, was utilized in this study to ascertain its removal from percolating water by Hawaiian Low Humic Latosols under laboratory conditions. Research concerned with movement of virus in ground water is an area which needs further study. Only recently has there been great concern regarding viruses in ground-water supplies.

## SUMMARY AND CONCLUSIONS

A laboratory study to determine the ability of three Oahu soils, Wahaiawa and Lahaina Low Humic Latosols and Tantalus cinder to remove Poliovirus Type 2 (Lansing) from percolating water at different thicknesses demonstrated that poliovirus is capable of passage through all soils. However, the study did not investigate removal mechanisms or other factors effecting the retention of the virus.

The major findings of this study are:

- 1) Short columns of Wahaiawa and Lahaina soils, representative of Low Humic Latosols overlying the basal-water lens of the island of Oahu, are ineffective in preventing the passage of Poliovirus Type 2 in concentrations of  $1.5 \times 10^5$  pfu/ml from percolating to the ground water. While virus removal has ranged from 96.6 to 99.3 percent over a period of five days for dosages of up to 20 bed-volumes of applied percolant, breakthrough of the virus was immediate.
- 2) Tantalus cinders, a natural granular material soil, proved ineffective in the prevention of passage of Poliovirus Type 2 with percolating water. Columns of cinders up to 15 inches in depth showed immediate breakthrough of the virus and only 22 to 35 percent of the virus was removed when dosed at levels of  $1.5 \times 10^5$  pfu/ml.
- 3) The mechanisms of virus movement through Oahu soils relevant to Poliovirus Type 2, an animal virus, have no direct relationship to that of bacteriophage. The mechanism of removal is affected by the properties of the viruses rather than their relative sizes. A simple straining action is not the major removal mechanism.
- 4) The results obtained from this experiment are valid only for poliovirus and does not apply to other enteroviruses.
- 5) Although virus work required highly technical knowledge, skill, and expense in laboratory experimentation, this study has demonstrated that the poliovirus group is an ideal enterovirus to work with because it is readily available, easily cultivated onto continuous cell lines, demonstrates characteristic cytopathic effects on tissue culture, and

researchers can be highly immunized against its pathogenicity.

#### ACKNOWLEDGEMENTS

The author wishes to thank Dr. Herbert K. Oie, Department of Microbiology, University of Hawaii, for supplying the virus and tissue culture and invaluable assistance during the entire course of the laboratory research.

The author is grateful to Mr. Sakuichi Nakamura, Soil Scientist, of the Soil Conservation Service for assistance with soil sampling sites and soil descriptions.

This report is based on a master's thesis by the senior author.

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## APPENDICES



## APPENDIX A: MEDIA

## EAGLE'S GROWTH MEDIUM (EGM)

To prepare 100 ml as follows:

1. BME with Hanks BSS.....88.0 ml.
2. BME essential amino acid mixture (100X).....1.0 ml.
3. NaHCO<sub>3</sub> (1.4%).....2.5 ml.
4. NaOH (1 N).....0.5 ml.
5. BME vitamin mixture (100X).....1.0 ml.
6. L-glutamine 200 mM (100X).....1.0 ml.
7. Penicillin-streptomycin mixture  
(10,000 units each/ml).....1.0 ml.
8. Calf serum.....5.0 ml.

## EAGLES'S MAINTENANCE MEDIUM (EMM)

Preparation is similar to EGM except calf serum is reduced to 2% (2.0 ml.).

## GLUCOSE POTASSIUM NITROGEN MEDIUM (GKN)

To prepare 10X concentration as follows:

1. Glucose.....10.0 gm.
2. KCl.....4.0 gm.
3. NaCl.....80.0 gm.
4. Phenol Red.....0.2 gm.
5. Chloroform.....5.0 ml.
6. Triple distilled water.....1000.0 ml.

At the time of use, dilute 10X with triple distilled water and autoclave at 15 psi (250°F) for 15 minutes.

## OVERLAY MEDIUM

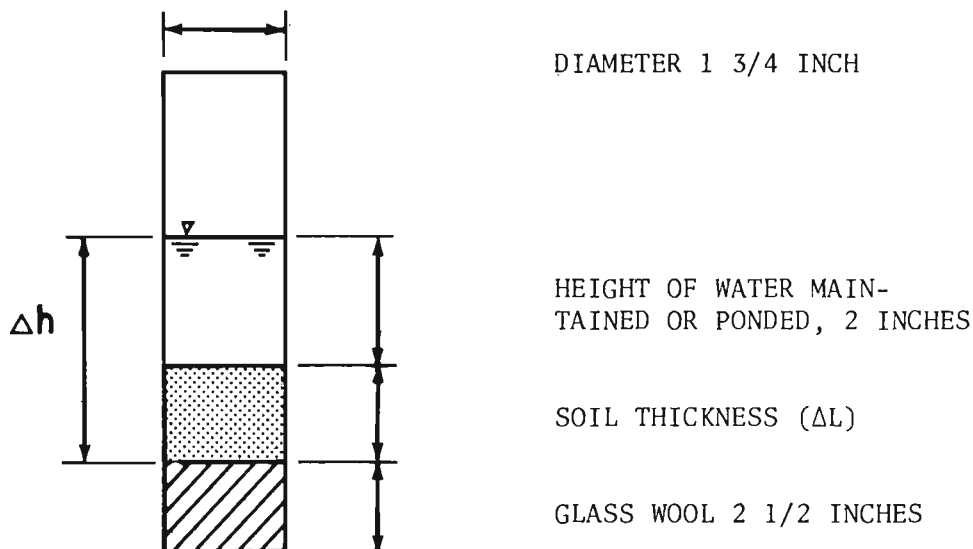
Prepare as follows:

1. Prepare Basal Medium Eagle (2X) with L-glutamine (2X), penicillin-streptomycin (2X), and calf serum (2%) and

incubate at 45° water bath.

2. Melt 1.5% agar and incubate at 45° water bath. Mix A and B in equal amounts and add the required amount to each bottle.

APPENDIX B: COMPUTATIONS ON PERMEABILITY  
OF LAHAINA AND WAHIAWA SOILS.



PERMEABILITY (K)

K as expressed as gal/day/ft<sup>2</sup>

$$K = \frac{Q}{A (\Delta h \Delta L)}$$

Where Q = flow rate in ml/sec

$\Delta h / \Delta L$  = hydraulic gradient

$$A = \text{area of circle} = (\pi r^2)$$

$$= (3.14) (0.785)$$

$$= 2.40 \text{ in}^2 \text{ or } 15.48 \text{ cm}^2$$

Conversions:

$$1 \text{ day} = 86,400 \text{ sec}$$

$$1 \text{ gal} = 3,785 \text{ ml}$$

$$1 \text{ ft}^2 = 929 \text{ cm}^2$$

## SAMPLE CALCULATIONS

Permeability of 6-inch soil columns:

$$\text{Lahaina: } \frac{(71.0/600)(929)(86,400)}{(15.48)(20.3/15.2)(3785)} = 121 \frac{\text{gal}}{\text{ft}^2\text{day}}$$

$$\text{Wahiawa: } \frac{(103.2/600)(929)(86,400)}{(15.48)(20.3/15.2)(3785)} = 177 \frac{\text{gal}}{\text{ft}^2\text{day}}$$