ASSESSING THE APPLICABILITY OF USEPA RECREATIONAL WATER QUALITY STANDARDS TO HAWAII AND OTHER TROPICAL ISLANDS

Roger S. Fujioka
and
Muruleedhara N. Byappanahalli

ASSESSING THE APPLICABILITY OF USEPA RECREATIONAL WATER QUALITY STANDARDS TO HAWAII AND OTHER TROPICAL ISLANDS

Roger S. Fujioka
and
Muruleedhara N. Byappanahalli

WRRC Project Completion Report: WRRC 96-01

July 1996

Prepared for:

The Department of Health
State of Hawaii
P. O. Box 3378
Honolulu, Hawaii 96801

Agreement Number: 94-517
Project Period: June 15, 1994 to June 30, 1996
Principal Investigator: Roger S. Fujioka

Water Resources Research Center
University of Hawaii at Manoa
2540 Dole Street
Honolulu, HI 96822

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

The Water Resources Research Center (WRRC) at the University of Hawaii has completed a series of studies beginning in the mid 1970's to evaluate the quality of recreational waters in Hawaii. These studies showed that many recreational water sites, especially fresh water streams exceed the USEPA recreational water quality standards based on concentrations of fecal bacteria. According to USEPA water quality standards, environmental waters containing elevated concentrations of fecal bacteria must be contaminated with sewage or other sources of feces of man or warm blooded animals and therefore this source of water is unsafe for swimming. However, sanitary survey of the streams in Hawaii did not support the extensive pollution of the streams in Hawaii which was indicated by the concentrations of fecal bacteria. Additional studies showed that unlike the continental USA, the soil environment of Hawaii contained high concentrations of fecal bacteria. Based on the results of our studies, we concluded that these fecal bacteria must be multiplying in the soil environment of Hawaii and therefore the USEPA recreational water quality standards which use these fecal bacteria as indicators of fecal contamination are not valid in the tropical environment of Hawaii. The results and conclusions reported by WRRC are contrary to the present understanding of water quality assessment as published in the books and enforced by USEPA. The present study was initiated to conduct the kinds of experiments which scientists from other universities and from USEPA have recommended to substantiate our previous findings.

The goals of the project were two-fold: First, to determine why USEPA recommended fecal indicator bacteria (fecal coliform, *E. coli*, enterococci) are not useful in determining the hygienic quality of recreational waters in Hawaii. Second, to suggest an alternative fecal indicator to establish recreational water quality standards that are more relevant to Hawaii.

Based on the results of the present study as well as past studies, the following conclusions were made:

1. Soil is the natural environmental source of fecal indicator bacteria in Hawaii and

this environmental and non-fecal source of fecal indicator bacteria is readily transported to streams in Hawaii by land run-off from rainfall.

2. The fecal indicator bacteria used by USEPA to establish recreational water quality standards are readily recovered from all seven major soil types in Hawaii. Thus, fecal indicator bacteria are prevalent and persist in all soil types in Hawaii.

3. Soil moisture, available nutrients and natural populations of soil microorganisms are the primary factors which control the populations of fecal indicator in the soil environments of Hawaii. Coliform bacteria have simpler nutritional requirements than enterococci bacteria. The diverse natural populations of soil microorganisms (bacteria, viruses, fungi, protozoa) are present in soil at concentrations exceeding a million times greater than fecal indicator bacteria. These soil microorganisms are very effective in obtaining nutrients from soil which they require to multiply. The growth of these soil microorganisms were shown to limit and to control the growth of fecal indicator bacteria most likely by competing for nutrients and additionally by producing by-products which inhibit the growth of fecal indicator bacteria.

4. Fecal indicator bacteria (fecal coliform, *E. coli*, enterococci) were shown to be capable of multiplying in the soil environment of Hawaii. In the natural soil environments of Hawaii, these fecal indicator bacteria have adapted themselves to grow in the soil environment and have established themselves as one of the indigenous populations of soil microorganisms. However, in the natural soil environment, these fecal indicator bacteria are at a disadvantage in their ability to grow. As a result, they probably grow opportunistically when conditions (available nutrients) allow them to grow. Although the populations of fecal indicator bacteria in soil grow slowly, these bacteria are able to persist and to wait for suitable conditions for their growth. Since time for rapid growth is not essential for the maintenance of this population of fecal indicator bacteria in soil, it appears that they have developed a successful strategy to maintain an active population in the soil environments of Hawaii.

5. The source of water for all streams in Hawaii is rainfall and rainfall carries soil-bound fecal indicator bacteria to streams resulting in high concentrations of fecal indicator bacteria in stream which often exceeds the recreational water quality standards as

established by USEPA. In the application of recreational water quality standards, USEPA assumes that there are no significant, environmental sources of fecal indicator bacteria and the fecal indicator bacteria recovered from natural waters represents contamination from a fecal source (sewage). However, this assumption is not applicable to Hawaii since fecal indicator bacteria are established as a natural, non-fecal and environmental source of fecal indicator. As a result, the same interpretations of water quality standards utilizing these fecal indicators are not valid in Hawaii.

6. Since the existing water quality standards are not applicable for Hawaii, alternative and more reliable recreational water quality standards should be used in Hawaii. Based on data and methodology considerations, *C. perfringens* is the most reliable and suitable fecal indicator to be used to establish recreational water quality standards in Hawaii as well as other tropical islands. *C. perfringens* was carefully evaluated as an indicator of recreational water quality and shown to fulfill all six of the criteria used to characterize an ideal indicator of fecal contamination.

The recommendations of this study are as follows:

1. Regulatory agencies such as USEPA and the State of Hawaii Department of Health should re-examine the usefulness of the existing recreational water quality standards as applied to the state of Hawaii and to other tropical islands as well.

2. We propose that *C. perfringens* be used to establish the hygienic quality of environmental waters in Hawaii. We propose the following standards based on geometric mean concentrations (CFU/100 ml) of *C. perfringens* using the mCP medium as developed by Bisson and Cabelli (1979):

(a) Inland waters for recreational use	< 50	CFU/ 100 ml
(b) Coastal beaches for recreational use	< 5	CFU/100 ml
(c) Near-shore marine waters which may become contaminated with sewage from ocean outfall or wastes from ship.	< 2	CFU/100 ml
(d) Pristine, uncontaminated waters	0	CFU/100 ml

CHAPTER 1

INTRODUCTION TO STUDY

I. Use of Fecal Indicators to Establish Recreational Water Quality Standards

The Clean Water Act (P.L. 92-500) was passed in 1972 as a federal law to ensure that every state meets the same minimal standard of treatment for wastewater and to ensure that the treated wastewater will not cause harmful pollution of environmental waters. The United States Environmental Protection Agency (USEPA) was established as the federal agency to implement the goals of the Clean Water Act. To meet these goals, USEPA established guidelines and regulations for minimal treatment of all wastewaters and established water quality standards based on the use of those environmental waters. For waters classified for recreational (swimming) use, USEPA identified fecal-borne microbial pathogens as the contaminants in water with the greatest potential for transmitting diseases. The primary source of fecal-borne pathogens is feces of human and warm blooded animals. The diseases that are transmitted as a result of ingestion of waters contaminated with these pathogens are referred to as waterborne diseases.

Ideally, waters should be tested for pathogens to determine whether the water is contaminated with pathogens. However, this is not practical nor feasible because there are numerous and different types of fecal-borne pathogens (bacteria, viruses, protozoans) and different methods are required to detect different pathogens. Moreover, the methods to detect for pathogens are characteristically expensive, inefficient or not available. Finally, even if a water sample is tested and determined to be negative for some pathogens, one cannot conclude that the water sample is free of all other pathogens. As a result, waters are not monitored for pathogens and USEPA has established recreational water quality standards based on the concentrations of bacteria which are normally present in feces in high concentrations. This group of bacteria is used as indicator for the presence of feces

and is therefore called fecal indicator bacteria. The theoretical basis for use of fecal indicator bacteria is that the recovery of such bacteria from a water sample indicates that the water is contaminated with feces. Moreover, the higher the concentrations of fecal indicator bacteria in the water, the greater the degree of fecal contamination and the greater likelihood that fecal-borne pathogens are present in the water. The ideal criteria for selecting a suitable group of fecal indicator bacteria to establish water quality standards have previously been reported by Dutka (1973) and can be summarized are as follows:

1. It must be consistently present in feces and at higher concentrations than fecal-borne pathogens.

2. It must not multiply outside the human intestinal tract.

3. It must be equal to or more resistant than the pathogens to environmental conditions and to disinfection.

4. It must be detected in water by simple and reliable methods.

Initially 1000 total coliform/100 ml was the recreational water quality standard used in the US. In 1972, USEPA for the first time established a national recreational water quality standard and recommended that the standard be 200 fecal coliform/100 ml because fecal coliform bacteria were more specifically associated with feces than total coliform bacteria. However, these coliform standards were not based on valid epidemiological studies and evidence to demonstrate that the risk to waterborne diseases to swimmers would proportionately increase as the concentrations of coliform bacteria in the water increased could not be obtained.

To address the limitation of the fecal coliform standard, USEPA conducted long term, intensive and well designed epidemiological and water quality studies (Cabelli, 1981; Dufour, 1984a) which resulted in the following two significant conclusions: (1) Concentrations of total and fecal coliform bacteria in recreational waters could not be used to predict incidences of waterborne diseases. (2) Concentrations of other indicator bacteria such as enterococci in marine waters and enterococci and *Escherichia coli* in fresh waters could be used to predict

incidences of waterborne diseases in recreational waters known to be contaminated with a sewage source. As a result of those studies, USEPA promulgated a new set of recreational water quality standards in 1986 based on concentrations of enterococci and *E. coli*. The advantages of these new standards were that they were based on the results of valid epidemiological studies and the concentrations of these new indicator bacteria were capable of predicting incidences of diseases and thereby risk levels to people using that water for recreational purposes. In 1986, USEPA recommended that all states establish two new sets of recreational water quality standards. For marine waters, the recommended standard was a geometric mean of 35 enterococci/100 ml and in fresh water the standard was a geometric mean of either 126 *E. coli*/100 ml or 33 enterococci/100 ml. All states were urged to accept these new standards which were based on risk levels. In 1990, the State of Hawaii concluded that the level of acceptable risk associated with the water quality standard as recommended by USEPA was too high and therefore, adopted a more stringent recreational water quality standard of 7 enterococci/100 ml for marine waters. However, the State of Hawaii retained its old standard of 200 fecal coliform/100 ml for its inland waters because of prevailing evidence that the inland waters in Hawaii could not meet the new freshwater recreational water quality standards.

II. Inherent Problems in Applying the Water Quality Standards to Freshwater Streams in Hawaii and other Tropical Islands.

Several studies conducted in Hawaii (Fujioka, 1983; Fujioka and Shizumura, 1985; Fujioka et al., 1988; Hardina and Fujioka, 1991) have demonstrated that the indicator bacteria (fecal coliform, fecal streptococci, *E. coli*, enterococci) used by USEPA to establish water quality standards are naturally present in high concentrations in streamwaters and consistently exceeded USEPA recreational water quality standards. High concentrations of these bacteria were also consistently recovered from numerous soil sites. It should be noted that there was no evidence of fecal or sewage contamination of these streams or soil sites.

Similar results were obtained when a comparable study was conducted in Guam. Other investigators have reported similar findings in other tropical islands such as Puerto Rico and the Virgin Islands (Hazen, 1988). The results in Hawaii led Hardina and Fujioka (1991) to conclude that soil in tropical islands is the major environmental source of these bacteria. These soil-bound bacteria are transported to streams by rainwater flowing over and through the soil before it empties into stream beds. Thus, stream waters in tropical islands may contain high concentrations of fecal indicator bacteria unrelated to sewage or fecal contamination and therefore, their presence in stream waters may not truly indicate the presence of fecal-borne pathogens.

The best explanation for the above observation is that fecal indicator bacteria were originally deposited on the soil of tropical islands by feces of man or animal. Under most conditions such as in temperate climate, the populations of indicator bacteria in the feces will die off once the feces are subjected to environmental conditions that are not conducive to growth of fecal indicator bacteria. On the other hand, environmental conditions in the tropical regions of the world are quite different from temperate regions of the world. Tropical environments are characterized by year round warm temperature, high humidity, and diverse soil types. These environmental soil conditions in the tropics are conducive for the establishment and multiplication of the fecal indicator bacteria. We therefore, hypothesize that due to favorable conditions in the tropics, a sub-population of fecal bacteria in the feces is able to establish and multiply in the soil.

III. Evaluation of the Assumptions Used in Water Quality Standards.

The reliability and accurate interpretation of water quality standards are based on scientific principles as well as on assumptions. If any of the assumptions used in establishing water quality standards are not valid, the interpretation and therefore the application of the water quality standards would not be valid. The three assumptions used by USEPA in the application of recreational water quality standards in every state are as follows:

A. Assumption one: *The data base used for establishing the water quality standard should be applicable to the environment where the standard is applied.*

USEPA mandated that the same water quality standards be equally applied to all states and US jurisdictions including tropical islands such as Hawaii, Puerto Rico, Virgin Islands, Guam, Samoa, and many other Pacific islands (USEPA, 1986). However, the data used to establish water quality standards by USEPA have traditionally been obtained from the continental USA, which is located in the temperate region of the world. These data have been confirmed by similar data obtained from other temperate countries (Canada, Northern Europe). Historically, data from tropical regions of the world have never been used by USEPA or WHO to establish water quality standards primarily because countries in the tropical region of the world are characterized as undeveloped with low economic base and unsanitary conditions. As a result, insufficient studies on water quality were conducted in tropical regions of the world and the few studies conducted usually used inferior methods.

In the application of recreational water quality standards, USEPA assumes that the water quality data obtained from temperate and continental USA are directly applicable to tropical island conditions as well. However, there is precedent that environmental conditions in temperate regions of the world differ greatly from tropical regions of the world. Accordingly, the data obtained from temperate region of the world may not be directly applicable to the tropical region of the world. The importance of the environment is a well-established principle in environmental ecology and environmental microbiology. For instance, it is well established that the climate and geographical location of countries select for flora and fauna which are adapted to that environment. As a result, people expect to see differences in the flora and fauna in temperate countries as compared to tropical countries. Although less obvious, the microbial populations in temperate regions of the world also differ from the microbial populations in the tropical regions of the world.

In summary, USEPA developed recreational water quality standards based on data gathered from temperate, continental USA. More specifically, the data to establish the new USEPA recreational water quality standards were obtained from studies conducted in coastal beaches of New York, Boston Harbor and Lake Pontchatrain in New Orleans as well as freshwater lakes in Pennsylvania and Oklahoma. The assumption made by USEPA is that these environments are similar enough to those of tropical islands, and therefore, the standards developed are directly applicable to tropical islands. However, there is compelling evidence that this assumption may not be valid because the environments of temperate and tropical countries of the world differ greatly. If this assumption is not valid, the application of the USEPA recreational water quality standards for tropical islands may not be valid.

B. Assumption two: *The source of fecal indicator bacteria and pathogens is feces of man and warm blooded animals.*

By analyzing the feces of human and animals throughout the world, it has been verified that fecal indicator bacteria and fecal-borne pathogens are consistently present in the feces of human and animal populations in every country. These results are to be expected as humans and warm-blooded animals have internal regulatory mechanisms that maintain the same body temperature of mammals whether they are in the temperate or tropical areas of the world. Thus, this assumption that the major source of fecal indicator bacteria and pathogens throughout the world is the feces of human and animals has been substantiated and is valid.

C. Assumption three: *There are no environmental sources of fecal indicator bacteria unrelated to contamination of the environment by human or animal feces.*

In the successful use of fecal indicators to determine whether environmental waters are contaminated with fecal matter, it is essential that the only major source of these fecal indicators is feces or sewage. This condition apparently exists in the temperate, continental USA as there has not been any

documentation of another major source of fecal indicator bacteria. It is therefore assumed by most scientists and regulatory agencies such as USEPA and WHO that this same condition exists in all environments throughout the world and therefore the fecal indicator concept are directly applicable throughout the world. However, the above assumption does not appear to be valid in tropical islands such as Hawaii, Guam and Puerto Rico (Fujioka et al., 1988, Hazen et al., 1988) where fecal indicator bacteria have been reported to be naturally present in the environment. Consequently, the presence of such environmental sources of fecal indicator bacteria will interfere with the reliable interpretation of using fecal indicator bacteria to assess water quality. This problem is magnified if the source of fecal indicator bacteria is well established in the environment such as in the soil and these environmental sources of fecal indicator bacteria can readily gain access to streams.

In summary, three assumptions are made in the application of water quality standards by USEPA and these standards are equally applied to all of the fifty states and jurisdictions of the USA. Of the fifty states, Hawaii is unique in that it is an island state which is located in the tropical region of the world. Compelling evidence has been presented to demonstrate that two of the three assumptions used by USEPA to establish water quality standards are not applicable to tropical island conditions. Thus, USEPA recreational water quality standards may not be applicable to Hawaii and other tropical islands.

IV. A Suitable Alternative Indicator for Tropical Islands

If the USEPA recommended fecal indicators and water quality standards are not applicable to Hawaii, there then is a need to find an alternative and suitable indicator of recreational water quality for Hawaii and other tropical islands. A review of the literature shows many other fecal indicator microorganisms which have been proposed as alternative indicators of fecal contamination. The most promising alternative indicators reported in the literature are one bacterium (*Clostridium perfringens*) and three viruses of bacteria (bacteriophages) which

include somatic DNA coliphage, male specific RNA coliphage and phages of bacteroides bacteria. Our laboratory is currently evaluating all of these alternative fecal indicators.

V. Project Goals

The first goal of this study is to re-evaluate the USEPA recreational water quality standards as applied to Hawaii by obtaining additional data to verify the prevalence, the persistence and the multiplication of the fecal indicator bacteria in the soil environment of Hawaii.

To address this goal, data will be obtained to answer the following questions raised during previous studies conducted by our laboratory:

1. Are the assumptions used by USEPA to interpret water quality standards not applicable to the state of Hawaii and thereby invalidate the usefulness of the USEPA recreational water quality standards in Hawaii?

2. Is soil the natural environmental source of fecal indicator bacteria which then serves as a non-fecal source of these indicator bacteria recovered routinely from streams in Hawaii?

3. How prevalent is the establishment of fecal indicator bacteria in the different soil environments of Hawaii?

4. What are the factors which control the persistence of populations of fecal indicator bacteria in the soil environments of Hawaii?

5. Do fecal indicator bacteria multiply in the soil environment of Hawaii and thereby invalidate the correlation used by USEPA that concentrations of fecal indicator bacteria in recreational waters are a good predictor for the presence of pathogens in water? This question is especially relevant because human viruses as well as protozoan parasites such as Giardia and Cryptosporidium are not capable of multiplying outside of human or animal host.

The second goal of this study is to re-evaluate the available data and to recommend recreational water quality standards which are more suitable and more reliable for conditions in Hawaii and other tropical islands.

CHAPTER 2

EVIDENCE FOR PREVALENCE, PERSISTENCE AND MULTIPLICATION OF FECAL INDICATOR BACTERIA IN THE SOIL ENVIRONMENT OF HAWAII

I. Objectives

The objectives of this phase of the study was to address the first goal of this study which was " to re-evaluate the USEPA recreational water quality standards as applied to Hawaii by obtaining additional data to verify the prevalence, persistence and multiplication of fecal indicator bacteria in the soil environment of Hawaii". The need to address this goal has been detailed in the introductory section of this report.

II. Materials and Methods

A. Selection of soil samples. To determine prevalence of fecal indicator bacteria in the soil environment of Hawaii, we decided to sample the different soil types from different parts of the island of Oahu. To select the soil samples, we consulted with Dr. Haruyoshi Ikawa, Soil Scientist and Director of Soil Classification Laboratory in the School of Agriculture, University of Hawaii. With Dr. Ikawa's assistance we selected soil samples representing the seven major soil types in Hawaii. A map of Oahu where these different soils can be found was obtained from Dr. Ikawa (see Figure 1)

B. Collection and processing of soil samples. Samples of these soil types were collected using sterile spatulas, the samples placed into sterile plastic bottles, and the samples transported back to the laboratory in a cooled ice chest. To determine the prevalence of fecal indicator bacteria in the soil environment of Hawaii, samples of surface soil were collected from various locations on the island of Oahu. The samples were assayed for concentrations of fecal coliform, *E. coli* and enterococci using two methods.

First, by eluting the soil with a buffer and analyzing the soil eluate for concentrations of fecal indicator bacteria using the membrane filtration method (Roll and Fujioka, 1993); second, by the most probable number (MPN) technique as described in the Standard Methods (1992).

C. Effect of soil moisture on the concentration and persistence of indigenous populations of E. coli and enterococci in soils of Hawaii. To find out the effect of soil moisture on the survival and persistence of *E. coli* and enterococci, soil samples were collected from the banks of Manoa Stream adjacent to the campus. A portion of the fresh soil was immediately assayed for moisture content as well as concentrations of *E. coli* and enterococci. The remaining soil was spread in a thin layer on a sheet of paper and the soil was left to dry at room temperature (23-25°C) for several days. During the next five days, samples were assayed for moisture content and counts of *E. coli* and enterococci using the MPN technique.

D. Multiplication of fecal Indicator bacteria in the soil environment. Studies relating to multiplication of fecal indicator bacteria in the soil were conducted under three different situations: (1) laboratory conditions, (2) greenhouse conditions, and (3) simulated field fields.

1. laboratory-based studies. The objective of laboratory-based study is to be able to control environmental conditions and thereby confidently interpret the data. In this experimental design, soil samples were initially collected from the Waimanalo Experimental Research Station of the University of Hawaii and thoroughly mixed. A portion of the soil was untreated while another portion was sterilized using cobalt irradiation using the facilities directed by Dr. James Moy of the Department of Food Science and Human Nutrition at the University of Hawaii. Personnel from Water Resources Research Center were certified to use the Cobalt Irradiator by Dr. James Moy. Sterilizing soil with cobalt irradiation is desirable because this process does not change the natural content and structure of the soil but will kill the indigenous soil microorganisms.

Diluted primary-treated sewage as source of fecal indicator bacteria was added to both the cobalt irradiated soil and the natural Waimanalo soil. These soil samples were then transferred to separate glass jars which were covered with perforated parafilm and

incubated under room temperature (23-25°C). The soils were maintained at about 60% of water holding capacity throughout the experimental period. Only, the natural Waimanalo soil received the basic nutrients (carbon, nitrogen and phosphorus) at minimal levels, as glucose, ammonium nitrate, and monobasic potassium phosphate at the rate of 1 g, 114.30 mg and 17.54 mg per 100 g of soil, respectively. Sub-samples were analyzed for fecal coliform and *E. coli* on a daily basis for nine days by the membrane filtration (MF) technique as described in the Standard Methods (1992).

2. Multiplication of fecal indicator bacteria under greenhouse conditions. The objective of this experimental design was to conduct experiment under more natural conditions where temperature and other conditions vary from day to day. To better approximate natural conditions and still maintain security of samples, an experiment was designed to determine whether fecal bacteria can multiply in the soil under greenhouse conditions at the University of Hawaii. Three pots were filled with soil and fecal bacterial combinations and kept in the greenhouse for four days while maintaining the moisture of the soil to approximately 60% of maximum water holding capacity. The three types of soil experiments included: (a) Natural Waimanalo soil containing indigenous soil bacteria and indigenous fecal indicator bacteria (*E. coli* and enterococci). (b) Natural Waimanalo soil deliberately contaminated with fecal indicator bacteria from primary-treated sewage effluent from Sand Island STP. (c) Sterilized (cobalt irradiated) Waimanalo soil deliberately contaminated with fecal indicator bacteria from primary-treated sewage. The air temperature within this enclosed greenhouse ranged from 26 to 36°C. Soil samples were assayed for *E. coli* and enterococci on a daily basis for four days by the most probable number technique (Standards Methods, 1992).

3. Multiplication of fecal indicator bacteria under secured, simulated field conditions. The objective of this experimental design was to conduct experiment to determine whether fecal indicator bacteria can multiply in the soil under conditions which were closer to natural conditions and still maintain security of our samples. To create this condition, a wooden rectangular growth chamber measuring 36" X 26" X 18.5" and completely covered with nylon mesh was built. The growth chamber had enough space to accommodate 5-6 small plastic pots containing experimental soil. The growth chamber

was placed on the outer walkway on the second floor of Holmes Hall. The area where the growth chamber was placed was exposed to natural conditions such as fluctuating temperature and sunlight. The nylon mesh prevented any external contamination by agents such as birds or insects, but provided constant air circulation.

To determine whether *E. coli* and enterococci can multiply under these secured, simulated field conditions, known concentrations of fecal indicator bacteria (fecal coliform, 2.00 X 10^3, *E. coli*, 1.81 X 10^3, and enterococci, 1.29 x 10^2, per g of soil) from sewage were added to a pot containing cobalt irradiated soil. To another plastic pot containing natural Waimanalo soil, an equal volume of the autoclaved sewage was added. The pots were transferred to the growth chamber and remained there until the experiment was terminated. The average day time (8.30 AM to 6.00 PM) air and soil temperature within the growth chamber were 32.1°C and 33.8°C, respectively. Soil samples were assayed for fecal coliform, *E. coli* and enterococci on a daily basis for 6 days by the MPN technique.

To demonstrate that soil enterococci require complex nutrients for their growth and multiplication, three one-hundred gram portions of natural Waimanalo soil containing native enterococci were weighed into three plastic pots. The soil in the three plastic pots received one of the following three treatments: (i) no treatment, (ii) peptone at the rate of 1 g per 100 g of soil and (iii) beef extract at the rate of 1 g per 100 g of soil. The pots were transferred to the growth chamber and remained there until the experiment was terminated. The soils were maintained at optimum moisture level throughout the experimental period. The average day time air temperature in the growth chamber was 30.1°C, while the soil temperature was 33.9°C. Sub-samples of soil from the three pots were collected on a daily basis for five days and analyzed for concentrations of enterococci by the MPN method.

III. Experimental Results

A. Prevalence of Fecal Indicator Bacteria in the Soil Environments of Hawaii.

Based on random samples, we had previously determined that fecal indicator bacteria are

naturally present in the soils of Hawaii. In order to ascertain whether the prevalence of fecal indicator bacteria is restricted to certain locations or they are widely distributed in the soils, a soil survey was undertaken on the island of Oahu. Soil samples were collected from various locations representing major soil types on Oahu. The study revealed that the fecal indicator bacteria (fecal coliform, *E. coli*, and enterococci) were prevalent in all the major soil types on this island (*figure 1 and table 1*). Enterococci were consistently recovered from all soil types and all but one soil sample. The concentrations of these bacteria ranged from 0-17,750 MPN/g of soil. On the other hand, fecal coliform and *E. coli* were recovered from all soil types, but not from all soil samples. The concentrations of fecal coliform ranged from 0-80,000 MPN/g of soil while the concentrations of *E. coli* ranged from 0-12,000 MPN/g of soil. The variations in concentrations of these bacteria may be attributed to the physical, chemical and biological properties of the soil at the time of collection. These results document that fecal indicator bacteria used by USEPA to determine the quality of recreational waters are naturally present and prevalent in all soil types of Hawaii.

B. Persistence of Fecal Indicator Bacteria in Soil Environments of Hawaii.

Although fecal indicator bacteria were recovered from all soil types in Hawaii, the concentrations of these fecal indicator bacteria in the various soil samples varied. The objective of this phase of the study was to determine the soil conditions which allow for the survival and persistence of fecal indicator bacteria in soil environments of Hawaii. Soil moisture is often the limiting factor in the growth of bacteria in soil and it has been established that soil microorganisms grow optimally at a moisture level of 50-70% of maximum water holding capacity (also known as field capacity).

The moisture content of soil in Hawaii varies considerably. However, most of the soil sites near streams maintain a fair moisture content due to overhanging trees, soil cover (mulch) and periodic rainfalls. A typical soil near Manoa Stream flowing adjacent to the campus was obtained to determine the effect of soil moisture on relative abundance of *E. coli* and enterococci in the soil. A portion of the sample collected was immediately assayed for moisture content and concentrations of *E. coli* and enterococci. The remaining soil was dried under laboratory conditions and concentrations of *E. coli* and enterococci

were monitored during this period. The results show that the soil moisture content which was 37.6% during day 0 was reduced to 13.1% after day 1 and to 10.93% by day 5 (*figure 2*). Under these conditions, the viable counts of enterococci in the soil remained stable, providing evidence that these bacteria are very resistant to soil moisture loss. In contrast, viable counts of *E. coli* were reduced by one log (90%) after one day. The counts of *E. coli* remained at that reduced level on day 2 and thereafter the counts of *E. coli* declined. When moisture was added back to soil, viable concentrations of *E. coli* approximating that of natural soil were recovered (data not shown). These results show that *E. coli* is much more sensitive to soil moisture loss than enterococci. These results also demonstrate that soil moisture is an important environmental variable that determines the concentrations of viable indicator bacteria.

C. Multiplication of Fecal indicator Bacteria in the Soil Environment of Hawaii

1. Laboratory-based experiments. Under controlled laboratory conditions, there was little or no evidence for multiplication of fecal coliform or *E. coli* in the natural Waimanalo soil during the first four days following sewage application (*figure 3*). Significant increases in the counts of these bacteria were evident only when simple nutrients such as glucose and salts were added to the soil. Upon addition of these nutrients, the concentrations of fecal coliform and *E. coli* reached as high as 9.77×10^5 and $1.95\ 10^5$, respectively, per g of soil. On the other hand, significant increases in the counts of fecal coliform and *E. coli* were evident starting from day 1 in the sewage-treated, cobalt irradiated soil (*figure 4*). The counts of these bacteria continued to increase over the next seven days and reached a peak at day 8.

2. Greenhouse experiments. Under greenhouse conditions, the indigenous populations of *E. coli* and enterococci in the natural Waimanalo soil increased slightly during day 1 and subsequently decreased during the next three days (*figure 5*). These results support our theory that indigenous soil bacteria will out compete fecal indicator bacteria for nutrients and as a result, growth of fecal indicator bacteria in natural soil is slow and controlled by the growth of soil bacteria.

When natural Waimanalo soil was deliberately contaminated with primary-treated sewage from Sand Island STP, the introduction of additional fecal indicator bacteria (*E. coli* and enterococci) into this soil did not result in further increases of these bacteria (*figure 6*). The counts of both the *E. coli* and enterococci remained unchanged after day one and subsequently declined during the next three days. These results support our hypothesis that growth of fecal indicator bacteria in soil is inhibited and controlled by the growth of indigenous soil bacteria.

When cobalt irradiated Waimanalo soil was deliberately contaminated with sewage, the populations of *E. coli* and enterococci from the sewage multiplied for two to four days and maintained their high concentrations (*figure 7*). The results of these experiments demonstrate that in the absence of indigenous (background) soil bacteria, fecal indicator bacteria are able to multiply in the soil.

3. Secured, simulated field conditions. In the natural Waimanalo soil, the counts of indigenous fecal coliform and *E. coli* decreased by about 1 and 1.5 log, respectively, after day 1 and declined further by day 3 (*figure 8*). When nutrients were added to the soil samples on day 4, there was a dramatic increase in the concentrations of fecal coliform and *E. coli.* These results are similar to those observed under laboratory conditions and support the hypothesis that the populations of fecal coliforms are restricted and controlled by indigenous soil bacteria which are better able to grow and obtain nutrients in soil environments. However, the addition of simple nutrients to soil will make these nutrients available to fecal bacteria and allow these bacteria to multiply.

A different response was observed when fecal bacteria was added to sterilized soil. Under these conditions, both the fecal coliform and *E. coli* showed a gradual increase in concentrations over the four day period and increased even faster after day 4 when additional nutrients were added (*figure 9*). These results are similar to those experiments conducted under laboratory conditions and indicate that in the absence of indigenous populations of soil bacteria, the populations of fecal indicator can readily multiply in the soil conditions of Hawaii.

Under the same set of conditions, there was little evidence that enterococci bacteria multiplied even after the addition of simple nutrients (glucose and salts) which had

stimulated the growth of fecal coliform and *E. coli*. These results are consistent with previous studies which had documented the simple growth requirements for fecal coliform and *E. coli* and the complex nutritional requirements for enterococci bacteria.

To demonstrate that enterococci will multiply in soil conditions in the presence of complex nutrients, a natural Waimanalo soil containing indigenous populations of enterococci was untreated (control) or treated with peptone or beef extract and held under secured, simulated field conditions. In the untreated Waimanalo soil, the concentrations of enterococci remained unchanged over three days and declined slightly after 5 days (*figure 10*). In soil treated with complex nutrients (peptone or beef extract) concentrations of enterococci increased by more than 1 log unit during the first 24 hours and remained relatively stable over the next four days.

In summary, fecal coliform and *E. coli* are known to have simple nutritional requirements and these coliform bacteria can readily multiply in the soil when available nutrients are present and especially in the absence of natural soil microorganisms. However, in the presence of natural soil microbial populations, these same coliform bacteria are not able to readily multiply most likely because soil microorganisms are much more efficient in obtaining nutrients and multiplying in soil conditions. In this regard, the heterogeneous populations of soil microorganisms (bacteria, fungi, actinomycetes) are present in concentrations approximately over 10,000 times greater than fecal indicator bacteria (*figure 11*). Therefore, under natural conditions, the abundant indigenous soil microflora appears to control the multiplication of the less abundant fecal bacteria through competition for available nutrients and perhaps in the production of inhibitors. Enterococci bacteria are known to require more complex nutrients than coliform bacteria. However, based on the available evidence, both coliform bacteria and enterococci bacteria are able to multiple and persist in the natural soil environments in Hawaii.

CHAPTER 3

APPROPRIATE RECREATIONAL WATER QUALITY STANDARDS FOR HAWAII

I. Objectives

The objectives of this phase of the study were to address the second goal of the project: "To re-evaluate the available data and to recommend recreational water quality standards which are more suitable and more reliable for conditions in Hawaii and other tropical islands."

II. Experimental Approach

C. perfringens and three bacterial viruses (DNA coliphages, male specific F-RNA phages, Bacteroides phages) have been described in the literature as the most suitable alternative indicators of water quality to those indicators (coliform, *E. coli*, enterococci) which are currently being used. Of these alternative indicators *C. perfringens* was determined to be most suitable to be used in Hawaii based on the scientific principles as well as the simplicity and reliability of the method to assay for *C. perfringens*.

C. perfringens was evaluated as a reliable indicator of fecal contamination of environmental waters in Hawaii by two approaches. First, to determine the concentrations of *C. perfringens* in various samples (human feces, sewage, pristine streams, streams receiving and not receiving sewage, storm drains, estuary waters, coastal waters, open ocean waters, feces of animals) obtained in Hawaii and to compare the data obtained with concentrations of the standard USEPA approved fecal indicators. Second, determination of how well *C. perfringens* meets the six major criteria of a suitable fecal indicator of water quality as previously proposed by others (Dutka, 1973; Bonde, 1977; Cabelli, 1978 and Dufour, 1984b). All microbial assays were done using standard membrane filtration

method or most probable number (MPN) method as described in Standard Methods for the Examination of Water and Wastewater (1992). *C. perfringens* was assayed by the method as described by Bisson and Cabelli (1979).

III. Evaluation of *C. perfringens* as an Ideal Indicator of Fecal Contamination

A. Criterion One. *The indicator must be consistently present in the feces of humans, must survive in sewage at concentrations greater than fecal-borne pathogens and must survive sewage treatment processes better than most pathogens.*

C. perfringens is considered a fecal indicator because its natural habitat is the intestinal tract of humans and other warm-blooded animals where it multiplies to very high levels. Since *C. perfringens* is a strict anaerobe, the vegetative cells of the bacterium are rapidly inactivated when exposed to aerobic environmental conditions. However, the spores of *C. perfringens* are very stable under environmental conditions. Thus, counts of *C. perfringens* from environmental samples (feces, sewage, streams, soil, sediment) mainly constitute spores of the bacterium. Cabelli (1978) summarized the comparative stability and concentrations of all fecal indicators including spores *of C. perfringens* in human and animal feces as well as in sewage (*table 2*). Data from our laboratory show that although concentrations of *C. perfringens* in raw sewage are less abundant than other fecal indicator bacteria, the spores are more abundant than other fecal indicator bacteria in the final disinfected sewage effluent, demonstrating the better survivability of spores as compared to other fecal indicator bacteria to sewage treatment processes including disinfection (*table 3*).

B. Criterion Two: *The method to isolate, identify and enumerate the indicator must be easy, inexpensive, rapid and reliable.*

The membrane filtration method to isolate, and to identify *C. perfringens* was first reported by Bisson and Cabelli in 1979. The reliability, feasibility and usefulness of this method has been documented by its use as an indicator of disinfection efficiency of drinking water (Payment and Franco, 1993), in determining whether environmental waters are contaminated with sewage (Fujioka and Shizumura, 1985) as well as to determine the

fate and movement of sewage sludge discharged into ocean (Hill et al., 1993). It has proven to be a relatively easy, inexpensive, rapid, and reliable method that can be easily adopted for use by most water quality laboratories. The outline of this two-step membrane filtration method is shown in *figure 12* and the reliability of this method when applied to field samples are summarized in *table 4* (Fujioka and Shizumura, 1985).

C. Criterion Three: *The stability of the indicator to environmental factors should be similar to or greater than sewage-borne pathogens.*

We (Fujioka et al., 1981) previously reported that sunlight is the primary factor controlling the survival of fecal indicator bacteria suspended in environmental waters. Fecal bacteria in environmental waters are rapidly inactivated to undetectable levels when exposed to sunlight (*figure 13*). Under these same conditions, pathogens such as viruses and spores of *C. perfringens* are more resistant to the cidal effects of sunlight. Thus, the expected survival of *C. perfringens* in environmental water more closely matches that of pathogens such as viruses, especially in the presence of sunlight (*figure 14*).

D. Criterion Four: *The only significant source of fecal indicators should be sewage or feces and waters uncontaminated with sewage or feces should not have significant concentrations of this indicator.*

Since all stream waters in Hawaii naturally contain high concentrations of the fecal indicator bacteria which are used to establish water quality standards, it is difficult to determine when a stream has actually been contaminated with sewage. However, streams generally contain low concentrations of *C. perfringens*. Therefore, an elevated level of *C. perfringens* is a reliable indicator that the stream water has been contaminated with sewage. Results to demonstrate these observations are summarized in *table 5* which show high concentrations of standard fecal indicator bacteria (fecal coliform and fecal streptococci) in the Kipapa Stream above and below the site where sewage is discharged into the stream. On the other hand, the concentrations of *C. perfringens* are lower in stream samples above the sewage discharge site but show an increase level below the sewage discharge site (Fujioka and Shizumura, 1985).

E. Criterion Five: *The indicator must not multiply in the environment since the concentration of indicator recovered from environmental samples is used to determine the time and extent of sewage contamination and because pathogens such as viruses and protozoa can not multiply in the environment.*

It is the vegetative cells of *C. perfringens* that actively metabolize and replicate, but only under anaerobic conditions. When these vegetative cells exit the human or animal host as part of the feces, they are rapidly killed upon exposure to the aerobic environment. Thus, under most environmental conditions (water, sewage, soil) which are aerobic, it is the spores of *C. perfringens* which are able to survive but not multiply. In this regard, the fate of *C. perfringens* is similar to pathogens such as viruses and protozoans that do not have the potential to multiply under environmental conditions and moreover, these pathogens exist in a resistant state (spore, virion, cyst) which allows these pathogens to survive much longer than other fecal indicator bacteria in environmental waters.

F. Criterion Six: *There should not be any significant environmental source of the fecal indicator bacteria.*

Soil was previously determined to be a significant source of fecal indicator bacteria that are used by USEPA to establish water quality standards. In contrast, the concentrations of *C. perfringens* in soil samples in Hawaii are generally low, although there are occasional soil samples with high concentrations of *C. perfringens*, most likely representing spot contamination with fecal droppings of animals such as cats or dogs. The average concentrations of fecal indicator bacteria in soil samples from three sites in Hawaii are summarized in *figure 15*. These results show that the average concentrations of *C. perfringens* in soil is much lower than other fecal indicator bacteria. These results support the theory that *C. perfringens* cannot multiply in the soil environment and also support the theory that standard fecal bacteria are able to multiply in the tropical soil environment. Finally, these results show that unlike the fecal indicators used by USEPA, soils are not a major environmental source of *C. perfringens*.

Storm drains represent another source of environmental contamination. Waters in the storm drains in Hawaii contain consistently high concentrations of most fecal indicator bacteria whereas concentrations of *C. perfringens* are sporadic, most likely representing

the sporadic contamination of feces from pets. Relative concentrations of various fecal indicator bacteria in some of Hawaii's storm drains are shown in *figure 16*. Animal feces represent another environmental source of all fecal indicator bacteria. The relative concentrations of all fecal indicator bacteria in animal feces are summarized in *table 6* and show high concentrations of *C. perfringens* in many but not all animals. It is significant that pigeon feces is a significant urban source of contamination affecting soil, sandy beaches, sidewalks, streams and storm drains. The absence of *C. perfringens* in the feces of pigeons eliminates a confounding source of this bacterium. Finally, counts of *C. perfringens* are generally low in the shoreline beach waters (*figure 17*) and generally absent in open ocean water. Since the background level of *C. perfringens* is low, it is much easier to determine when an environmental water is contaminated with sewage.

In summary, *C. perfringens* is a superior indicator of fecal contamination of waters in Hawaii, and therefore, *C. perfringens* should be used to determine the hygienic quality of recreational waters in Hawaii and other tropical islands.

CHAPTER 4

PROJECT SUMMARY AND RECOMMENDATIONS

I. Summary and Conclusions

Historically, concentrations of fecal indicator bacteria (total coliform, fecal coliform, *E. coli*, enterococci) have been used to establish recreational water quality standards in the US. In using these fecal indicators as an index of water quality, it is assumed that the source of these indicator bacteria is human or animal feces, that there are no major environmental sources of these bacteria and that these indicator bacteria do not multiply in the environment. However, in Hawaii, we have documented that all of the USEPA recommended fecal indicators (total coliform, fecal coliform, *E. coli*, enterococci) of water quality are naturally present in high concentrations in the natural environments (streams, soil). The goals of this project were two-fold: First, to determine why USEPA recommended fecal indicator bacteria (fecal coliform, *E. coli*, enterococci) are not useful in determining the hygienic quality of recreational waters in Hawaii. Second, to suggest an alternative fecal indicator to establish recreational water quality standards that are more relevant to Hawaii. The important findings of this study are as follows:

1. Soil is the natural environmental source of fecal indicator bacteria in Hawaii and this environmental and non-fecal source of fecal indicator bacteria is readily transported to streams in Hawaii by land run-off from rainfall.

2. The fecal indicator bacteria used by USEPA to establish recreational water quality standards are readily recovered from all seven major soil types in Hawaii. Thus, fecal indicator bacteria are prevalent and persist in all soil types in Hawaii.

3. Soil moisture, available nutrients and natural populations of soil microorganisms are the primary factors which control the populations of fecal indicator in the soil environments of Hawaii. Coliform bacteria have simpler nutritional requirements than enterococci bacteria. The diverse natural populations of soil microorganisms (bacteria,

viruses, fungi, protozoa) are present in soil at concentrations exceeding a million times greater than fecal indicator bacteria. These soil microorganisms are very effective in obtaining nutrients from soil which they require to multiply. The growth of these soil microorganisms were shown to limit and to control the growth of fecal indicator bacteria most likely by competing for nutrients and additionally by producing by-products which inhibit the growth of fecal indicator bacteria.

4. Fecal indicator bacteria (fecal coliform, *E. coli*, enterococci) were shown to be capable of multiplying in the soil environment of Hawaii. In the natural soil environments of Hawaii, these fecal indicator bacteria have adapted themselves to grow in the soil environment and have established themselves as one of the indigenous populations of soil microorganisms. However, in the natural soil environment, these fecal indicator bacteria are at a disadvantage in their ability to grow. As a result, they probably grow opportunistically when conditions (available nutrients) allow them to grow. Although the populations of fecal indicator bacteria in soil grow slowly, these bacteria are able to persist and to wait for suitable conditions for their growth. Since time for rapid growth is not essential for the maintenance of this population of fecal indicator bacteria in soil, it appears that they have developed a successful strategy to maintain an active population in the soil environments of Hawaii.

5. The source of water for all streams in Hawaii is rainfall and rainfall carries soil-bound fecal indicator bacteria to streams resulting in high concentrations of fecal indicator bacteria in stream which often exceeds the recreational water quality standards as established by USEPA. In the application of recreational water quality standards, USEPA assumes that there are no significant, environmental sources of fecal indicator bacteria and the fecal indicator bacteria recovered from natural waters represents contamination from a fecal source (sewage). However, this assumption is not applicable to Hawaii since fecal indicator bacteria are established as a natural, non-fecal and environmental source of fecal indicator. As a result, the same interpretations of water quality standards utilizing these fecal indicators are not valid in Hawaii.

6. Since the existing water quality standards are not applicable for Hawaii, alternative and more reliable recreational water quality standards should be used in

Hawaii. Based on data and methodology considerations, *C. perfringens* is the most reliable and suitable fecal indicator to be used to establish recreational water quality standards in Hawaii as well as other tropical islands. *C. perfringens* was carefully evaluated as an indicator of recreational water quality and shown to fulfill all six of the criteria used to characterize an ideal indicator of fecal contamination.

II. Recommendations

A. Regulatory agencies such as USEPA and the State of Hawaii Department of Health should re-examine the usefulness of the existing recreational water quality standards as applied to the state of Hawaii and to other tropical islands as well.

B. We propose that *C. perfringens* be used to establish the hygienic quality of environmental waters in Hawaii. We propose the following standards based on geometric mean concentrations (CFU/100 ml) of *C. perfringens* using the mCP medium as developed by Bisson and Cabelli (1979):

1. Inland waters for recreational use	< 50	CFU/ 100 ml
2. Coastal beaches for recreational use	< 5	CFU/100 ml
3. Near-shore marine waters which may become contaminated with sewage from ocean outfall or wastes from ship.	< 2	CFU/100 ml
4. Pristine, uncontaminated waters	0	CFU/100 ml

REFERENCES

American Public Health Association. 1992. Standard Methods for the Examination of Water and Wastewater. 18th edition.

Bisson, J. W., and V. J. Cabelli. 1979. Membrane filtration enumeration method for *Clostridium perfringens*. Appl. Environ. Microbiol. **37:**55-56

Bonde, G. J. 1977. Bacterial Indication of water pollution, p. 273-365. *In* M. R. Droop and H. W. Jannasch (ed.), Advances in Aquatic Microbiology.

Cabelli, V. J. 1978. New standards for enteric bacteria, p. 233-271. *In*, R. Mitchell (ed.), Water Pollution Microbiology, Vol. 2. Wiley Interscience.

Cabelli, V. J. 1981. Health effects criteria for marine recreational waters. EPA-600/1-80-031, U.S. Environmental Protection Agency, Washington, D.C.

Dufour, A. P. 1984a. Health effects criteria for fresh recreational waters. EPA-600/1-84-004.

Dufour, A. P. 1984b. Bacterial indicators of recreational water quality. Can. J. Public Health. **75:**49-56.

Dutka, B. J. 1973. Coliforms are an inadequate index of water quality. J. Environ. Health. **36:**39-45.

Fujioka, R. S. 1983. Stream water quality assessment based on fecal coliform and fecal streptococcus analysis. Water Resources Research Center, University of Hawaii, Technical Memorandum Report No. 70, pp. 1-37.

Fujioka, R. S., and L. K. Shizumura. 1985. *Clostridium perfringens*, a reliable indicator of stream water quality. J. Water Poll. Control Fed. **57:**986-992.

Fujioka, R. S., H. H. Hashimoto, E. B. Siwak, and R. H. F. Young. 1981. Effect of sunlight on survival of indicator bacteria in sea-water. Appl. Environ. Microbiol. **41:**690-696.

Fujioka, R. S., K. Tenno, and S. Kansako. 1988. Naturally occurring fecal coliforms and fecal streptococci in Hawaii's freshwater streams. Toxicity Assessment **3:**613-630.

Hardina, C. M., and R. S. Fujioka. 1991. Soil: The environmental source of *E. coli* and enterococci in Hawaii's streams. Environmental Toxicology and Water Quality **6:**185-195.

Hazen, T. C. 1988. Fecal coliforms as indicators in Tropical Waters: A Review. Toxicity Assessment **3:**461-477.

Hill, R. T., I. T. Knight, M. S. Anikis, and R. R. Colwell. 1993. Benthic distribution of sewage sludge indicated by *Clostridium perfringens* at a deep-ocean dump site. Appl. Environ. Microbiol. **59:**47-51.

Payment, P., and E. Franco. 1993. *Clostridium perfringens* and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. Appl. Environ. Microbiol. **59:**2418-2424.

Roll, B. M., and Fujioka, R. S. 1993. Microbiological Assessment of Kaelepulu Stream and the Impact of Discharge in Kailua Bay (KB-3). Water Resources Research Center, University of Hawaii, Project Report No. PR-94-06 , pp. 1-101.

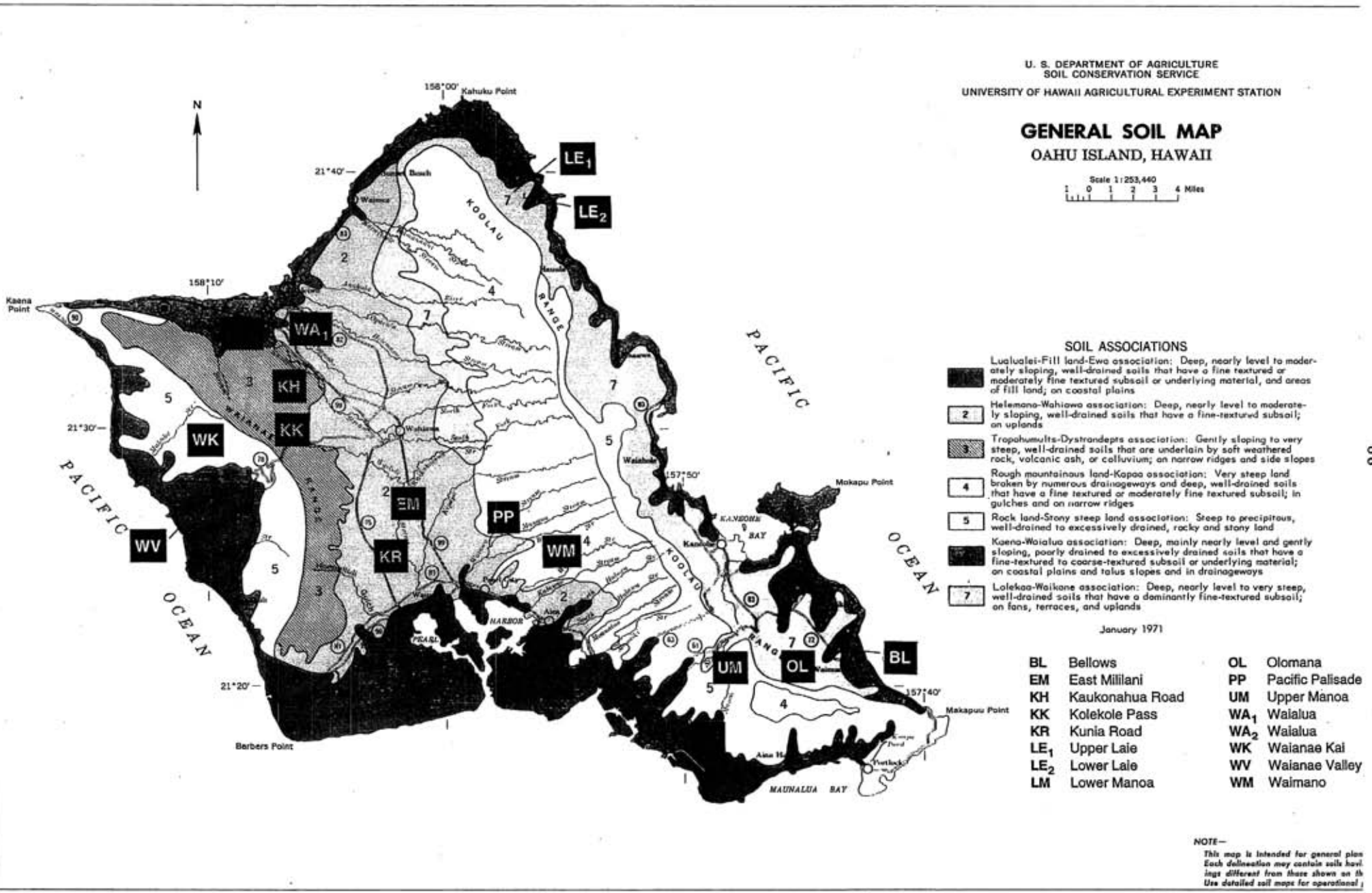


Figure 1. Occurrence of fecal indicator bacteria in the major soil types in Oahu

Fig. 2. Effect of Soil Moisture on Survival of *E. coli* and Enterococci Indigenous to Soil

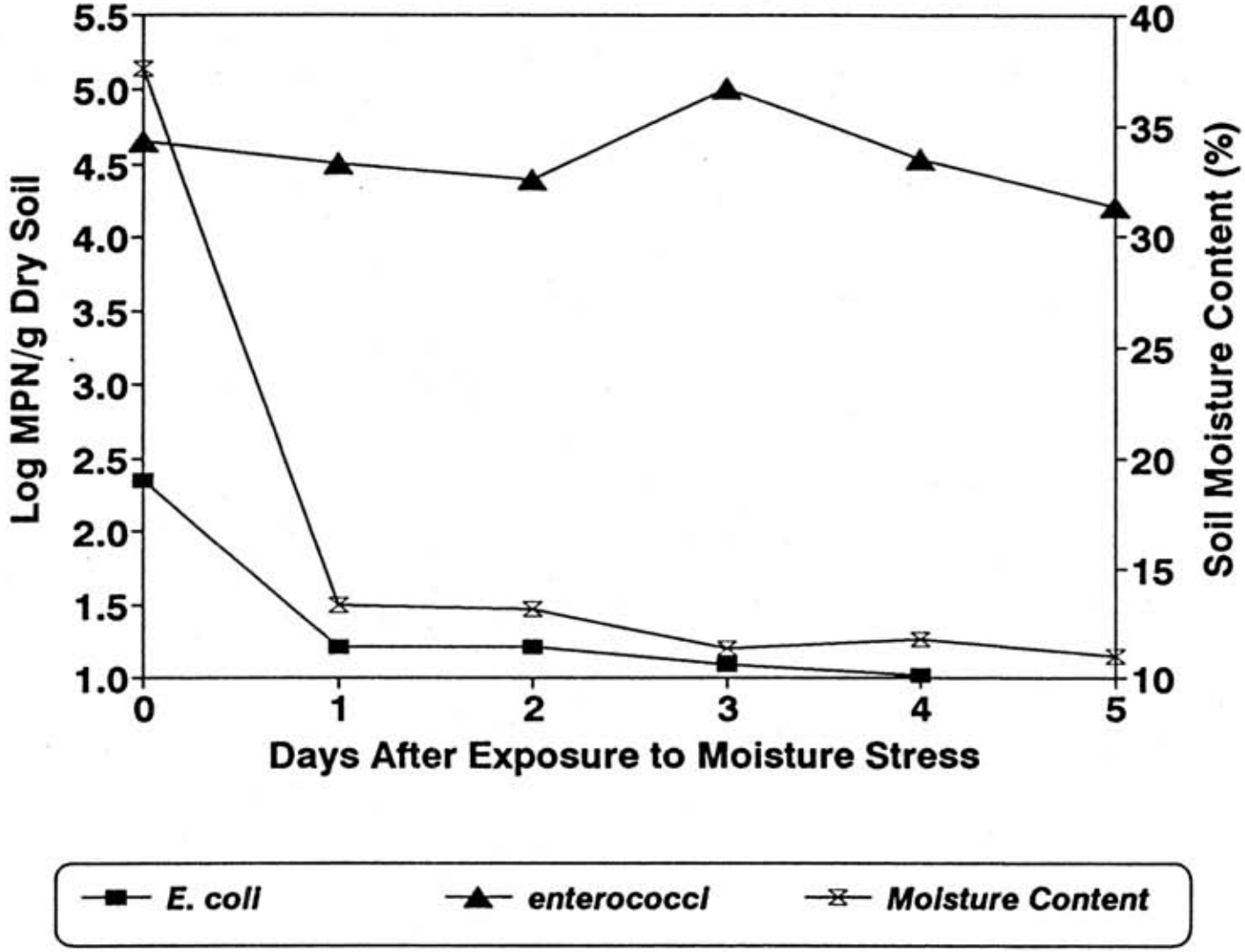


Fig. 3. Fate of Fecal Indicator Bacteria from Sewage Introduced into Natural Soil

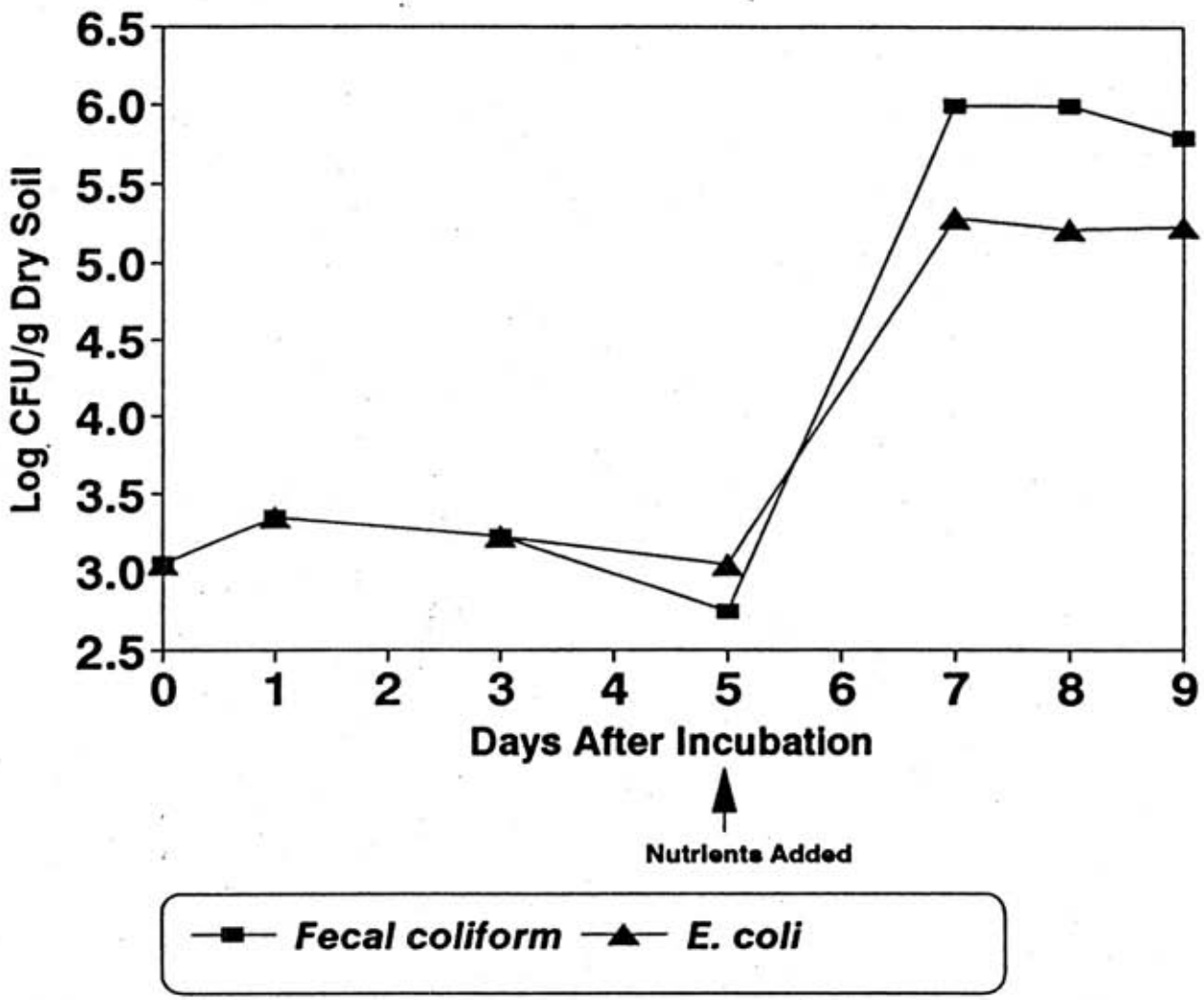


Fig. 4. Fate of Fecal Indicator Bacteria from Sewage Introduced into Cobalt Irradiated Soil

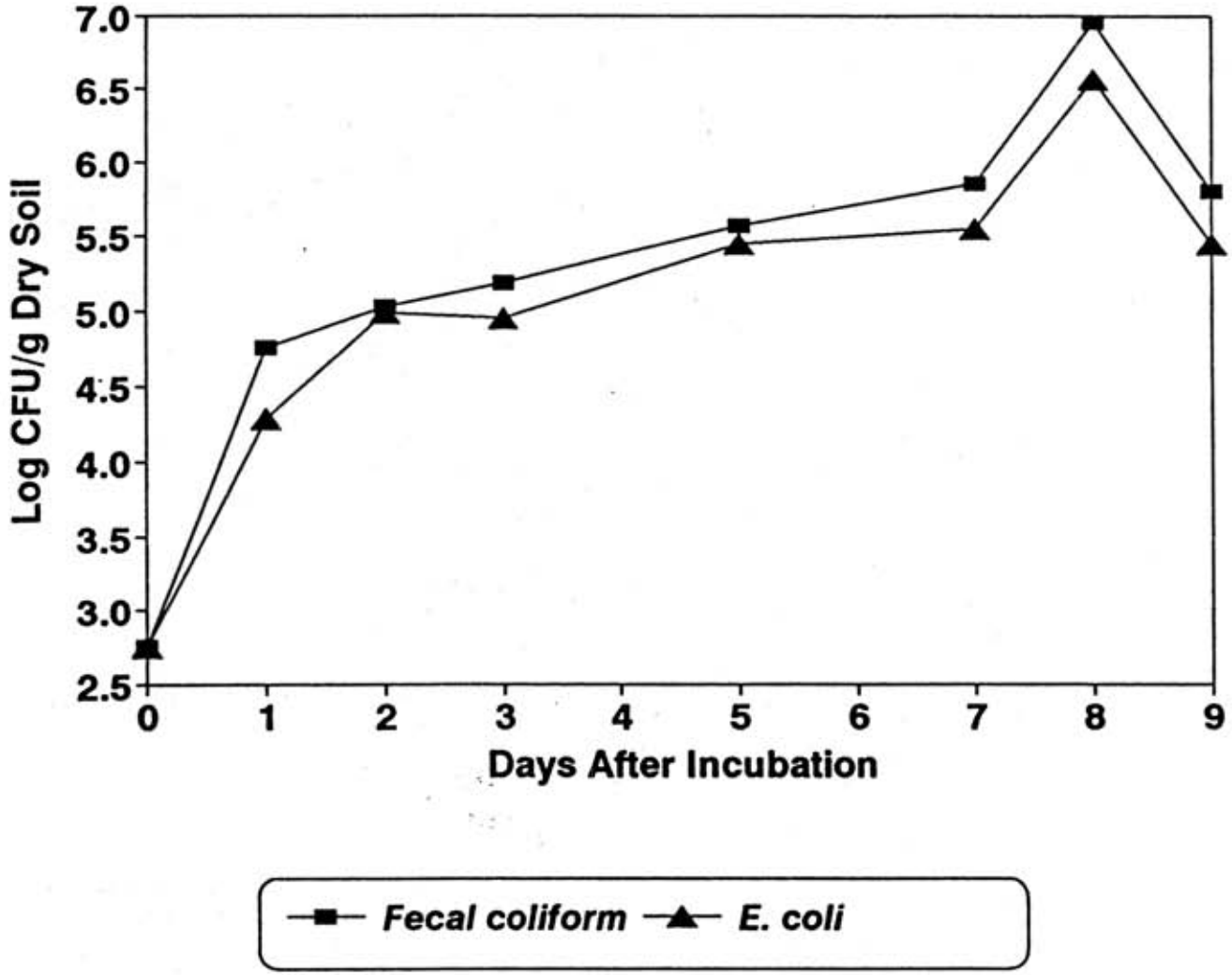


Fig. 5. Changes in the Populations of *E. coli* and Enterococci in Natural Soil Held Under Greenhouse Conditions

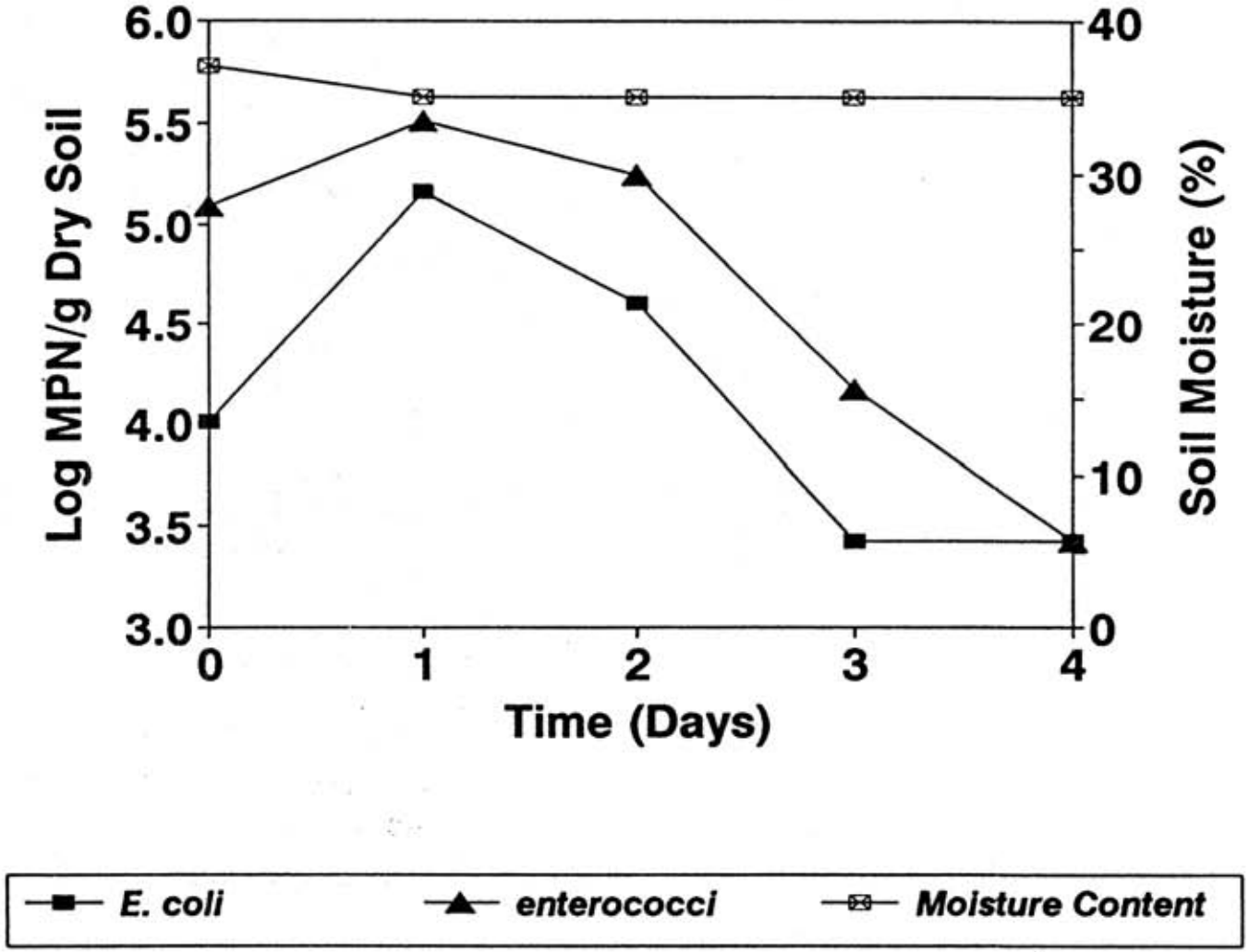


Fig. 6. Fate of Fecal Indicator Bacteria in Soil Held Under Greenhouse Conditions: Natural Waimanalo Soil Deliberately Contaminated with Sewage

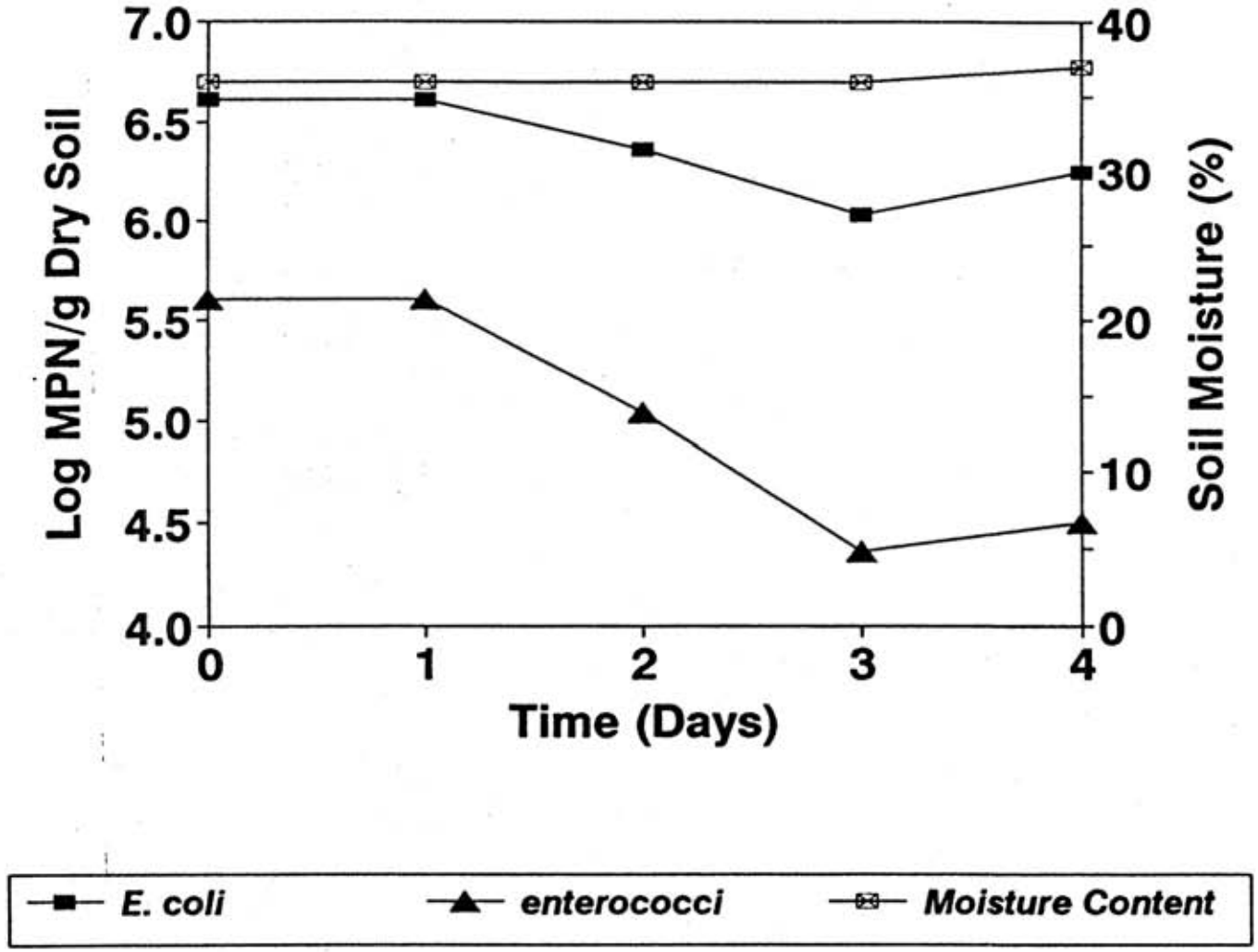


Fig. 7. Fate of Fecal Indicator Bacteria in Soil Held Under Greenhouse Conditions: Cobalt Irradiated Waimanalo Soil Deliberately Contaminated with Sewage

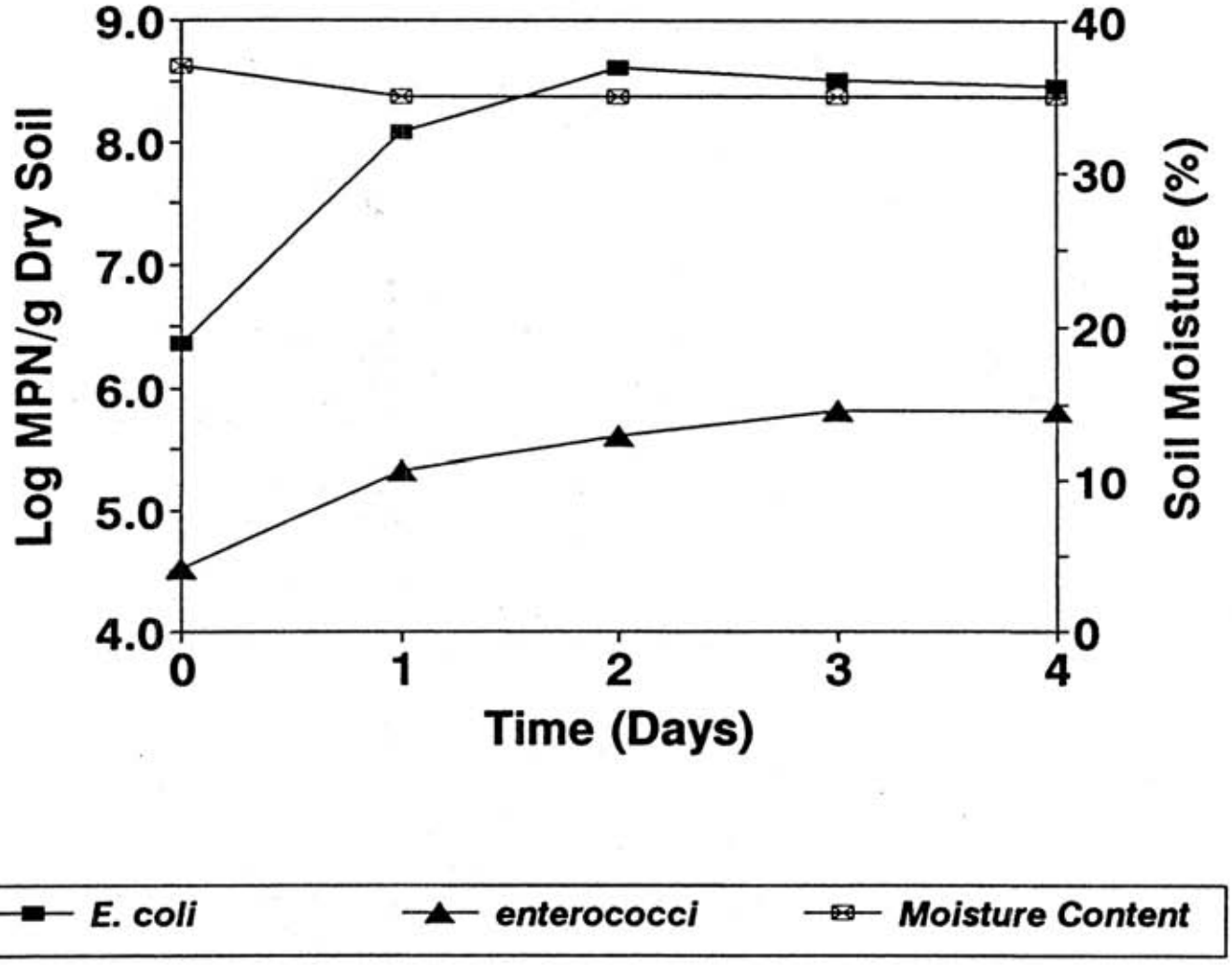


Fig. 8. Changes in the Counts of Fecal Indicator Bacteria Indigenous to the Waimanalo Soil Held Under Secured, Simulated Field Conditions

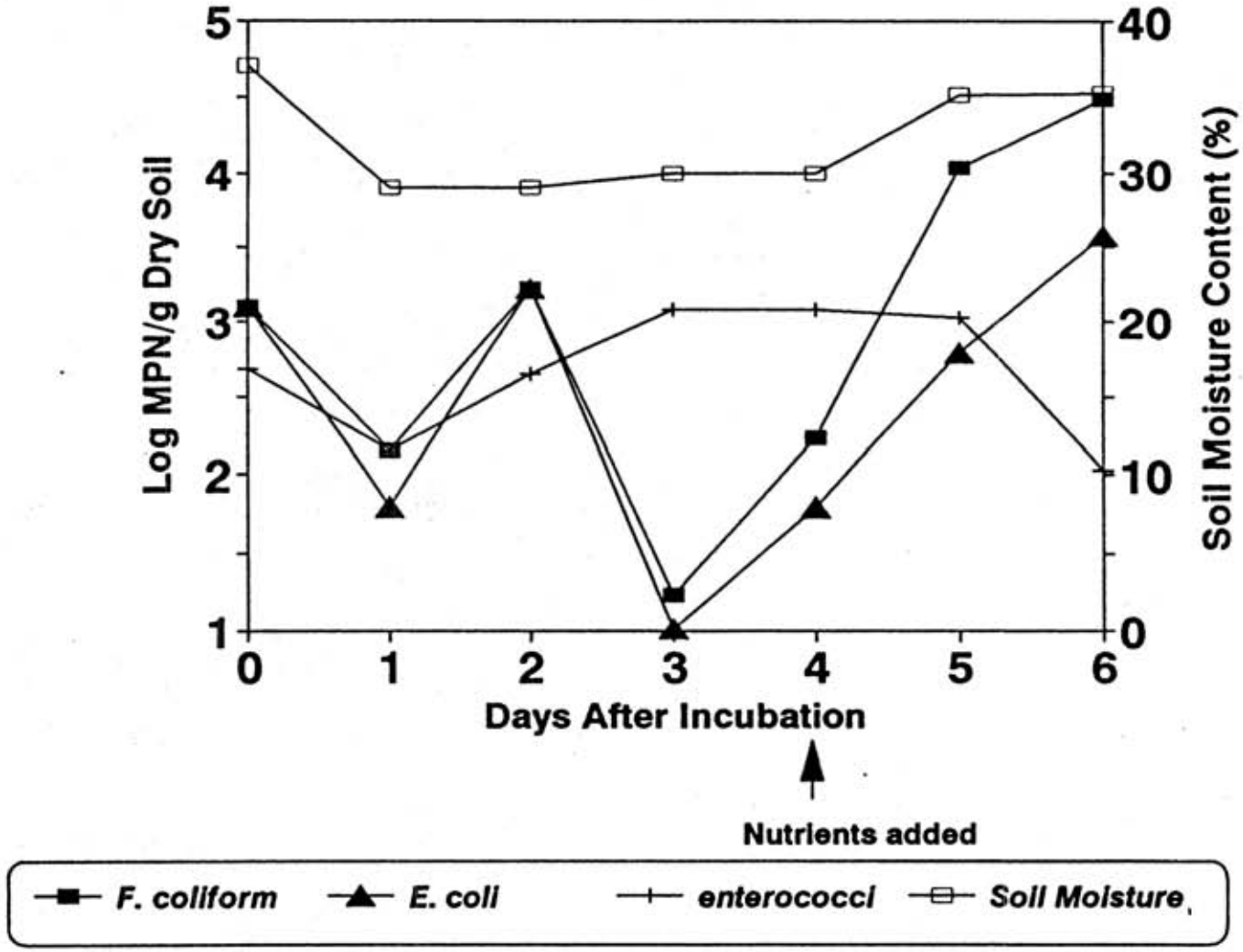


Fig. 9. Fate of Fecal Indicator Bacteria from Sewage Introduced into Cobalt Irradiated Soil Held Under Secured, Simulated Field Conditions

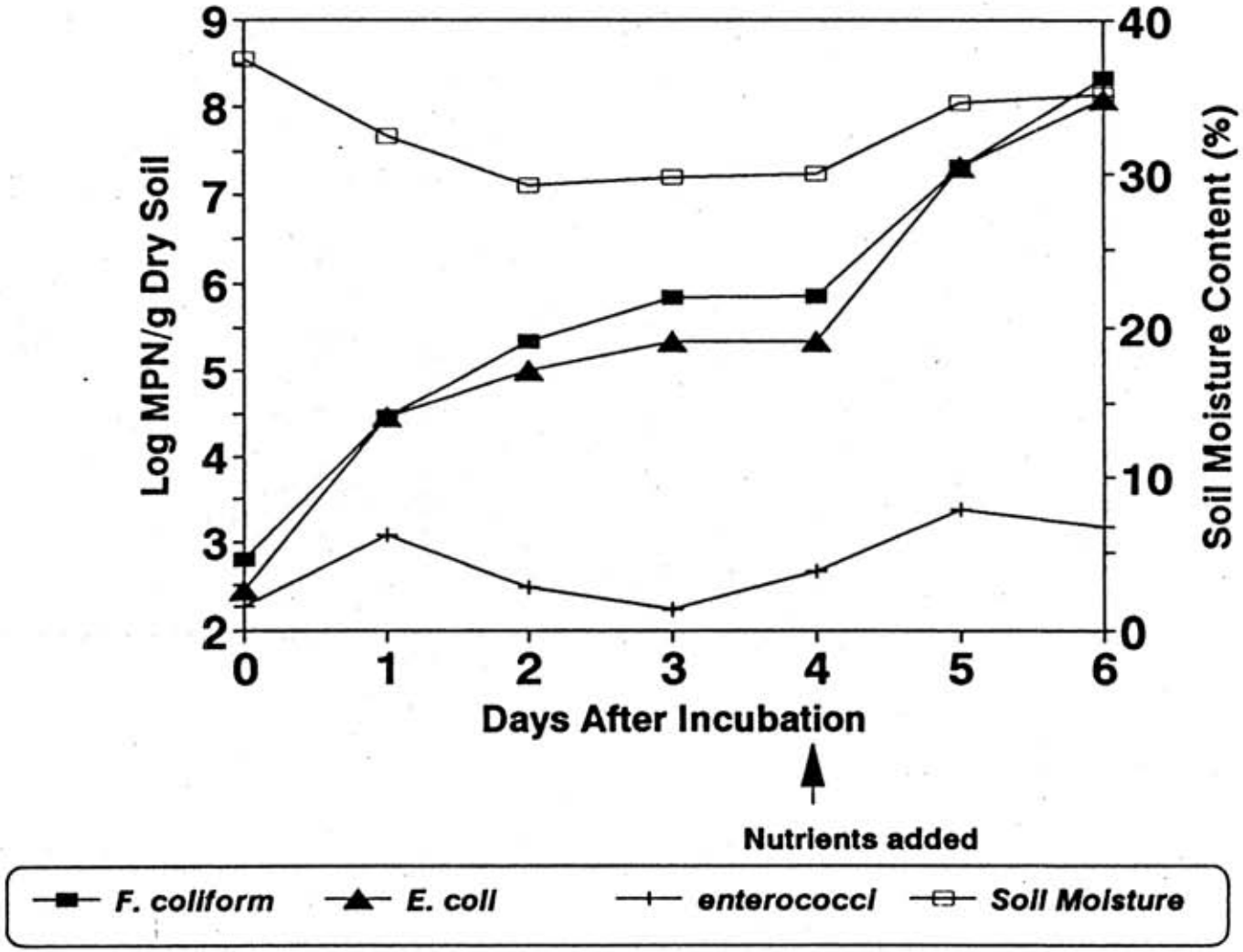


Fig. 10. Growth of Enterococci in Soil
in the Presence or Absence of Nutrients

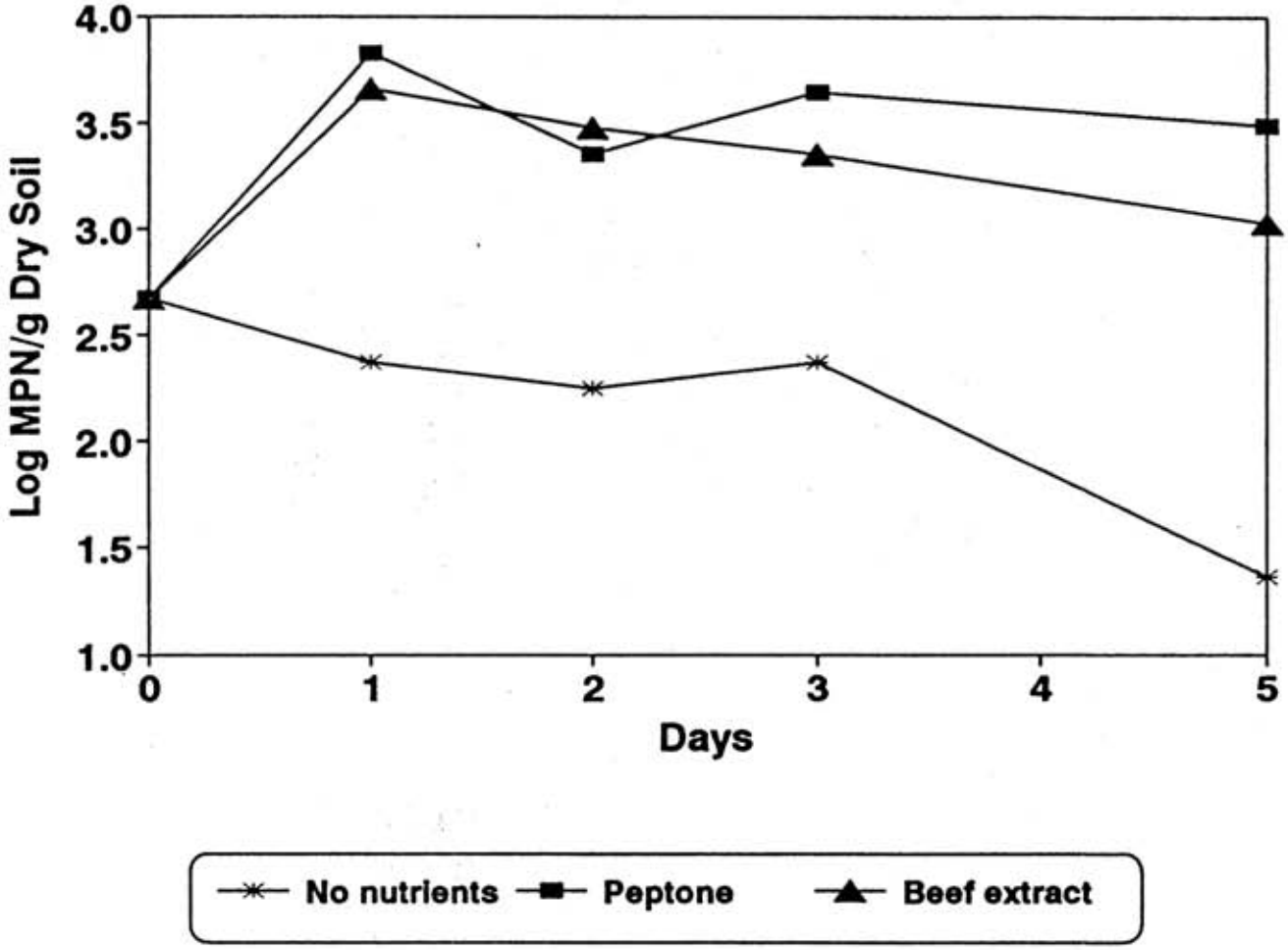
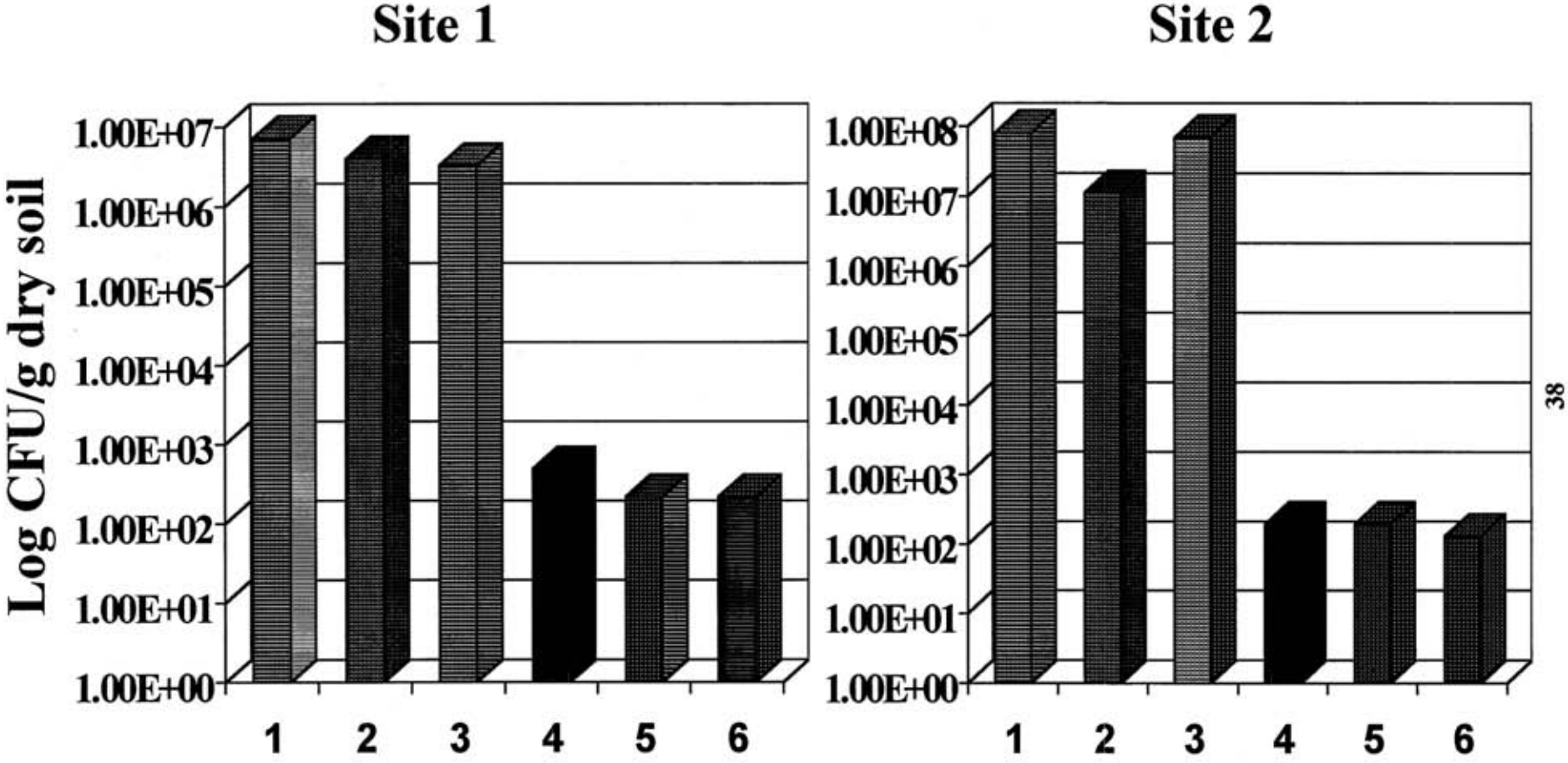


Fig. 11. Abundance of Fecal Indicator Bacteria in Soil Relative to Total Bacteria



1. Total bacteria
2. Gram+
3. Gram-
4. Fecal coliform
5. *E. coli*
6. enterococci

Fig. 12 Flow Chart of Bisson & Cabelli (1979) Method to Enumerate *Clostridium perfringens*

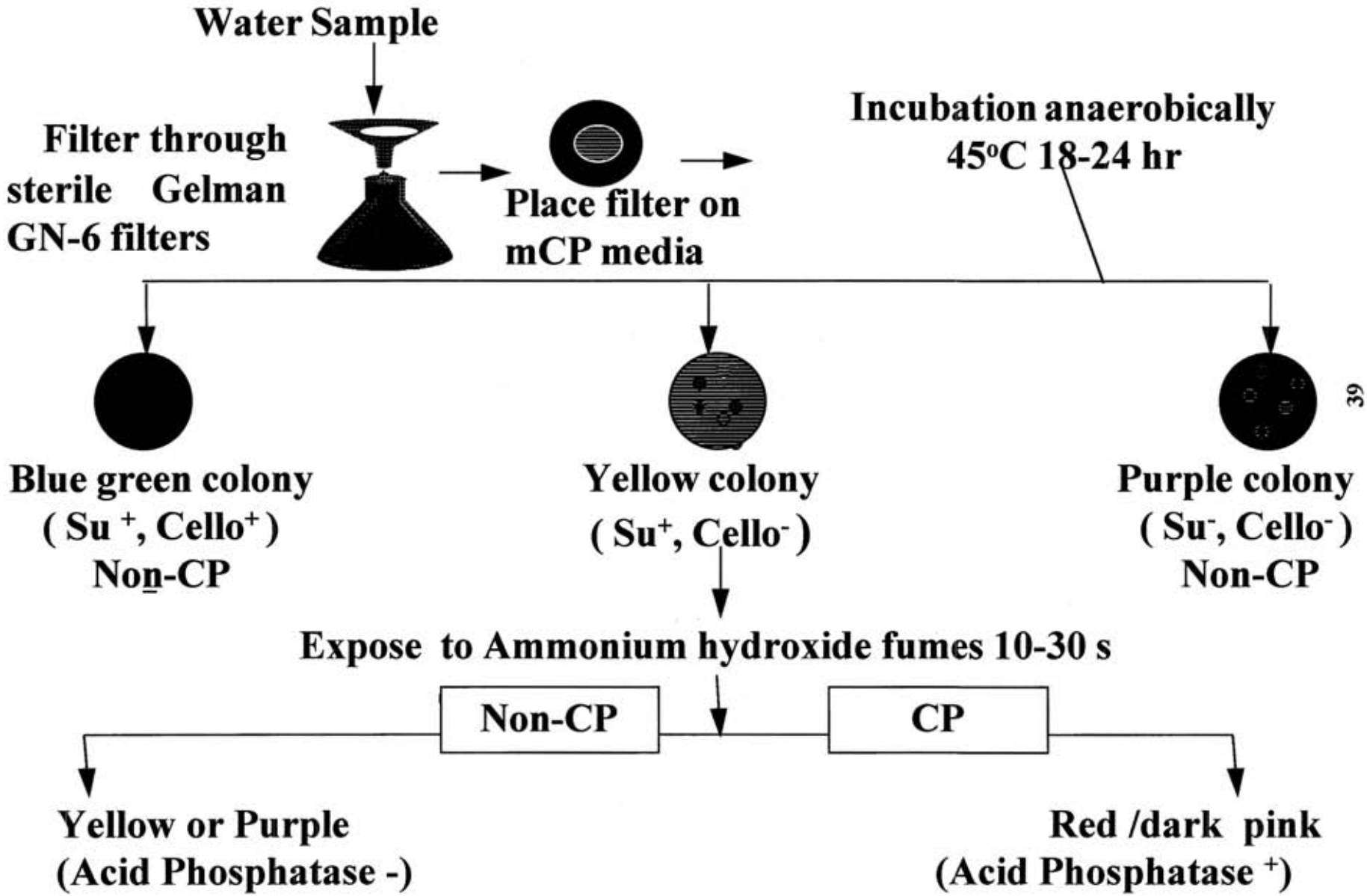


Fig. 13 Rapid Inactivation of Bacteria (*E. coli*) Versus Slow Inactivation of Viruses (MS2, Poliovirus) Suspended in Seawater and Exposed to Sunlight

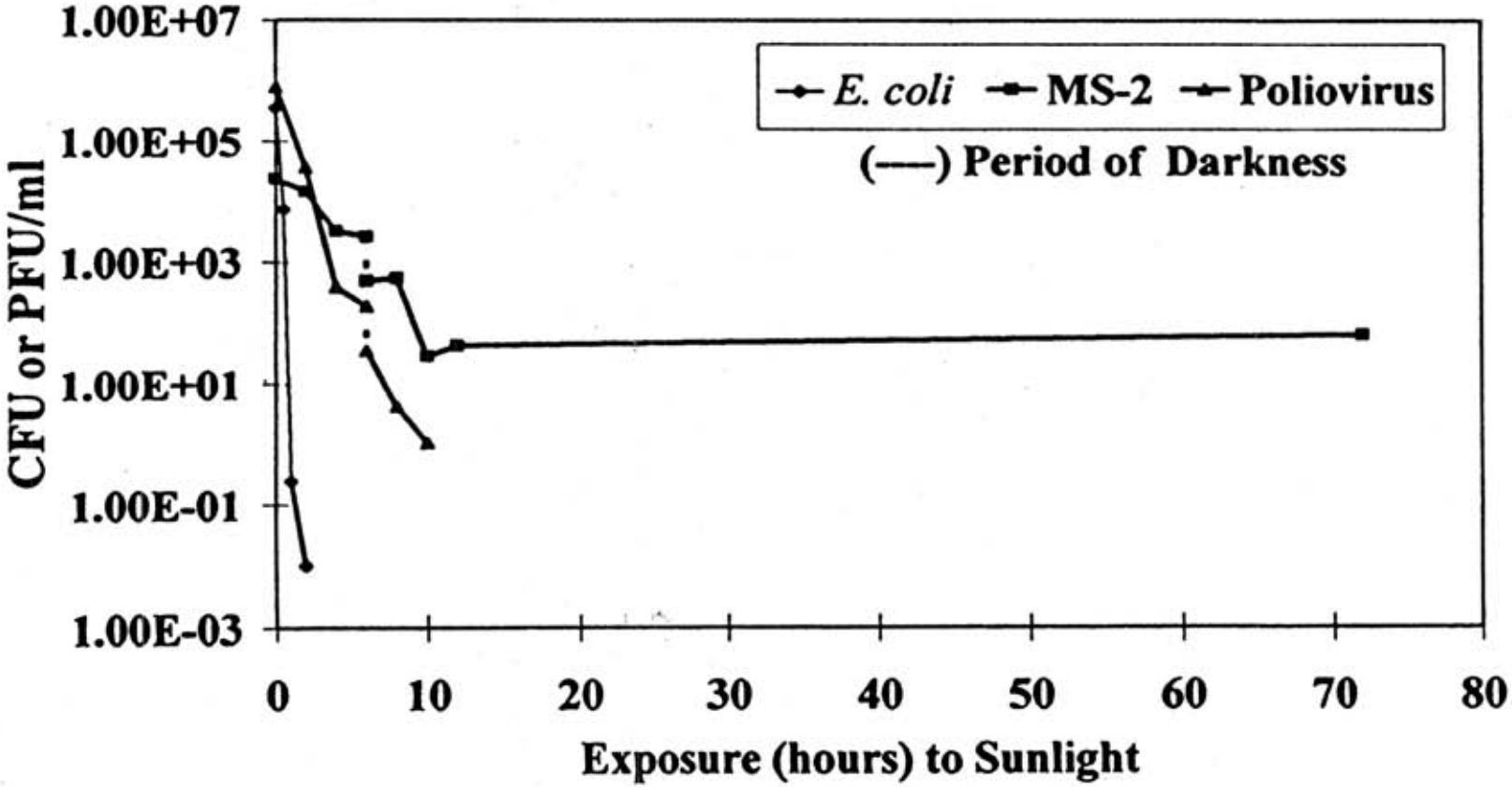


Fig. 14 Comparative Survival of Fecal Indicator Bacteria in Water Exposed to Sunlight

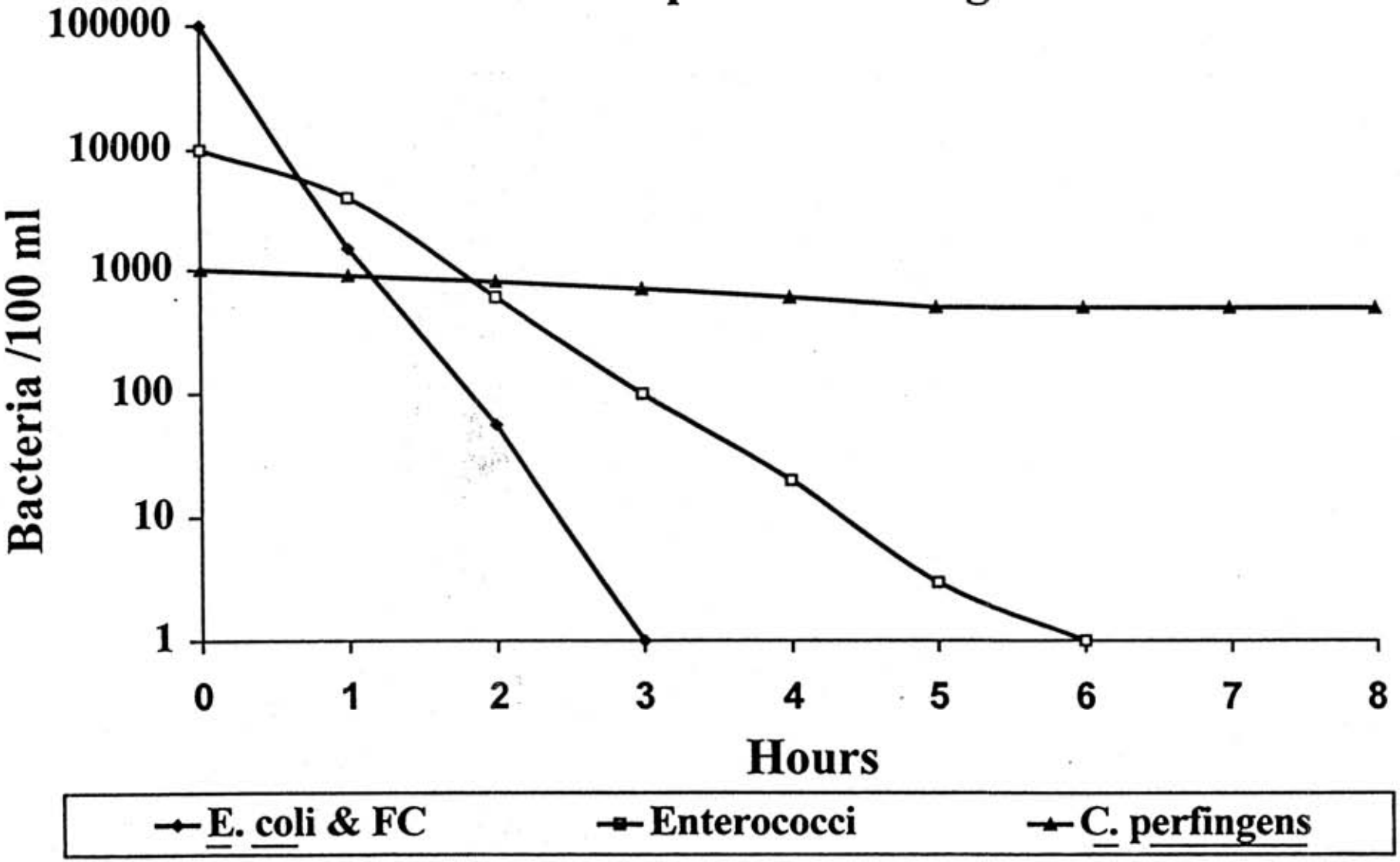


Fig. 15 Indicator Bacteria in Soils Samples

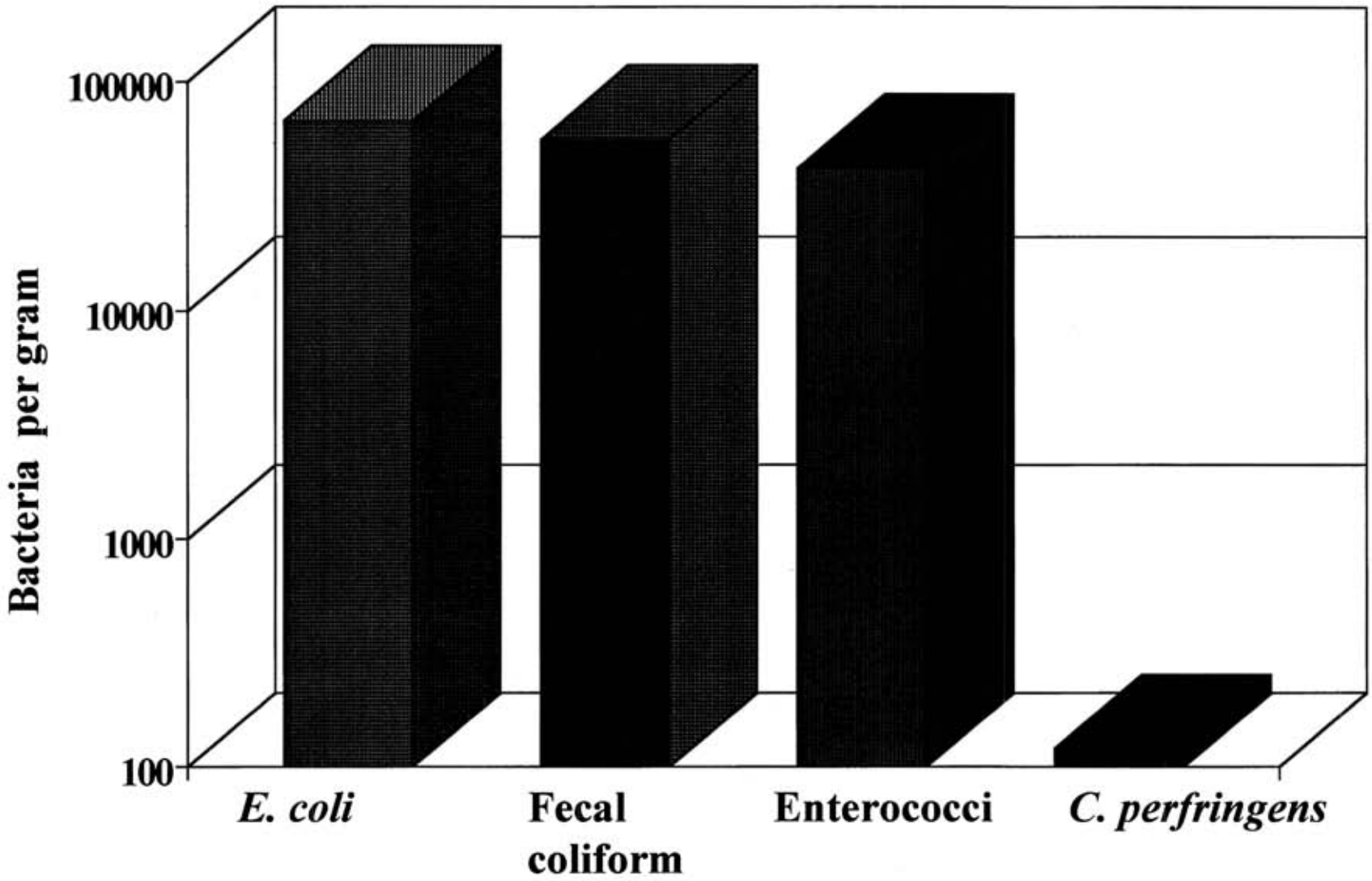


Fig. 16 Geometric Mean Concentrations of Fecal Indicators in Storm Drains

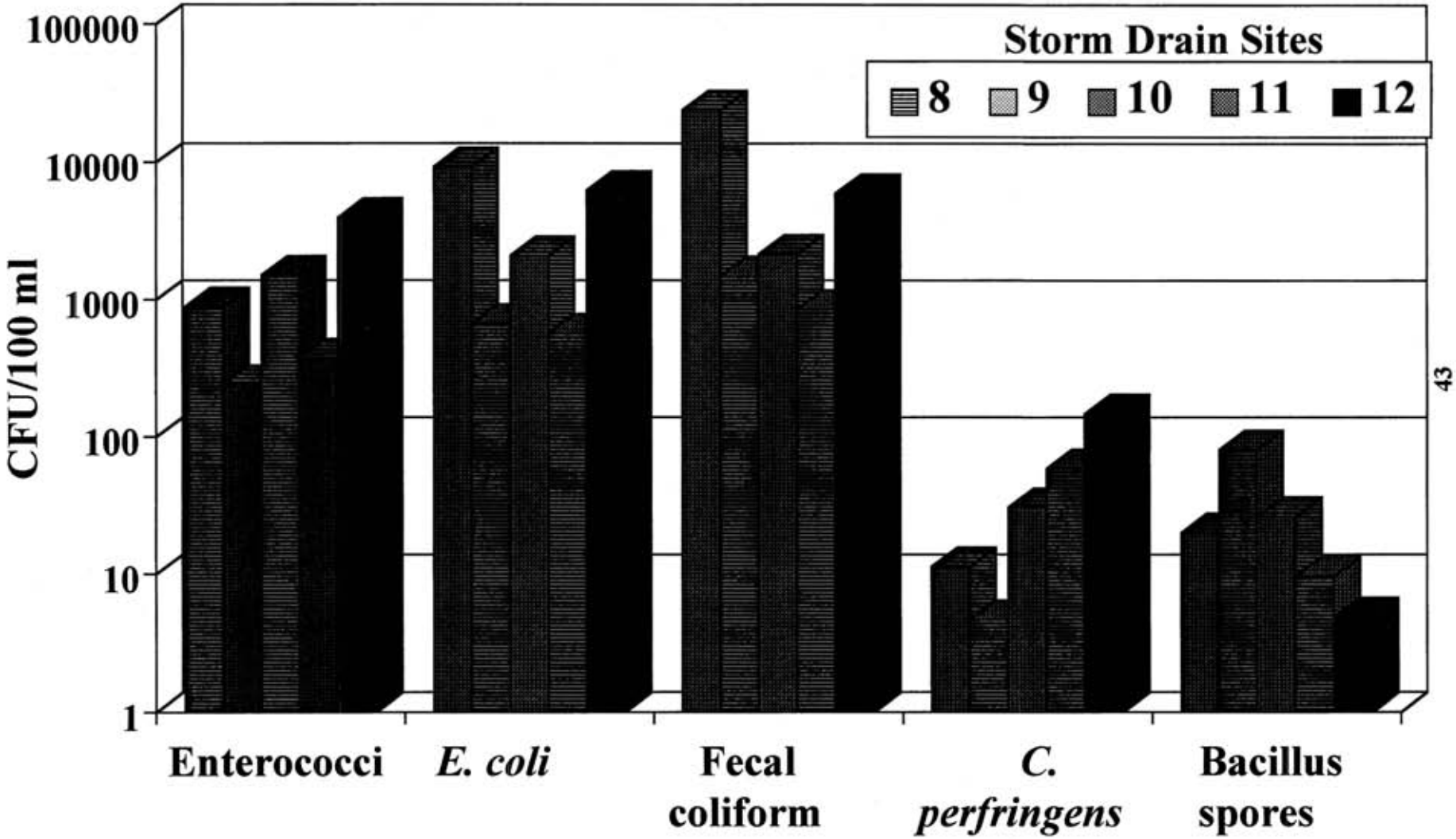


Fig. 17 Geometric Mean Concentrations of *C. perfingens* in Storm Drain Sites & in Shore Line Water at Popular Beaches

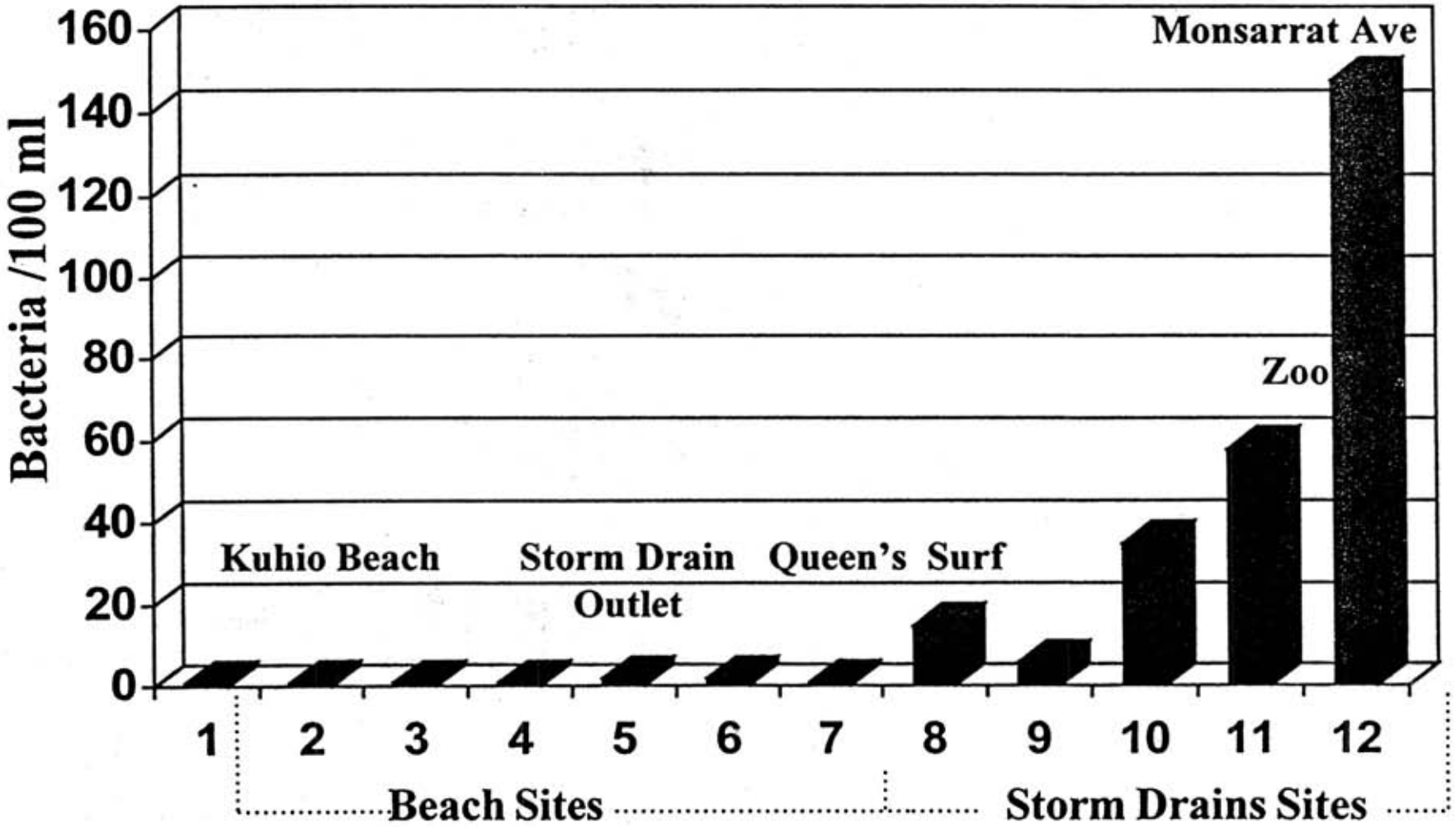


Table 1. Recovery of Fecal Indicator Bacteria from the Major Soil Types Found on the Island of Oahu.

Soil Type	Location	N		Concentration of Indicator Bacteria (MPN/g Soil)		
				Fecal Coliform	*E. coli*	enterococci
1	Lower Manoa	07	GM	270	<1	1255
			Range	0-80000		200-17750
2	East Mililani	03	GM	65	23	985
			Range	0-1200	0-1200	35-8000
3	Kolekole Pass	03	GM	67	<1	3236
			Range	0-920		1150-12000
4	Pacific Palisades	04	GM	50	1	452
			Range	0-5500	0-10	40-5500
5	Upper Manoa	07	GM	26	26	793
			Range	0-400	0-400	0-14000
6	Bellows	03	GM	1711	143	3831
			Range	250-12000	0-12000	1350-12000
7	Olomana	04	GM	851	172	1297
			Range	115-12000	0-12000	130-12000

N = Number of Samples; GM = Geometric Mean

Table 2. Concentrations, Sources and Relative Stability of Fecal Indicator Bacteria

INDICATOR BACTERIA	LOG 10 DENSITY Feces/g		Sewage/100 ml		RELATIVE SURVIVAL	EXTRA FECAL SOURCES
	Human	Animal	Influent	Cl*		
Bifidobacteria	7-8	1	7	<1	+	None
Total Coliform	6-7	4-6	6-7	<1	++	soil; vegetation and industrial effluent
Fecal Coliform	6-7	4-6	6-7	<1	++	vegetation and industrial effluent
E. coli	6-7	4-6	6-7	<1	++	None
Enterococci (*S. faecalis* & *S. faecium*)	3-4	2-3	5	1	+++	*S. faecalis* biotypes from insects and vegetation
C. perfringens	3-4	2-3	4	4	++++	Spore collection in soil and sediment with possible multiplication

* Chlorinated

Source: Adapted from Cabelli (1979).

Table 3. Affect of Sewage Treatment Processes on Concentrations of Fecal Indicators

PLANT	EFFLUENT	INDICATOR BACTERIA (CFU/100 ml)			
		Fecal Coliform	*E. coli*	Enterococci	*C. perfringens*
Wahiawa STP*	Raw	1.7×10^7	1.8×10^7	4.1×10^6	1.6×10^5
	Primary	3.2×10^7	2.7×10^7	3.2×10^6	1.7×10^5
	Secondary	7.9×10^4	8.0×10^4	9.6×10^3	1.2×10^3
	Chlorinated	4	4	<4	1.5×10^3
Mililani STP*	Secondary	2.8×10^4	---	3.5×10^3	3.8×10^3
	Chlorinated	3	---	15	2.3×10^3

*STP: Sewage Treatment Plant

Table 4. Confirmation of Natural Isolates from mCP Media as *Clostridium perfringens*

Date	Stream	# CP isolates	# Confirmed	# Non-CP Isolates	Confirmed
06/03/81	Kipapa	19	13	13	1
06/10/82	Manoa	19	19	-	-
06/24/81	Kipapa	18	17	3	0
06/30/81	Ahuimanu	12	12	3	0
07/07/81	Ahuimanu	10	9	5	0
08/04/81	Manoa	10	10	-	-
10/14/81	Kipapa	10	9	5	0
Total		98	89	29	1
Percent confirmed			91		3

Table 5. Concentrations of Fecal coliform (FC), Fecal streptococcus (FS), and *C. perfringens* (CP), in Kipapa Stream Upstream & Downstream of Mililani WWTP Discharge Site

--------(CFU/100 ml)-------

SAMPLING SITE	SAMPLING DATE	INDICATOR BACTERIA		
		FC	FS	CP
K-0 (remote Kipapa, 6.2 miles upstream)	06/03/81	170	100	<1
K-1 (rural Kipapa, 1.2 miles upstream)	06/03/81	150	390	5
	10/14/81	430	840	3
K-2 (urban Kipapa, 300 yd upstream)	06/03/81	14000	6500	40
	06/17/81	3700	8000	46
	06/24/81	39000	18000	41
K-3 (Mililani WWTP effluent discharge)	06/17/81	760	650	2700
	06/24/81	2000	1700	3400
	10/14/81	3900	9400	2300
K-4 (lower Kipapa, 300 yd downstream)	06/17/81	2800	2800	2100
	06/24/81	2600	1900	1500
	10/14/81	1800	5300	1800

Table 6. Concentrations of Indicator Bacteria in Animal Feces

SAMPLE	BACTERIA (CFU/gram)			
	Fecal Coliform	*Escherichia coli*	Enterococci	*C. perfringens*
Guinea Pig	8.0×10^3	1.17×10^3	1.57×10^3	<67
Rat	1.08×10^7	1.03×10^7	6.33×10^6	<67
Chicken	1.62×10^8	1.62×10^8	3.81×10^7	<67
Cat	1.32×10^6	1.46×10^6	3.57×10^5	5.5×10^4
Rabbit	1.55×10^7	1.57×10^7	3.23×10^6	<67
Mice	2.03×10^6	3.83×10^6	1.99×10^6	<67
Monkey	1.45×10^7	2.35×10^7	1.35×10^5	<67
Pig	5.73×10^7	5.8×10^7	9.33×10^6	1.73×10^5
Quail	5.4×10^7	3.4×10^7	5.8×10^6	<67
Sheep	3.47×10^5	2.8×10^5	1.67×10^6	4.07×10^4
Cow	1.87×10^6	1.87×10^6	4.27×10^4	2.67×10^2
Dog	6.47×10^4	7.13×10^4	5.33×10^4	1.47×10^4
Pigeon	1.87×10^7	1.71×10^7	5.0×10^6	<67
Duck	7.6×10^5	1.6×10^6	1.4×10^6	2.9×10^5