AN EVALUATION OF A MODIFIED MEMBRANE FILTER TECHNIQUE FOR THE RECOVERY OF FECAL COLIFORMS EXPOSED TO SELECTED HEAVY METALS

by

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The author wishes to
dedicate this work to
his parents,
Mr. and Mrs. E. B. Gayle Jr.
for their immeasurable
assistance and support.

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I. INTRODUCTION

Industrial growth and expansion and rising urban populations have led to an ever increasing demand for clean water. Yet, increased pollutant loadings from point sources, such as industrial and domestic discharges, and nonpoint sources, such as storm water runoff, have made the maintenance of suitable water supplies difficult. Often, industrial and domestic waste treatment plants discharge treated effluents into waterways that later become municipal water supplies for communities down stream. With groundwater supplies dwindling and waste discharges reducing the quality of our surface water supplies, clean water promises to be an issue of continuing importance in years to come.

Water is known to be a vector for the spread of communicable diseases. Fecal contamination of water by warm-blooded animals may introduce a variety of enteric pathogens including bacteria, viruses, and intestinal parasites. Detection of most enteric pathogens by current methods is much too difficult and cumbersome for routine use; therefore, tests for the detection and enumeration of indicator organisms are traditionally performed instead. At present, the most widely used indicator organisms are those of the coliform group. Coliforms are found in the gut of all warm blooded animals, and their detection in significant numbers is considered an indication of the possible presence of enteric pathogens. Not all coliforms originate in the gut. The preferred indicators of sanitary quality of a water are those coliforms known to be of fecal origin.

Standard Methods (1) recommends the use of two techniques, the "membrane filter" and "multiple tube fermentation" procedures, for the enumeration of fecal coliforms. The former involves filtration of a sample followed by direct plating of the filter on selective agar. After incubation, the fecal coliforms may be counted and by taking into consideration the dilution of the sample, the fecal coliform density may be determined. The multiple tube fermentation procedure is a statistical technique that relies on the fermentation of lactose with resulting gas production as the criterion for detection of fecal coliforms. Serial dilutions of a sample are inoculated into fermentation tubes and incubated. The number of positive tubes (those showing gas production) at each dilution may then be compared to statistical tables to arrive at a most probable number (MPN) of fecal coliforms yielding the observed results. Both techniques employ inhibitory media and elevated incubation temperatures to obtain the desired selectivity. The MPN procedure, however, is more efficient in the recovery of coliforms, in certain instances, because it includes an enrichment step in which the coliforms are allowed to grow at a lower temperature in a nonselective medium prior to exposure to either elevated temperatures or selective agents.

Bacteria that enter the aquatic environment are subject to many adverse environmental factors such as extreme fluctuations in temperature and pH, exposure to irradiation, and contact with disinfectants or toxic substances, especially heavy metals. Prolonged exposure to these and other antagonistic influences may cause stress in microbial populations (2), and many of these stressed organisms may be undetected when the recovery procedure involves further stress through exposure to the selective conditions of the test. In recent years there have been many studies to improve the recovery of stressed organisms. There have been several methods developed, including modifications of both the membrane filter technique and multiple tube fermentation procedure.

The purpose of this study was twofold. The effects of heavy metal stress on the recovery of fecal coliforms was examined, while two methods of enumeration were compared for their efficiency in recovering the stressed organisms. The methods compared were a modified membrane filter technique (3) and the standard membrane filter technique as proposed by Standard Methods (1).

II. LITERATURE REVIEW

Significance of Pathogens and Coliforms

Municipal water supplies, in some situations, may serve millions of people. While many problems are associated with water treatment systems of this size, the detection and control of waterborne pathogens is of greatest importance. Sources of contamination are ubiquitous, and much work is needed, not only to improve methods of pathogen detection but also to eliminate these organisms in finished water.

Weibel and associates (4) summarized the available information on waterborne disease outbreaks between 1946 and 1960. They reported 228 outbreaks resulting in 25,984 individual cases. General gastroenteritis was by far the most common ailment, being associated with 126 outbreaks and over 13,000 cases. Typhoid and infectious hepatitis were implicated in 39 and 23 outbreaks, respectively. Each, however, was involved in a relatively small number of cases: 930 for hepatitis and only 506 for typhoid. Diarrhea and shigellosis, while accounting only for a small number of outbreaks, each were responsible for over 5,000 cases.

Whenever a water is exposed to fecal contamination, the possible presence of intestinal pathogens arises. The most common pathogens include strains of *Salmonella*, *Shigella*, *Leptospira*, enteropathogenic *Escherichia coli* (*E. coli*), *Pasteurella*, *Vibrio*, *Mycobacterium*, human enteric viruses, cysts of Endamoeba histolytica, and hookworm larvae (5). Concern with such contamination is associated with both point and nonpoint sources. While sewage treatment plants and other related point discharges are of obvious interest, nonpoint discharges, such as storm water runoff, have recently received much attention as significant sources of fecal contamination.

In a study conducted by Geldreich and his associates (6), nonhuman sources of bacterial contamination were confirmed in storm water runoff and the existence of a potential health hazard was demonstrated by isolating a Salmonella species. Also, the results of a study conducted by Butler and Busbee (8) found Salmonella and Shigella species to be present in a significant percentage of family pet dogs studied. Salmonella species also were isolated by Faddoul and his associates (9) from the fecal material of several species of wild birds including gulls, sparrows, and cowbirds.

The actual detection of pathogens by present methods is much too difficult and time consuming for routine use. For this reason, indicator organisms are commonly used to monitor the sanitary quality of water. Historically, coliforms have been used as indicators of fecal pollution. These organisms are part of the normal fecal flora of all warm-blooded animals. Hence, their detection implies the possible presence of intestinal pathogens. There are, however, many members of the coliform group which are nonfecal in origin. Recently, there has been interest in using fecal coliforms, those known to be of fecal origin, as the preferred indicators of fecal pollution. This concept, however, has been met with some resistance because it is less conservative than the "total coliform" evaluation.

The coliform group is defined as all aerobic and facultatively anaerobic, gram-negative, nonspore-forming, rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35 C. This definition may be applied to fecal coliforms that also ferment lactose with gas formation at 44.5 C (1).

One of the problems of using indicator organisms to predict the presence of pathogens is the differential survival time of the two groups. One study conducted to determine the time required to obtain a 90 percent die-off of *Salmonella* and several indicator organisms found the die-away patterns of *Salmonella typhimurium* and *E. coli*, a fecal coliform, were very similar at 0, 5, 10, and 20 C. *Enterobacter aerogenes*, a nonfecal coliform, was much less hardy at all temperatures (10).

When water of drinking water quality is examined with regard to USEPA standards, it is expected that more than 95 percent of all samples yield negative results (2). Regardless of standards, however, it is difficult to predict at what threshold level of coliform density the danger of enteric pathogens begins. Because *Salmonella* is the only pathogen easily detected with existing methods that are adaptable to field studies, there is great interest in establishing a threshold level for fecal coliforms below which *Salmonella* would be found infrequently (11). The results of a study conducted to correlate coliform densities with the presence of *Salmonella* indicated low total and fecal coliform values are not sufficient to ensure the absence of pathogens. Salmonella was isolated from waters containing total coliform densities of less than 1000/100 ml. and fecal coliform densities of less than 150/100 ml. (12).

Weibel's summary of disease outbreaks not only confirms water as a major disease vector but also indicates the public health significance of the pathogens themselves. The isolation of human enteric pathogens from the fecal material of house pets, wild birds, and rodents has caused increased interest in urban runoff as a source of enteric pathogens. The use of the coliform group as indicators of this type of contamination is bound in tradition, yet studies have indicated that low coliform densities, obtained using present methods, do not necessarily indicate the absence of pathogens. As these points indicate, there is a tremendous need for improved methods of water quality examination.

Standard Fecal Coliform Recovery Methods

There are two methods currently recommended by Standard Methods (1) for the recovery of fecal coliforms: the multiple tube fermentation procedure and the membrane filter technique. Both involve elevated temperatures and inhibitory media to obtain the desired selectivity. The former is a statistically based procedure that is often referred to as the "most probable number" (MPN) method. The membrane filter technique allows for the determination of fecal coliform densities by direct plate counts.

The MPN procedure involves three steps: the presumptive, confirmed, and completed tests. In the presumptive test, serial dilutions of a sample are incubated in fermentation tubes containing a lactose-based medium. These tubes are incubated at 35 C, and gas production within 48 hours (hr) is considered a positive test.

In the confirmed test, elevated temperatures and an inhibitory medium are used to obtain the desired selectivity of fecal coliforms. All positive presumptive tubes are inoculated into fermentation tubes containing EC medium. This medium is a lactose-based broth that contains bile salts as an inhibitory agent. The fermentation tubes are incubated at 44.5 C for 24 hr and all tubes showing gas production are considered positive. At this time, a statistical table may be consulted from which one may determine a most probable number of organisms that would yield the observed combination of positive tubes.

If further confirmation of fecal coliform presence is necessary, one may continue with the completed test. In this final step, one petri of either Endo agar or Eosin Methylene Blue (EMB) agar is streaked from each positive EC tube. After incubation at 35 C for 24 hr, isolated colonies are inoculated into fermentation tubes containing lactose broth. These tubes are then incubated at 35 C and gas production is expected within 48 hr. A Gram stain is also performed in this step, and the confirmation of gram-negative, rod shaped bacteria, along with gas production, constitutes a positive completed test. The completed test often is deleted. Only in the examination of finished water is it required and then only on sufficient samples to establish the validity of the confirmed test.

The membrane filter procedure for fecal coliform analysis involves filtration of a sample and direct plating of the filter on MFC medium. This medium consists of a lactose broth base with various indicator and inhibitory agents including aniline blue, bile salts, and rosolic acid. The volume of the sample filtered depends on the expected organism density and the suspended solids concentration of the water. Volumes filtered should yield from 20 to 60 fecal coliform colonies per filter. Turbidity may, in some cases, be a problem by preventing filtration of sufficiently large sample volumes. In such situations the MPN procedure should be used.

After filtration, the filter is placed on a MFC agar plate and incubated at 44.5 C for 24 hr. All blue colonies are considered to be of fecal origin. These colonies are enumerated and by taking the sample size and dilution into consideration, the number of fecal coliforms in the original sample may be determined.

Both methods are widely used; however, there are advantages and disadvantages to each. Problems associated with the MPN procedure include a lack of precision and an extended period of time required to obtain results. Because the method is based on statistical probabilities, some inherent error would be expected, and MPN values have been found to vary over a ten-fold range on identical samples (13, 14). The method also requires from two to four days to obtain fecal coliform densities. The discovery of contamination four days after the fact would be of little value in emergency situations. The direct counting involved in the membrane filter technique allows for greater precision, and also, fecal coliform densities may be obtained within 24 hr.

The major disadvantage associated with the membrane filter procedure is its decreased recovery efficiency on samples containing stressed organisms. The MPN and membrane filter procedures have been found to be comparable for analysis of settled sewage that has been disinfected by heat or ultrasonic vibration. When chlorine disinfection is used, however, the densities obtained using the membrane filter procedure have been found to be much lower than those of the MPN method. The superior recovery of the MPN method is thought to be due to the partial reversibility of the chemical stresses more readily in the MPN broth than on the membrane filter (15).

Green and associates (16) found recoveries of fecal coliforms obtained using the standard membrane filter technique for the analysis of primary and secondary chlorinated effluents to be only eight percent of those obtained with the MPN method.

Because the MPN method has been proven to be the superior method, its use is recommended by the EPA for the analysis of chlorinated samples and those thought to contain stressed organisms.

Stress

Stress, as it is used here, refers to any sublethal factor or combination of factors that may injure or impair the growth of microorganisms. Exposure to adverse environmental conditions in both aquatic and terrestrial ecosystems may lead to stress in microbial populations. Survival of indicator organisms and associated intestinal pathogens is related to many factors including exposure to sunlight, temperature and pH extremes, toxic substances, and organic matter. Survival may also be affected by the presence of competing or antagonistic organisms (18).

In parallel studies conducted by Geldriech and associates (6, 7) seasonal variations in the survival of indicator organisms in both soil and storm water were observed. Coliforms were found to persist in greater numbers during winter studies (10C) than summer studies (20C), presumably due to increased irradiation during the summer months.

Heavy metals, which are often present in significant concentrations in both aquatic and terrestrial environments, may also cause stress in microbial populations. Many heavy metals are essential nutrients and required in trace amounts for optimum bacterial growth. However, at higher metal concentrations, toxic effects are often observed.

Cadmium (Cd) toxicity in *E. coli* is characterized by a change in cell morphology, involving the development of large, intracellular vacuoles and an abnormally long lag phase. In a study using a defined medium containing glucose and salts including 3×10^{-6} M (0.144 mg/l) Cd it was discovered that 95 percent of early lag phase cells failed to form colonies when plated on nutrient agar containing NaCl. The cells later became acclimated, their morphology returned to normal, and their rate and extent of growth were normal once growth began (19).

Cells grown in the presence of Cd accumulate high concentrations of the ion. In acclimated cells, 56 percent of the metal is associated with the cell wall, 13 percent with the cell membrane, and 31 percent in the cytoplasm. In unacclimated cells the values are 2, 75, and 23 percent, respectively. The activity of alkaline phosphatase, a zinc-metallo enzyme that is inhibited by Cd, is not abnormally low in acclimated cells, suggesting that Cd is compartmentalized in these cells. It appears acclimination to Cd in E. coli involves exclusion of the ion from the cell and repair of the damage caused by exposure to it (19).

Chromium (Cr), in its hexavalent form (chromic oxide, chromate, and dichromate), is a strong oxidant, and the known toxicity of the chromates is due largely to this oxidizing action. Also tri- and hexavalent chromium have been found to inhibit bacterial urease activity. The magnitude of chromium toxicity toward bacteria depends on the concentration of the metal ion as well as the type of bacteria. In general, concentrations in excess of 1 mg/l of medium are toxic, particularly in the case of the hexavalent form (20).

Heavy metals, such as zinc (Zn), have been found to precipitate proteins by altering their solubility. Also, Zn may tie up the active sites on enzymes at concentrations below those required for precipitation (21).

Lead (Pb), unlike many heavy metals, has been found to accumulate to high concentrations in bacterial cells without causing death. As much as 400 mg Pb/g bacterial mass has been detected in viable cells (22).

The recovery of fecal coliforms seems to be particularly influenced by stress. The failure of current methods of fecal coliform recovery to enumerate injured cells is thought to be the result of hypersensitivity to secondary stresses such as the presence of selective agents (23) and the initial shock of elevated temperatures (16). A study conducted by Scheusner and associates (24) to determine the extent to which selective agents in various media affect the recovery of E. coli found significant differences in the inhibitory effects of various dyes and salts. The dye "brilliant green" was found to be inhibitory to both stressed and unstressed E. coli. "Crystal violet" and "neutral red" were found to be inhibitory to the same extent while E. coli grown on agar containing "eosin Y" exhibited no inhibition. Variations were found between the effects of different bile salts but in all cases they were found to be very inhibitory. Hill (25) found the inhibition of bile salts to be attributable to the dissolving action they incur upon cell membranes. Presswood and Strong (26) found that the elimination of rosolic acid, a common component of selective media, allowed higher recoveries of fecal coliforms without any loss of selectivity.

Hufham (27) conducted a study comparing various media at 35 C and 44.5 C and found no significant difference between the recoveries of E. coli cultured at 35 C on solid media containing plate count, total coliform, and MFC broths. At elevated temperatures, however, the recoveries on MFC agar were much less than on the other media tested.

Braswell and Hoadley (28) found E. coli cells injured during chlorination of secondary sewage failed to produce colonies on MFC medium or to grow and produce gas in lactose broth when both were incubated at 44.5 C. The total viable count, determined on trypticase-soy agar at 35 C, was found to be significantly greater than the recoveries obtained at the elevated temperature.

A study conducted by Klein and Wu (29) to compare the efficiency of two plating procedures on agar (the "pour-plate" and "spread-plate" technique), found that aquatic organisms were stressed by their exposure to the warmer agar used in the pour-plate technique. The recoveries obtained using pour-plate agars at 42 C, 45 C, and 50 C were, in all cases, found to be lower than those obtained using the spread-plate technique. The results of this study also indicated that starvation of a microbial population, which may be postulated to occur in a low-nutrient aquatic-environment, can lead to increased susceptibility to the secondary warming stress caused by the warm pour-plate agar. Hoadley and Cheng (30) examined the effects of various dilution waters on the recovery of _E_. _coli_. Of the dilution waters tested, tap water was found to be highly toxic to all indicator organisms. Marginal recoveries were obtained using phosphate buffered dilution water and distilled water while 0.1 percent peptone dilution water was found to permit the highest recoveries of _E_. _coli_.

Often when working with solutions, sample, or media containing metal ions, chelating agents such as EDTA are used. In microbiological studies, however, EDTA has been found to exhibit a bactericidal effect on several species (31). It is thought that EDTA complexes metal ions essential to the bacteria for maintaining cell wall integrity.

Many factors, both in aquatic and terrestrial environments and in the laboratory, may cause stress in microbial populations. Many studies have been conducted confirming stress as a factor in the recovery of microorganisms. Since the recovery of indicator organisms is crucial to the effectiveness of water quality determinations, a number of stressed organism recovery methods have been proposed.

Stressed Organism Recovery Methods

In recent years, much work has been done to improve stressed organism recoveries. Variations in the membrane filter technique including different media, incubation schedules, and filter pore size have been evaluated. Also, there have been studies to improve the MPN procedure and to develop an automated enzyme-assay method for the detection of E. coli.

Low specificity, poor precision, and prolonged time of analysis required for the MPN method of fecal coliform enumeration led Delaney and associates (32) to develop a membrane filter technique for the recovery of fecal coliforms in water. It was discovered that E. coli could produce indole from tryptophane and that differentiation of this indicator organism from other organisms in a sample could be accomplished by first plating the sample on a medium containing tryptophane and subsequently staining the colonies to determine which were indole formers.

McCarthy and associates (33) compared an enrichment and agar substitute (A&E) technique with a previously used, one-step endo broth method. The A&E technique was found to yield higher recoveries and also to exhibit a more pronounced and longer lasting colony sheen. The need for enrichment was postulated when it was found that the fucshin and sulfite present in the endo broth were detrimental to coliforms. Successive reductions in these components proved that concentrations low enough to prevent bacterial stress failed to produce a sheen on the colonies formed. Media stability and sheen development were improved with the agar substrate.

Speck (34) found that recoveries of coliforms associated with frozen foods could be enhanced with the use of a modified plating procedure. Coliforms on a nonselective medium, trypticase-soy agar (TSA), were first incubated for four to five hours at 37 C, then covered with an overlay of violet red bile agar (VRB) and incubated for an additional 18 hr.

Hartman and co-workers (35) obtained increased recoveries of coliforms with a modified VRB agar as compared to standard VRB agar. The formula of the modified VRB agar was altered to allow recovery of injured cells before exposure to selective or inhibitory agents.

Sladek and associates (36) found a critical factor in the recovery of coliforms to be the pore morphology of the membrane filter used. They obtained improved recoveries with a membrane filter having a surface opening of 2.4 μm as compared to the presently used filter with a 0.45 μm retention. Others (37, 38) have also conducted similar studies that confirmed Sladek's findings.

A study conducted by Martin and his co-workers (23) found the addition of catalase to the surface of selective agar media enhanced the recovery of stressed microorganisms. Catalase prevented the accumulation of hydrogen peroxide in and around injured cells, implicating

H_2O_2 as a factor in the failure of stressed organisms to grow on selective media.

Rose and associates (3) have developed a two-layer agar modification of the membrane fecal coliform technique that consistently yielded higher recoveries of fecal coliforms than the presently used membrane filter procedure. The method involves the use of a nonselective resuscitation medium containing lactose broth, as an overlay, and a selective base medium containing MFC broth. After filtration, the membrane filter is placed on the lactose overlay and incubated at 35 C for 2 hr, after which it is incubated at 44.5 C for an additional 22 to 24 hr. It is postulated that the resuscitation medium, in conjunction with the lower incubation temperature, allows for the partial recovery of injured organisms. In a few hours, the selective agents in the base medium are able to diffuse through the overlap and contact the membrane filter. This eventual exposure to inhibitory agents, along with the elevated temperature (44.5 C), allows for the necessary selectivity. Comparisons involving 61 water samples, including chlorinated effluents, confirmed a 93 percent increased recovery over the standard membrane filter procedure.

Green and associates (16) obtained improved recoveries of stressed fecal coliforms by modifying the incubation schedule used in the standard membrane filter technique. Chlorinated effluent samples were filtered and incubated at 35 C for varying time intervals prior to incubation at the higher temperature (44.5 C). Pre-incubation for five hours was found to yield the highest recoveries of stressed fecal coliforms, and the densities obtained were comparable to those of the MPN procedure. Longer pre-incubation periods, however, were found by Brown (39) to permit excessive growth of nonfecal background organisms that interfere with the enumeration of coliforms.

Lanz and Hartman (40) have developed a modification of the standard MPN method for coliform enumeration. Timed-release capsules containing brilliant green and oxgall were added at the time of inoculation of a lactose broth medium. The selective agents diffuse through the capsule imparting selectivity to the originally nonselective medium. Concentrations of brilliant green did not reach inhibitory levels for at least two hours, thus allowing for the recovery of injured cells. Recovery of heat-injured E. coli was found to occur using the capsule method but not in brilliant green bile broth or on VRB agar. There were no significant differences found between the recoveries obtained using the capsule method and the standard MPN procedure. It was suggested by the authors that the capsule method could be used as a combined presumptive and confirmed test, thus reducing the time needed to obtain results similar to those obtained using the standard MPN procedure.

Recently, information was published concerning an automated method for measuring fecal pollution (41). A system has been developed that detects E. coli by analyzing for the enzyme glutamate decarboxylase. This technique was first used in the foods industry to detect fecal coliforms in milk and is now being used with water to a limited extent. No other organisms common to water have been found to produce this enzyme. Therefore, the test is specific for _E. coli_. Moran and Wither (41) found coliform recoveries by this automated method to be higher than those obtained by the standard MPN procedure.

In a later study concerning this automated technique (42), heavy metal ions, phosphates, and sulfates were found to inhibit the synthesis of glutamate decarboxylase thereby biasing the results obtained. A conclusion of the study, however, was that, at concentrations normally encountered, the results of the automated test would not be significantly affected.

The Occurrence, Transport, and Chemistry of Heavy Metals

Heavy metals may be introduced into the aquatic environment in many ways. Obviously, mining and smelting operations and various industrial discharges may be significant contributors. The effects of these sources, however, tend to be localized. Metals may also be introduced in much smaller concentrations yet over a much broader area via other modes.

Significant amounts of heavy metals have been detected in the contaminant material collected from street surfaces and parking lots (18) and also in storm water runoff from these areas (43, 44). When metals concentrations in urban runoff were compared to those in domestic waste, loadings of 10 to 100 times that of sewage were found (18).

Metals such as Cd, Cr, Pb, and Zn are used as additives in petroleum products and are deposited through motor vehicle exhaust emissions (44). Combusted tetraethyl lead in gasoline constitutes a major source of this metal found in runoff from urban areas. With the introduction of no-lead petroleum products, these concentrations may be expected to decrease in the future. Zinc and Pb are also used in the manufacture of automobile tires and are deposited through abrasion of the tire on the street surface (44). Chromium (Cr) is commonly used as a rust inhibitor and is also found in storm water runoff (45).

Analytical methods for the determination of metals concentrations have improved in recent years. Much of the published data, however, disagrees on ambient aquatic concentrations. Both sampling techniques and analytical efficiency may be sources of error. The range of environmental concentrations varies greatly and, therefore, data represented as "average concentration" often do not reflect the concentration to be expected in any discrete sample.

In one study (46) Cr concentrations in waters in the United States were found to range from one to 112 μg/l with a mean level of 14 μg/l. Cadium was found in only three percent of the samples with a mean concentration of 10 μg/l when detected. Concentrations of Zn were found to range from two to 1183 μg/l with a mean of 64 μg/l. Lead was observed in 20 percent of the samples taken and usually at levels below 90 μg/l. Concentrations as high as 800 μg/l were found near mining operations, however, and a mean concentration of 23 μg/l was calculated for the composite of the samples.

Hydrous oxides of aluminum (Al), iron (Fe), and manganese (Mn) play an important role in the deposition of heavy metals such as Cd, Pb, and Zn. These relationships have been known for years. Many of the methods used to concentrate heavy metals for analysis involve coprecipitation with ferric or aluminum salts. Hydrous metal oxides may arise from a variety of sources such as the weathering of various mineral species. The uptake and release of heavy metals from hydrous metal oxides is a function of ion concentration, pH, and the amount and type of organic and inorganic complex formers available. Although it is evident hydrous metal oxides play a significant role in the deposition and transport of heavy metals, the magnitude of this role is not known for natural water systems (47).

Pita and Hyne (48) studied the transport and accumulation of Zn, Pb, and Cd in reservoirs. They found all three metals to be associated with the sediments and Pb and Zn with particulate matter in the form of organo-metallic compounds. It is believed that adherence of Zn and Pb ions, or organo-metallic compounds to clay particles may cause flocculation and deposition in the sediments. Transport was believed to occur in the ionic form or as organo-metallic complexes.

The chemistry of heavy metals in water is extremely complex and not very well understood at the present time. Heavy metals in solution are highly reactive and may be removed by a variety of physical and chemical processes. The solubility of most heavy metals is affected by pH and tends to increase as pH decreases. The concentrations of heavy metals in surface waters is highly variable but in most cases very low (48).

III. MATERIALS AND METHODS

It is believed that heavy metals may cause stress in coliform populations and inhibit their enumeration by the present membrane filter technique. This technique employs an inhibitory medium (MFC) and it is believed that during enumeration coliforms may be further stressed by these selective agents, causing the number of organisms recovered to be deceptively low. Standard Methods (1) specifies the use of a stressed recovery membrane filter technique for the examination of chlorinated effluents, but no stressed membrane filter technique is recommended for the recovery of fecal coliforms that may have been exposed to heavy metals or other stress inducing factors. It may in fact be true, when the effects of stress on microbial populations are more fully known, that all fecal coliforms having been exposed to the aquatic environment, are stressed to some extent by one factor or another.

Rose et al. (3) have developed a two-layer agar technique that was a base medium of MFC agar with an overlay of a resusitation medium containing lauryl tryptose broth. This overlay is added within one hour prior to plating the filters. It is believed that within a few hours the selective agents in the base medium are able to diffuse through the overlay and contact the filter. The fecal coliforms, however, are subject to the favorable environment of the resusitation medium for a few hours prior to exposure to the inhibitory agents in the MFC base medium. This overlay, along with a preincubation at 35 C (for two hours in the experiment by Rose (3) but for five hours in this study), is believed to allow for improved recoveries by this technique.

The objectives of this study were to determine the effects of heavy metal stress on fecal coliforms and to evaluate the efficiency of two methods of enumerating fecal coliform populations. The test organism used was *Escherichia coli* (*E. coli* ATCC strain 9637), and the recovery methods used included the standard fecal coliform membrane filter technique, as recommended by *Standard Methods* (1), and a modification of a two-layer technique as proposed by Rose et al. (3).

Culture of Test Organism

A pure culture of *E. coli* ATCC strain 9637 was obtained and stored on a stock nutrient agar (Difco) slant at 4 C. To obtain a standard inoculum, one loop of the stock culture was transferred to 10 ml of lauryl tryptose broth (Difco) and incubated at 35 C for 17 hr. From this standard inoculum, 0.2 ml was transferred to 50 ml of lactose broth (Difco) and incubated at 35 C for four hours. A growth curve, based on turbidity and cell counts was constructed, and it was determined that a four-hour old culture of *E. coli*, grown in this manner, yielded approximately 10^8 cells/ml. A five-ml sample of this cell suspension was then centrifuged for 25 minutes in a Fisher centrifuge, Model 28158H, washed in 0.1 percent peptone in glass-distilled water, and resuspended to 5 ml in the same peptone water. This procedure was repeated three times.

Reactor and Medium Preparation

Polyethylene, 500-ml screw-capped bottles were used as reactors. They were cleaned to rid them of any residual metal ions by soaking them in an acid bath consisting of equal parts of 1:1 HNO_3 and 1:1 HCl, and then rinsing first with tap water and then three times with glass-distilled water. The reactor medium contained the following inorganic salts: 3.66×10^{-2} M (60 mg/l) $Ca(NO_3)_2$, 7.06×10^{-2} M (60 mg/l) $NaNO_3$, 9.20×10^{-3} M (16 mg/l) K_2HPO_4, 8.80×10^{-3} M (25 mg/l) $Na_2SiO_3 \cdot 9H_2O$, 4.93×10^{-2} M (100 mg/l) $MgCl_2 \cdot 6\ H_2O$, 1.67×10^{-2} M (20 mg/l) $MgSO_4$, and 1.49×10^{-1} M (125 mg/l) $NaHCO_3$ in glass-distilled water. The pH of this medium was adjusted with HCl to obtain a final pH, after autoclaving (121 C for 15 minutes), of approximately 7.0.

Two reactors were used in each experiment, each containing 400 ml of reactor medium. The 10^8 cells/ml cell suspension obtained through the culture preparation procedure was diluted to 10^5 cells/ml using milk dilution bottles containing 99 ml of autoclave-sterilized (121 C for 15 minutes) peptone (0.1 percent, Fisher) in glass-distilled water. Each reactor was inoculated with 0.4 ml of the 10^5 cells/ml, resulting in an approximate cell density in each reactor of 10^4 cells/100 ml. One reactor, designated a "control," was left unchanged; to the other a known quantity of one heavy metal was added. Zinc (Zn), lead (Pb), cadmium (Cd), and Chromium (Cr) were used at the following concentrations: Zn, 12.0 and 1.0 mg/l; Pb, 1.5 and 0.5

mg/l; Cd, 1.0 and 0.1 mg/l; Cr, 1.0 and 0.1 mg/l. Fisher atomic absorption standard metal solutions were used as the metal source. The reactors were incubated at 20 C, and samples were taken from each at 6, 24, and 48 hour intervals following inoculation. Serial dilutions were made of each sample in 160 ml milk dilution bottles containing 99 ml of autoclave-sterilized (121 C for 15 minutes) peptone (0.1 percent, Fisher) dilution water. Dilutions made ranged from 10^0 to 10^{-4}.

Fecal Coliform Enumeration

Two methods of enumeration were used in this study: the standard fecal coliform membrane filter technique (1) and an improved membrane filter technique (3) for the recovery of fecal coliforms. For the standard membrane filter technique, 10 ml of sample was filtered through a Millipore type HC membrane filter (0.7 μm retention) on a multiple funnel, vacuum filtration apparatus that was constructed in the laboratory (see Figure 1). The device consisted of six Gelman magnetic filter funnels (47 mm) mounted on a six-place vacuum manifold that was connected to a Gelman Model 13152 vacuum pump. This apparatus allowed for the filtration of up to six samples simultaneously. The filtering of each sample was followed by a thorough rinse of the apparatus and filter with sterile, glass-distilled water.

Four replicate aliquots from each dilution were filtered. The filters were then transferred to 50 mm Petri dishes containing MFC medium (BBL MFC broth plus 1.5% Difco agar plus 1% MCB rosolic acid in 0.2N NaOH). The Petri plates were then placed in waterproof

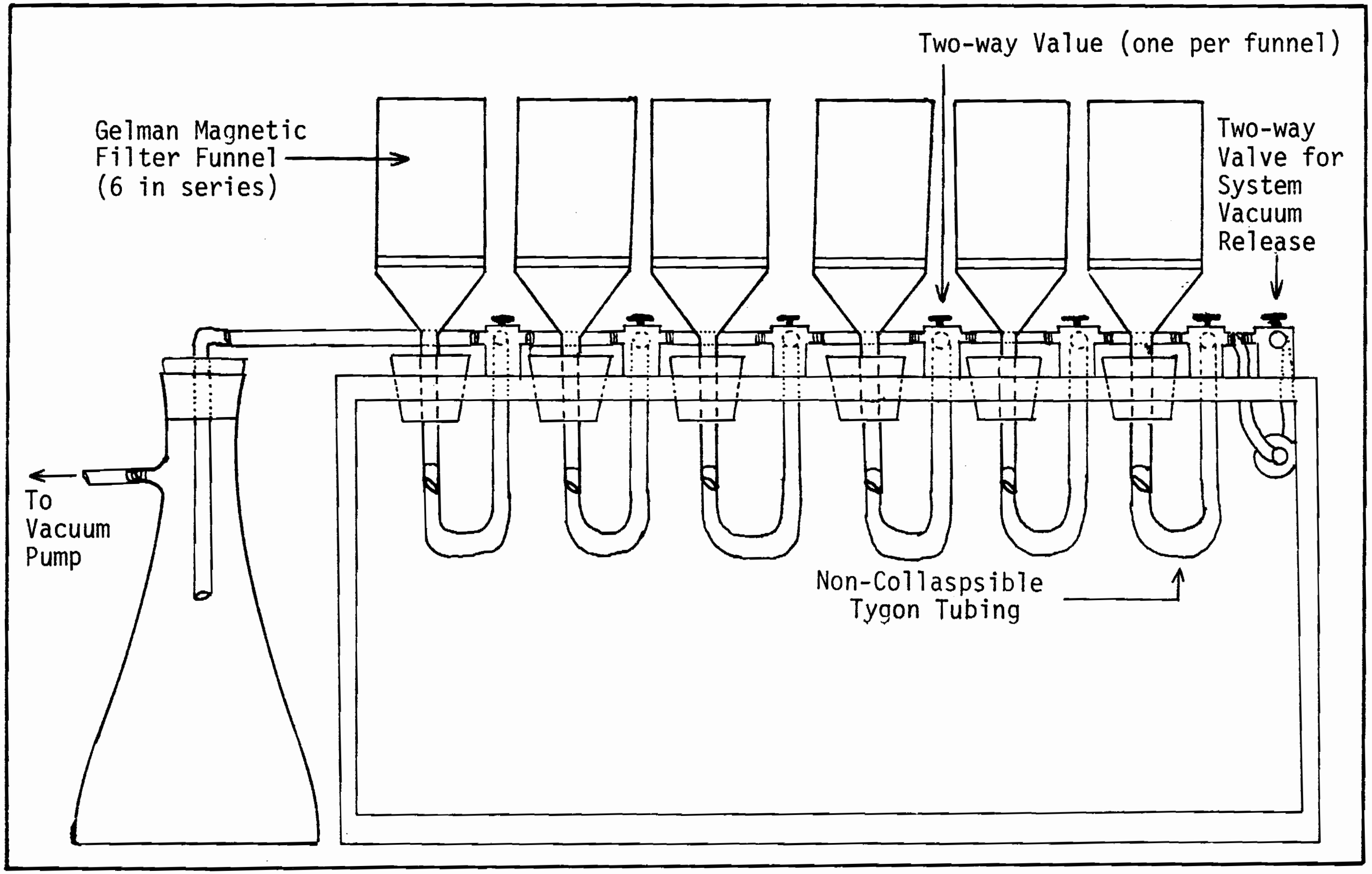

Figure 1. Membrane filter apparatus--after Brown (39)

plastic bags (NASCO) and incubated for 24 hr. in a 44.5 C circulating water bath. Colonies growing in these plates were counted with the aid of a 15-power Baush and Lomb dissecting microscope and fluorescent light source. It is preferable to select plates for counting that have 20 to 60 fecal coliform colonies. The plates with counts closest to this range were counted in each case.

For enumeration using the improved membrane filter technique (3) samples were filtered in the same manner as above. Four replicate filters were then placed in Petri dishes (50 mm) containing 5.0 ml of MFC medium (as before) with an overlay of 2.0 ml laurgl tryptose broth (Difco) plus 1.5 percent agar (Difco). The overlay was added within one hour of the time the filters were placed on the agar. These plates were then placed in waterproof plastic bags (NASCO), incubated at 35 C for five hours, and then transferred to a 44.5 C circulating water bath for 19 hr. The plates with colony numbers closest to the 20 to 60 range were then counted as previously described.

Samples from each reactor were filtered and enumerated using both membrane filter techniques. The control sample (no heavy metal added) was filtered, highest dilutions first, and plates using both techniques. The filter apparatus was then UV sterilized. The sample taken from the reactor containing the heavy metal was then filtered, as above, and plates using both techniques. A control was established to check the sterility of the membrane filter, wash water, and filter apparatus. A sample of sterile wash water was filtered and plates at the beginning and end of each filter run. These "control" plates were incubated along with those of the improved membrane filter technique (3).

Heavy Metal Analysis

After each three day experiment, 50 ml samples were taken from each reactor, placed in clean acid-washed (as before) polyethylene 50 ml containers, and acidified with concentrated HCl. The concentrations of Cd, Cr, Pb, and Zn were then determined in each sample using a Perkin-Elmer Model 403 atomic absorption spectrophotometer.

Data Analysis

Data analysis was done on an IBM 370 computer using the Statistical Analysis System (SAS76). Using this program, the raw data (the number of colonies counted on each Petri plate) was fed into the computer on cards and a ratio was constructed by dividing the recoveries obtained from the reactor containing the metal by the recoveries from the control reactor. These ratios were constructed for each repetition and represent the percent recoveries obtained by each method relative to the control for that method. A four-way procedure ANOVA (analysis of variance) with nesting was performed with the levels (main effects) being method, time, metal, and concentration nested within metals. A procedure MEANS was also done for each main effect and every combination of interactions possible. A three-way procedure ANOVA was also done for each metal separately to eliminate the main effect for metals. The levels used were method, time, and concentration.

IV. RESULTS AND DISCUSSION

General

During a two-month period, a total of fourteen experiments were completed, each involving the use of Cd, Cr, Pb, or Zn at either of two concentrations. Cadmium and Cr were tested for their effects on *E. coli* at both 1.0 and 0.1 mg/l. Zinc was tested at 12.0 and 1.0 mg/l and Pb at 1.5 and 0.5 mg/l. Metal concentrations were chosen to be representative of actual environmental levels, using urban runoff data (Table 1) as a guideline. Lead and Zn were tested at concentrations which closely approximated the maximum and mean levels detected for each in urban runoff. Cadmium and Cr, however, were tested at concentrations somewhat higher than those suggested by the available data concerning urban runoff. The use of Cd and Cr at levels an order of magnitude or more higher than ambient concentrations was needed in order to induce an acute response. A replicate of the experiment involving Cr at 1.0 mg/l was performed using phosphate-buffered dilution water, rather than peptone dilution water, in the washing and preparation of the test organism culture. The use of peptone dilution water introduces a small quanity of organic carbon (1.0 mg/l as peptone) into the reactor medium. It was found, however, that microbial death occurred in less than 48 hr in the absence of carbon, probably due to starvation. The plate counts and average recoveries for all experiments are listed in Table A-I.

Table 1

Concentrations of Pb, Zn, Cr, and Cd Found in Urban Runoff in Northern Virginia

(unpublished data)

	Metal Concentration (mg/l)			
Source	Pb	Zn	Cd	Cr
Max. runoff	1.32	12.6	0.033	0.097
Mean runoff	0.45	1.7	0.0067	0.014
Max. DWS[1]	0.05	5.0[2]	0.01	0.05

[1] Drinking Water Standards (USEPA).

[2] Suggested Limit; No Maximum.

Following each experiment, the exact concentrations in both reactors (the reactor containing the metal and the control reactor) were determined for Cd, Cr, Pb, and Zn by atomic absorption spectrophotometry. These values (Table 2) indicate the heavy metals at concentrations that affected the recovery of the test organism in each situation.

The data were analyzed by computer (IBM 370) using the Statistical Analysis System (SA 576). A ratio (R) was constructed for each data set by dividing the number of colonies recovered from the reactor containing the metal by those recovered from the control reactor. These ratios were constructed for each repetition and represent the percent recoveries obtained by each method relative to the control for that method. A four-way analysis of variance (PROC ANOVA) with nesting was performed on R with the "levels" being method, time, metal, and concentration nested within metals. Levels or main effects are factors (variables) that may influence the results of an experiment (i.e., method, time, metal, concentration). We say that concentration is nested within metals because the concentrations used were unique for each metal.

The effects of each metal on recovery were also examined by performing a three-way PROC ANOVA on R for each metal independently. The levels used were method, time, and concentration. Concentration is no longer nested within metals because metal is not a main effect when using the three-way analysis of variance.

Table 2

Post Experiment Metal Concentrations Determined for Each Reactor Using A Perkin-Elmer 403 Atomic Absorption Spectrophotometer

Source	Metal Concentration (mg/l)			
	Cd	Cr	Zn	Pb
Control Pb 0.5 ppm	0	0	0.02	0
Pb 0.5 ppm	0	0	0.05	0.53
Control Pb 1.5 ppm	0	0	0.05	0
Pb 1.5 ppm	0	0	0.02	1.22
Control Cd 0.1 ppm	0	0	0.07	0
Cd 0.1 ppm	0.11	0.02	0.02	0
Control Cd 1.0 ppm	0	0.01	0.01	0
Cd 1.0 ppm	0.92	0	0.01	0
Control Zn 12.0 ppm	0	0	0.02	0
Zn 12.0 ppm	0	0	12.2	0
Control Zn 1.0 ppm	0	0.01	0.02	0
Zn 1.0 ppm	0	0.01	0.94	0
Control Cr 1.0 ppm	0	0.01	0.03	0
Cr 1.0 ppm	0	1.16	0.04	0
Control Cr 1.0 ppm	0	0.01	0.04	0
Cr 1.0 ppm	0	1.16	0.06	0
Control Cr 0.1 ppm	0	0.01	0.02	0
Cr 0.1 ppm	0	0.11	0.04	0

A PROC MEANS analysis was also performed on R using the same SAS 76 program. This analysis computes the means of two or more groups relative to one or more variables. In this analysis the main effect method was held constant and average recoveries (R) were computed for each method (all other main effects were pooled). This PROC MEANS analysis was performed on each main effect and every combination of main effects, generating a large amount of data (Tables B I-XIV). These data are commonly referred to as "descriptive statistics" and are useful in determining trends and understanding the interaction between main effects.

Throughout this experiment no die-away was ever observed in the control reactors (i.e., growth was always evident over the entire 48 hr test period). In many cases (Figure 2) growth was also evident in the metal reactor. Die-away, however, was observed with the higher concentrations of some metals. The difference between the number of organisms recovered from control and metal reactors was the parameter being measured in each case. The ratio R (percent recovery) was chosen as the unit to be analyzed because it represented the difference in recoveries being examined.

The following sections present the interpretation of the statistical analyses performed on the data.

Discussion of Statistical Results

This experiment was designed to determine the effect of method, time, metal, and concentration of metal on the recovery of

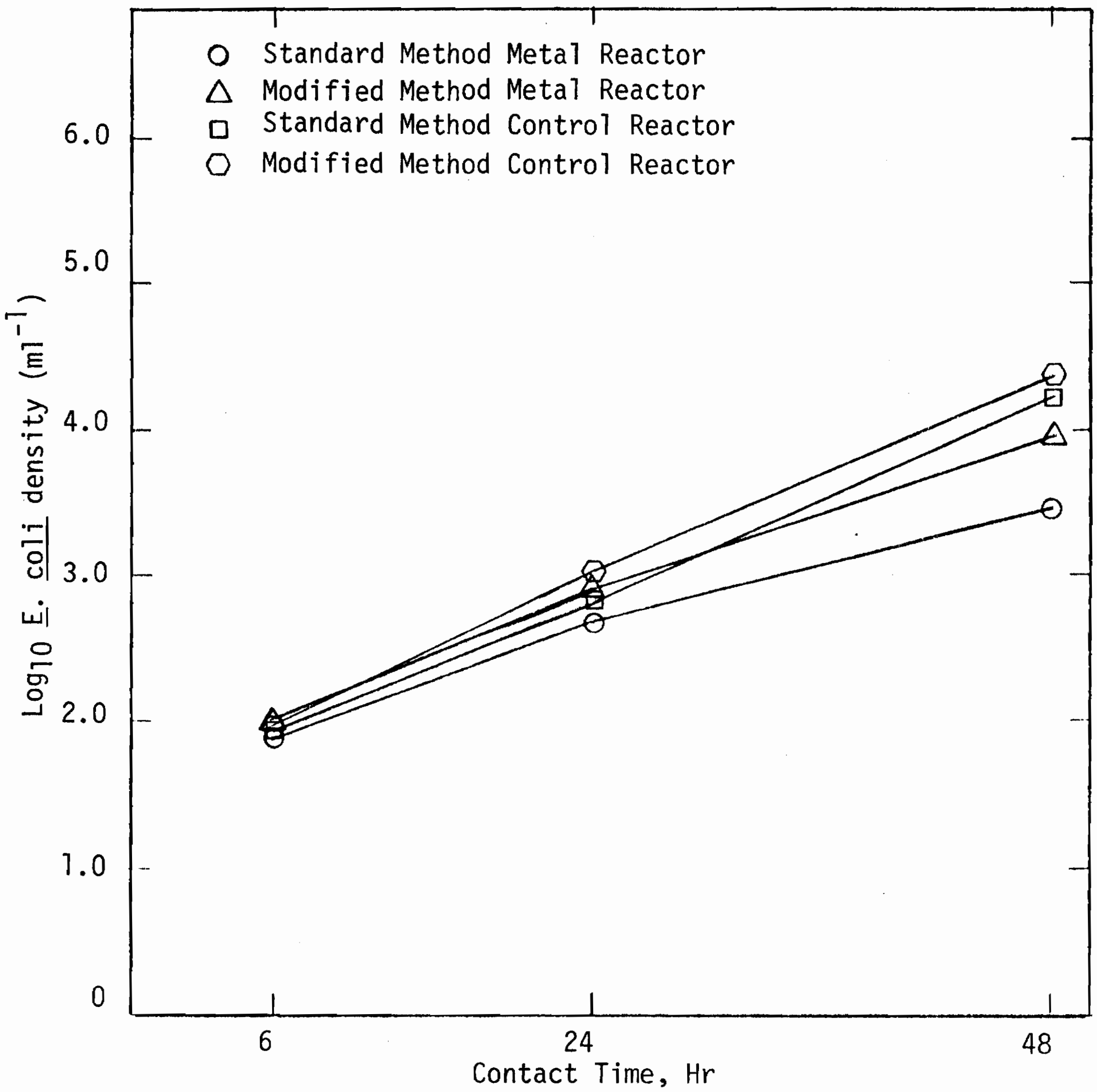

Figure 2. Log of *E. coli* recovered from each reactor as a function of time of contact with 1.5 mg/l Pb.

E. coli, a fecal coliform. Two recovery methods (standard (1) and modified (3) membrane filter techniques), three times (6, 24, and 48 hr), four metals (Cd, Cr, Pb, and Zn), and two unique concentrations of each metal were tested. The four-way analysis of variance on R (Table C-I) revealed all levels to be highly significant ($\alpha < 0.01$), indicating that each of the variables had an effect on the recovery of the test organism.

In several, the modified method (M-MF) was found (by PROC MEANS on method) to yield higher recoveries than the standard method (S-MF). The M-MF recovered 64 percent of the test organisms as compared to 50 percent for the S-MF. However, as Figure 3 indicates, the superior recoveries, obtained with M-MF, were most obvious when the organisms were in contact with the metal for longer times. At 48 hr the M-MF recovered 61 percent as compared to 26 for the S-MF. The interactions between each method and the detention times, metals, and concentration of metals were found significant using the four-way ANOVA model. In general, as concentrations and contact times increased, the recovery efficiencies decreased for each method. It was also noted that as concentration (metals pooled) increased, the difference between the recovery efficiency by each method became smaller (Figure 4). The superiority of the M-MF at the lower concentration is obvious (96 percent by M-MF, 70 percent by S-MF), while at the higher concentrations the percent recoveries of the two methods are very similar (35 percent by M-MF, 30 percent by S-MF).

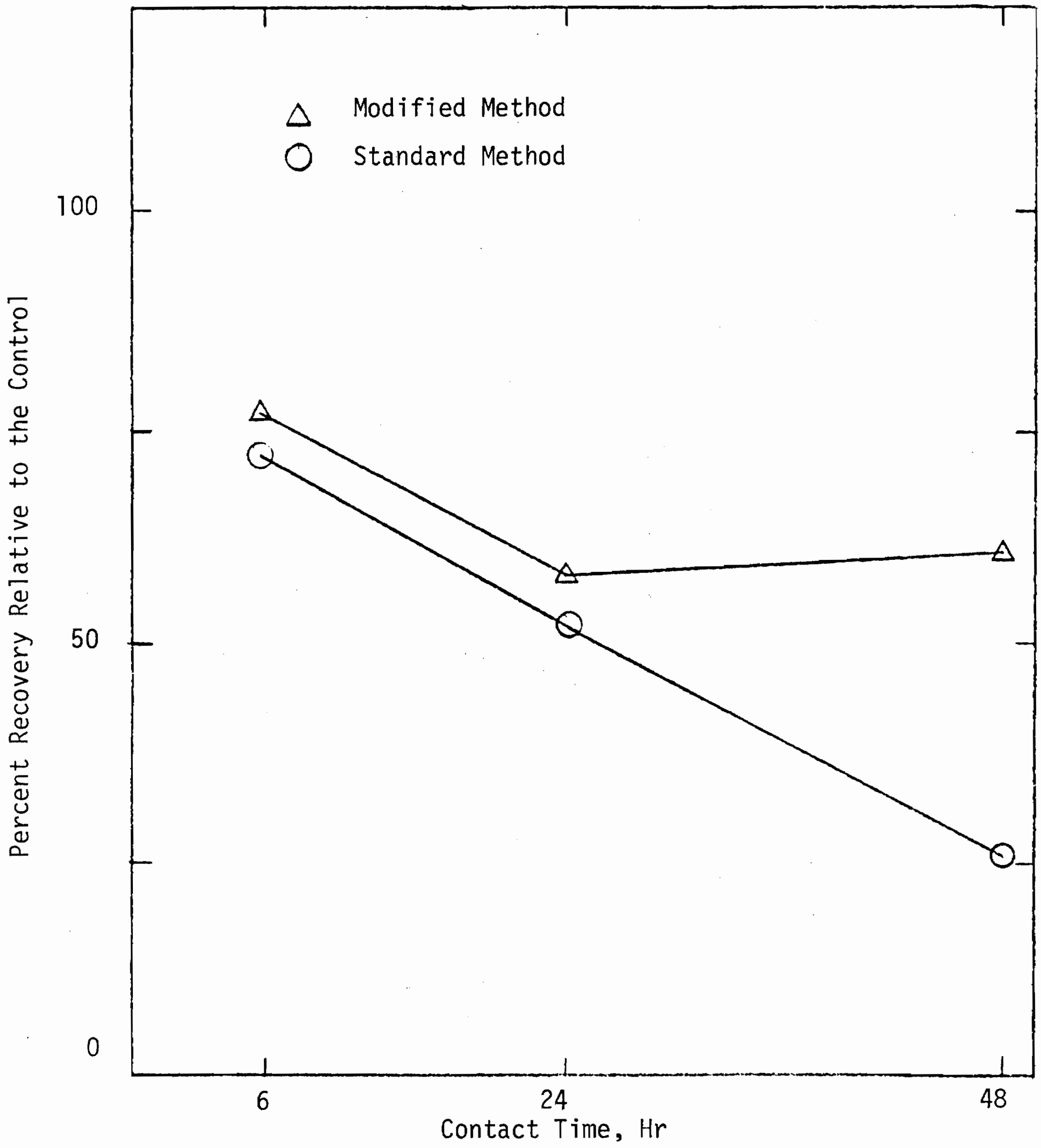

Figure 3. Average *E. coli* recoveries by each membrane filter method as a function of time with metal.

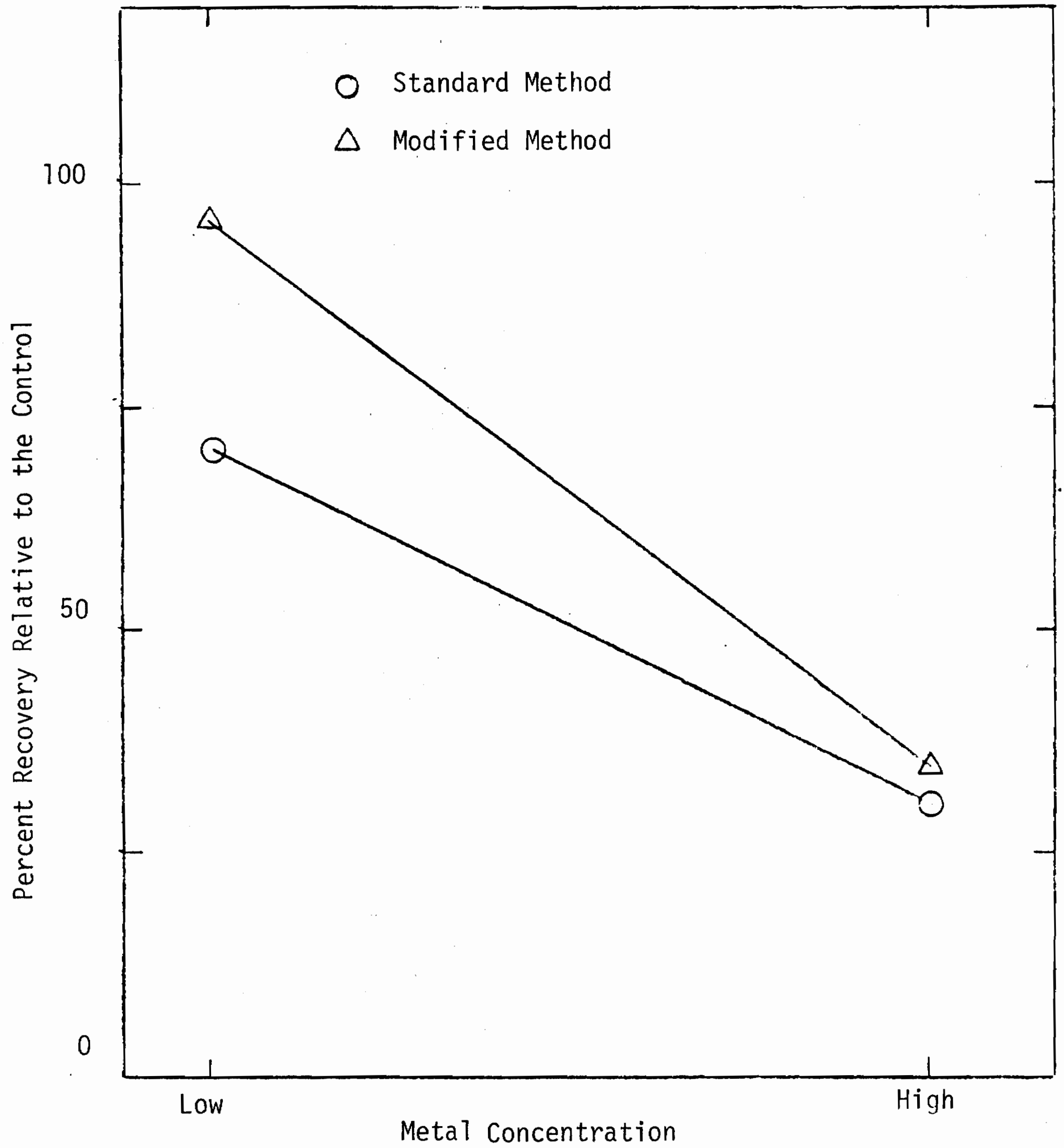

Figure 4. Average percent recovery by each membrane filter method of *E. coli* as a function of concentration of metal.

Time was found to have cumulative effect on recovery; that is, as time increased the recovery efficiency (all methods pooled) decreased. The percent recoveries after 6, 24, and 48 hr were 74, 55, and 44 percent, respectively. Both the three-way and four-way ANOVA tests also revealed that time was a highly significant factor on the recovery of fecal coliforms. Times significance is important from an environmental monitoring stand point. Indicator organisms released into a low nutrient aquatic environment or deposited in the terrestrial system (subject to contamination of stormwater runoff) may be stressed by various factors. Under such unfavorable conditions the recovery efficiency using the two methods studied would be expected to decrease with increasing time.

The differences in the recoveries, as functions of the particular metals were found to be highly significant. In general, the average recovery obtained using Cr (contact times, concentration, and methods pooled) was 73 percent; Pb, 72 percent; Cd, 28 percent; and Zn, 0 percent (death in less than 48 hr).

The recoveries observed in experiments involving different metals depended largely on the concentration of the specific metal being tested; therefore, in order to rank the metals according to their relative toxicities in a way that would have "environmental" significance, one should consider the concentrations that are similar to those known to occur under some "natural" conditions. In this study, "natural" was considered to be the maximum and average

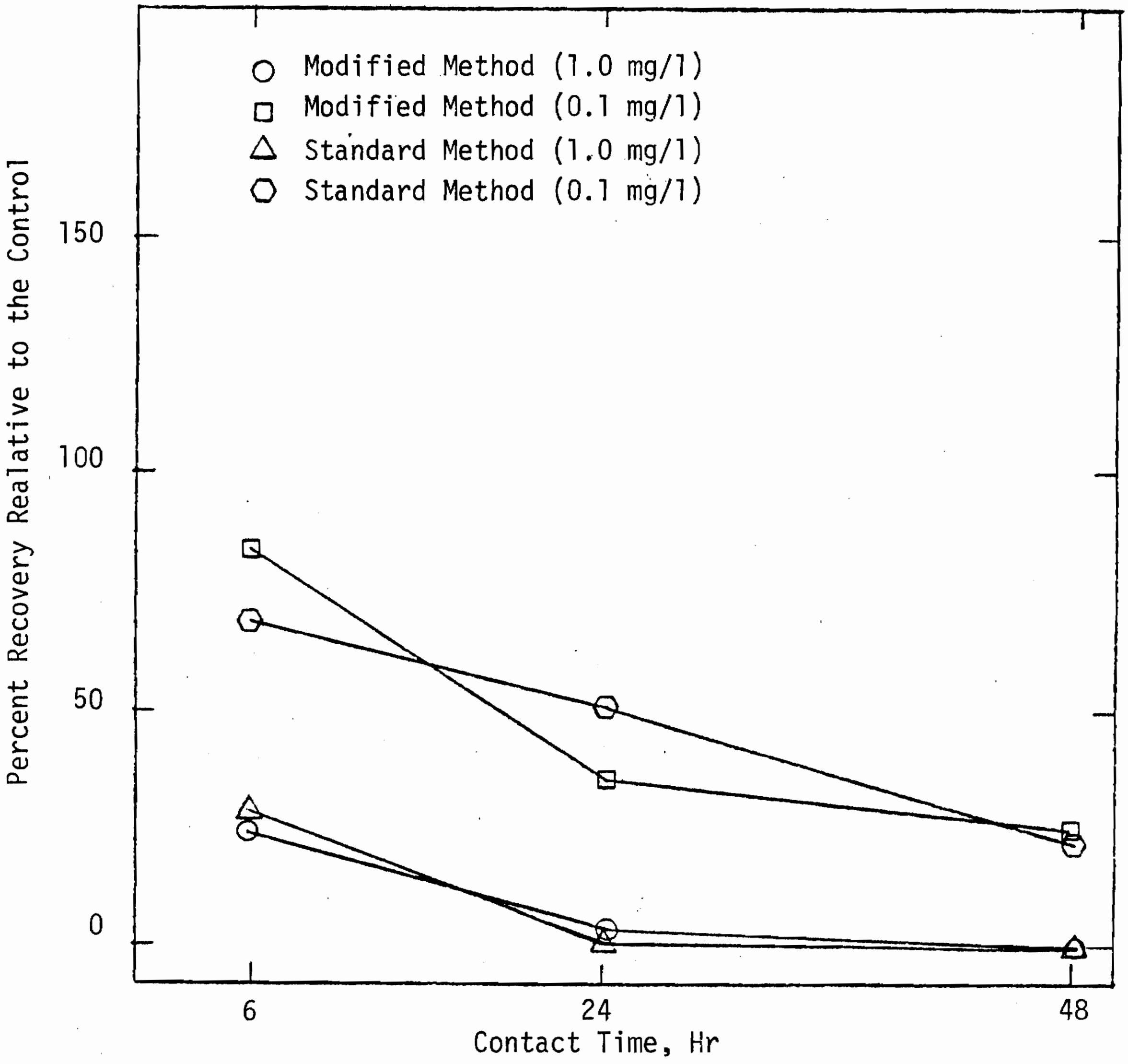

Figure 5. Percent recovery of E. coli by each membrane filter method as a function of time of exposure to Cd at two concentrations.

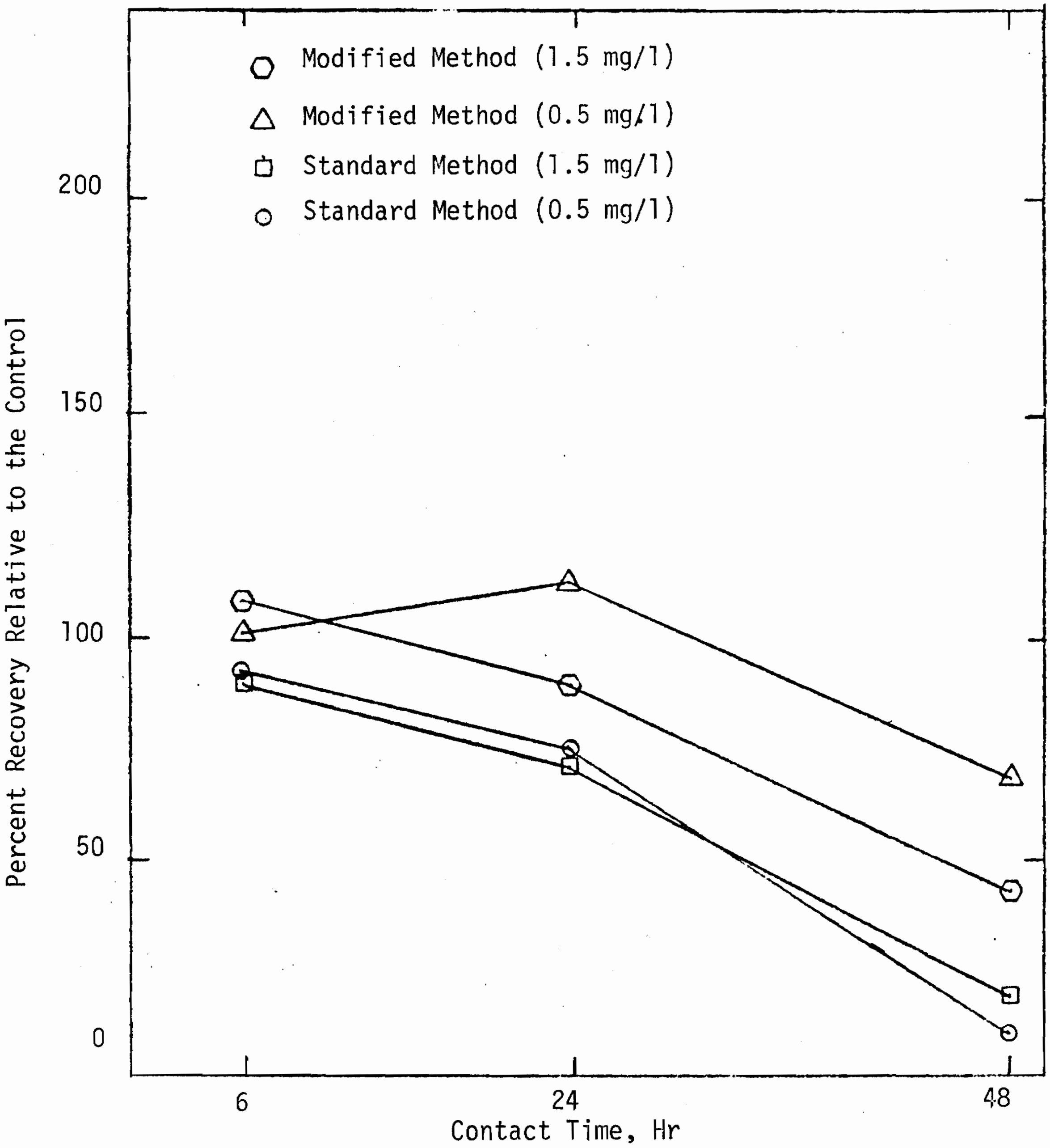

Figure 6. Percent recovery of *E. coli* by each membrane filter method as a function of time of exposure to Pb at two concentrations.

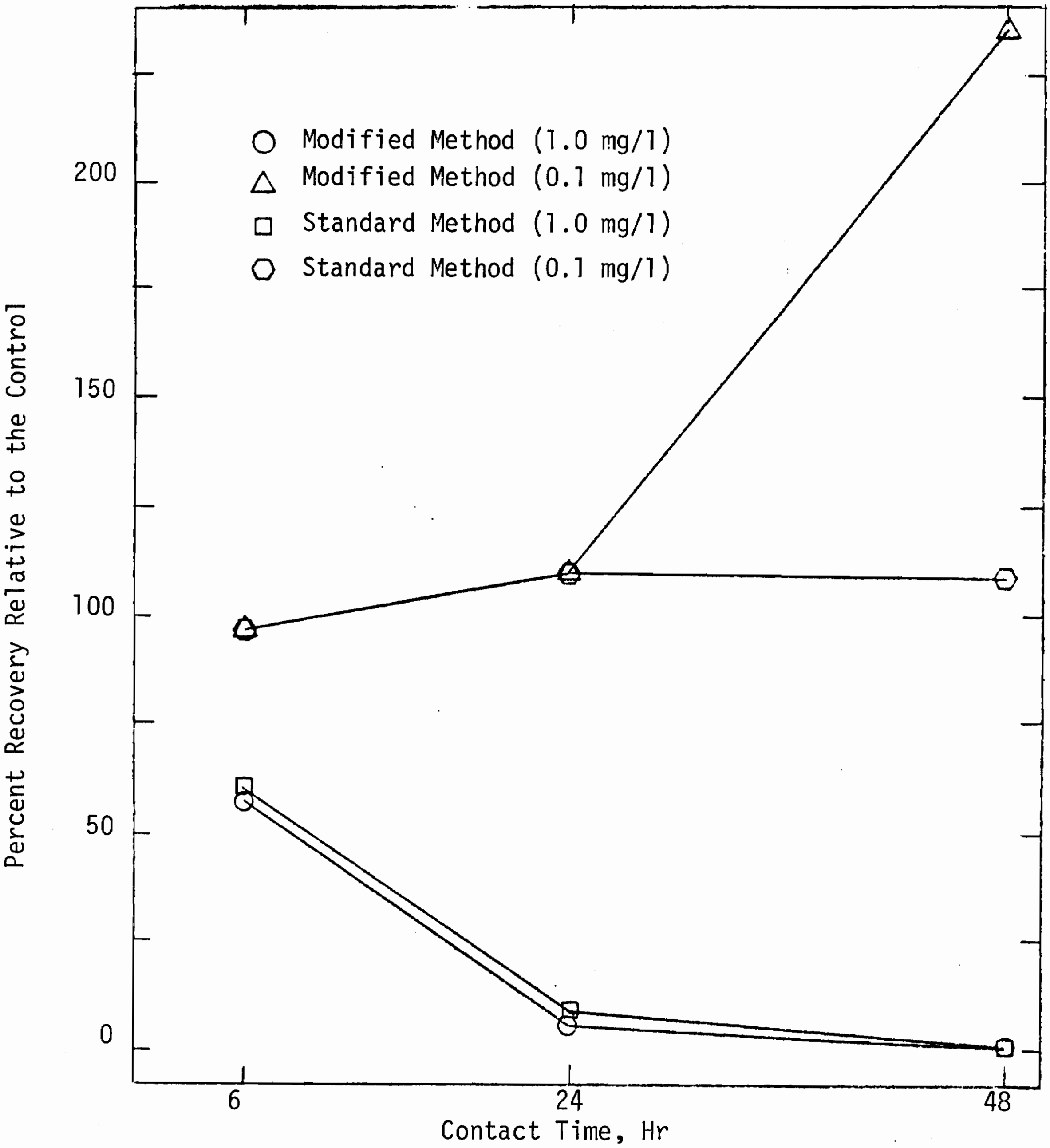

Figure 7. Percent recovery of E. coli by two membrane filter methods as a function of time of exposure to Cr at two concentrations.

concentrations observed in runoff from areas in northern Virginia. When concentration of each metal was fixed at the observed naturally occurring maximum (northern Virginia data), the ranking is as follows: Cr, 125 percent recovery; Pb, 68 percent; Cd, 47 percent; and Zn, 0 percent (death in less than 48 hr). Chromium seemed to be somewhat stimulatory at this concentration (0.1 mg/l) while Zn was found to be the most toxic under the conditions of the study. No explanation can be given for the stimulatory effects of Cr because it is normally toxic to organisms in higher trophic levels.

Metals Analysis

Following each experiment the medium in each reactor was analyzed for Cr, Cd, Pb, and Zn using the atomic absorption (AA) technique (Table 2). It was intended for each metal reactor to contain only one heavy metal at a known concentration and control reactors were to be free of all heavy metals. The results of the AA analysis however, indicated Cr and Zn contamination in the 10 μg/l range was found in almost all reactors, including the controls (these contaminant concentrations determined were well within the detection limits for each metal). In all cases but one, the contamination detected was between one and ten percent (as Cr and Zn) of the total metal concentration. In the case of Cr at 0.1 mg/l the Zn contamination was found to be 27 percent of the total metal concentration. While an explanation is not available, it is interesting to note that the Cr, 0.1 mg/l, experiment with the high Zn contamination, found this combination of metals to be stimulatory to the growth of *E. coli*.

This discovery of contamination changed the perspective from which the findings were evaluated. This study was originally designed to determine the effect on recovery of a single metal at a known concentration. The presence of two or more metals in any one reactor raised additional questions, such as whether there were synergistic affects by the metals on *E. coli*. These affects cannot be estimated, however, the occurrence of "contamination" was uniform and relatively low throughout the study.

V. SUMMARY AND CONCLUSIONS

This experiment was designed to evaluate the efficiency of two membrane filter techniques for the recovery of *E. coli* and determine the effects on recovery of the heavy metals Cd, Cr, Pb, and Zn, at various concentration. The experiment was conducted in fourteen parts with only one metal at one concentration being examined in each part. During each experiment, the recoveries obtained for each method, metal, and concentration were determined for these contact times (6, 24, and 48 hr). Ultimately, the data collected during this study revealed the effects of recovery method, contact time, four heavy metals, and various concentrations of each metal on the enumeration of *E. coli*.

From the results of this study the following conclusions may be derived:

1. The heavy metals examined were all found to have an effect on the recovery of the test organism. Each metal was examined at a concentration similar to the maximum concentration found for that metal in urban runoff. On this basis the metals may be ranked in order of increasing toxicity to *E. coli* as follows: Cr, Pb, Cd, and Zn.

2. The modified membrane filter method was found to be superior to the standard membrane filter method for the recovery of the metal stressed, *E. coli* used in this study.

3. The concentration of each metal as well as the contact time for which the *E. coli* was exposed to the metal were both found to significantly effect the recovery of *E. coli*.

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APPENDICES

Key:

M = Modified Method of Recovery

S = Standard Method of Recovery

H = High Concentration of Metal

L = Low Concentration of Metal

X = Number of organisms recovered from the reactor containing a heavy metal

Y = Number of organisms recovered from the control reactor

R = X/Y; percent recovery of organisms relative to the control

APPENDIX A

TEST ORGANISM RECOVERIES

TABLE A-I
PLATE COUNTS AND AVERAGE RECOVERY PER PLATE

OBS	METHOD	TIME	METAL	CONC	X	Y	R
1	M	6	CD	H	40.0	260	0.153846
2	M	6	CD	H	44.0	170	0.258824
3	M	6	CD	H	47.0	190	0.247368
4	M	6	CD	H	35.0	130	0.269231
5	M	6	CD	L	106.0	114	0.929825
6	M	6	CD	L	86.0	109	0.788991
7	M	6	CD	L	100.0	117	0.854701
8	M	6	CD	L	103.0	135	0.762963
9	M	24	CD	H	6.1	1700	0.003588
10	M	24	CD	H	5.1	2500	0.002040
11	M	24	CD	H	5.5	2000	0.002750
12	M	24	CD	H	6.3	1400	0.004500
13	M	24	CD	L	800.0	1900	0.421053
14	M	24	CD	L	790.0	2800	0.282143
15	M	24	CD	L	680.0	2300	0.295652
16	M	24	CD	L	600.0	1500	0.400000
17	M	48	CD	H	1.8	75000	0.000024
18	M	48	CD	H	1.5	66000	0.000023
19	M	48	CD	H	1.4	68000	0.000021
20	M	48	CD	H	1.7	76000	0.000022
21	M	48	CD	L	25000.0	101000	0.247525
22	M	48	CD	L	31000.0	111000	0.279279
23	M	48	CD	L	23000.0	105000	0.219048
24	M	48	CD	L	27000.0	104000	0.259615
25	S	6	CD	H	36.0	115	0.313043
26	S	6	CD	H	28.0	115	0.243478
27	S	6	CD	H	41.0	124	0.330645
28	S	6	CD	H	32.0	137	0.233577
29	S	6	CD	L	90.0	105	0.857143
30	S	6	CD	L	83.0	116	0.715517
31	S	6	CD	L	77.0	166	0.463855
32	S	6	CD	L	78.0	115	0.678261
33	S	24	CD	H	2.4	1500	0.001600
34	S	24	CD	H	1.6	1900	0.000842
35	S	24	CD	H	3.1	1600	0.001937
36	S	24	CD	H	2.6	2200	0.001182
37	S	24	CD	L	640.0	1100	0.581818
38	S	24	CD	L	670.0	1400	0.478571
39	S	24	CD	L	650.0	1100	0.590909
40	S	24	CD	L	730.0	2000	0.365000
41	S	48	CD	H	0.6	57000	0.000011
42	S	48	CD	H	0.3	63000	0.000005

TABLE A-I
PLATE COUNTS AND AVERAGE RECOVERY PER PLATE

OBS	METHOD	TIME	METAL	CONC	X	Y	R
43	S	48	CD	H	0.0	66000	0.00000
44	S	48	CD	H	0.3	65000	0.00000
45	S	48	CD	L	20000.0	91000	0.21978
46	S	48	CD	L	15000.0	86000	0.17442
47	S	48	CD	L	20000.0	86000	0.23256
48	S	48	CD	L	21000.0	93000	0.22581
49	M	6	CR	H	111.0	200	0.55500
50	M	6	CR	H	124.0	160	0.77500
51	M	6	CR	H	110.0	250	0.44000
52	M	6	CR	H	122.0	240	0.50833
53	M	6	CR	L	86.0	94	0.91489
54	M	6	CR	L	99.0	91	1.08791
55	M	6	CR	L	95.0	90	1.05556
56	M	6	CR	L	70.0	88	0.79545
57	M	24	CR	H	180.0	4200	0.04286
58	M	24	CR	H	190.0	4700	0.04043
59	M	24	CR	H	300.0	5700	0.05263
60	M	24	CR	H	270.0	5500	0.04909
61	M	24	CR	L	530.0	490	1.08163
62	M	24	CR	L	450.0	580	0.77586
63	M	24	CR	L	560.0	450	1.24444
64	M	24	CR	L	680.0	560	1.21429
65	M	48	CR	H	96.0	470000	0.00020
66	M	48	CR	H	92.0	500000	0.00018
67	M	48	CR	H	117.0	520000	0.00022
68	M	48	CR	H	93.0	440000	0.00021
69	M	48	CR	L	17000.0	7500	2.26667
70	M	48	CR	L	16000.0	7000	2.28571
71	M	48	CR	L	12000.0	6800	1.76471
72	M	48	CR	L	22000.0	7200	3.05556
73	S	6	CR	H	110.0	210	0.52381
74	S	6	CR	H	94.0	180	0.52222
75	S	6	CR	H	127.0	190	0.66842
76	S	6	CR	H	96.0	140	0.68571
77	S	6	CR	L	71.0	82	0.86585
78	S	6	CR	L	82.0	73	1.12329
79	S	6	CR	L	76.0	72	1.05556
80	S	6	CR	L	61.0	76	0.80263
81	S	24	CR	H	126.0	4200	0.03000
82	S	24	CR	H	111.0	4200	0.02643
83	S	24	CR	H	920.0	3700	0.24865
84	S	24	CR	H	104.0	4400	0.02364

TABLE A-I
PLATE COUNTS AND AVERAGE RECOVERY PER PLATE

OBS	METHOD	TIME	METAL	CONC	X	Y	R
85	S	24	CR	L	560	520	1.07692
86	S	24	CR	L	510	530	0.96226
87	S	24	CR	L	570	440	1.29545
88	S	24	CR	L	480	500	0.96000
89	S	48	CR	H	42	430000	0.00010
90	S	48	CR	H	48	330000	0.00015
91	S	48	CR	H	35	370000	0.00009
92	S	48	CR	H	34	340000	0.00010
93	S	48	CR	L	8800	6700	1.31343
94	S	48	CR	L	8100	6900	1.17391
95	S	48	CR	L	7400	6600	1.12121
96	S	48	CR	L	5900	7800	0.75641
97	M	6	PB	H	94	92	1.02174
98	M	6	PB	H	98	100	0.98000
99	M	6	PB	H	97	97	1.00000
100	M	6	PB	H	99	96	1.03125
101	M	6	PB	L	79	80	0.98750
102	M	6	PB	L	80	82	0.97561
103	M	6	PB	L	74	72	1.02778
104	M	6	PB	L	73	73	1.00000
105	M	24	PB	H	710	870	0.81609
106	M	24	PB	H	790	850	0.92941
107	M	24	PB	H	770	840	0.91667
108	M	24	PB	H	740	840	0.88095
109	M	24	PB	L	530	430	1.23256
110	M	24	PB	L	710	720	0.98611
111	M	24	PB	L	660	510	1.29412
112	M	24	PB	L	620	640	0.96875
113	M	48	PB	H	8500	28000	0.30357
114	M	48	PB	H	8100	25000	0.32400
115	M	48	PB	H	9500	18000	0.52778
116	M	48	PB	H	7000	14000	0.50000
117	M	48	PB	L	7400	12500	0.59200
118	M	48	PB	L	8300	12100	0.68595
119	M	48	PB	L	7600	10400	0.73077
120	M	48	PB	L	7700	11400	0.67544
121	S	6	PB	H	67	90	0.74444
122	S	6	PB	H	69	82	0.84146
123	S	6	PB	H	75	91	0.82418
124	S	6	PB	H	84	74	1.13514
125	S	6	PB	L	74	63	1.17460
126	S	6	PB	L	56	59	0.94915

TABLE A-I
PLATE COUNTS AND AVERAGE RECOVERY PER PLATE

OBS	METHOD	TIME	METAL	CONC	X	Y	R
127	S	6	PB	L	54.0	67	0.80597
128	S	6	PB	L	47.0	66	0.71212
129	S	24	PB	H	520.0	560	0.92857
130	S	24	PB	H	420.0	630	0.66667
131	S	24	PB	H	490.0	720	0.68056
132	S	24	PB	H	390.0	740	0.52703
133	S	24	PB	L	330.0	440	0.75000
134	S	24	PB	L	330.0	570	0.57895
135	S	24	PB	L	500.0	490	1.02041
136	S	24	PB	L	290.0	460	0.63043
137	S	48	PB	H	2900.0	21000	0.13810
138	S	48	PB	H	2600.0	17000	0.15294
139	S	48	PB	H	2800.0	15000	0.18667
140	S	48	PB	H	2500.0	11000	0.22727
141	S	48	PB	L	800.0	10200	0.07843
142	S	48	PB	L	900.0	7600	0.11842
143	S	48	PB	L	600.0	8900	0.06742
144	S	48	PB	L	900.0	8800	0.10227
145	M	6	ZN	H	0.0	380	0.00000
146	M	6	ZN	H	0.0	180	0.00000
147	M	6	ZN	H	0.0	320	0.00000
148	M	6	ZN	H	0.0	270	0.00000
149	M	6	ZN	L	8.7	30000	0.00029
150	M	6	ZN	L	9.4	30000	0.00031
151	M	6	ZN	L	11.8	30000	0.00039
152	M	6	ZN	L	8.4	30000	0.00028
153	M	24	ZN	L	0.0	280000	0.00000
154	M	24	ZN	L	0.0	310000	0.00000
155	M	24	ZN	L	0.0	360000	0.00000
156	M	24	ZN	L	0.0	290000	0.00000
157	S	6	ZN	H	0.0	170	0.00000
158	S	6	ZN	H	0.0	190	0.00000
159	S	6	ZN	H	0.0	230	0.00000
160	S	6	ZN	H	0.0	220	0.00000
161	S	6	ZN	L	4.3	30000	0.00014
162	S	6	ZN	L	4.0	30000	0.00013
163	S	6	ZN	L	3.1	30000	0.00010
164	S	6	ZN	L	4.4	30000	0.00015
165	S	24	ZN	L	0.0	230000	0.00000
166	S	24	ZN	L	0.0	230000	0.00000
167	S	24	ZN	L	0.0	230000	0.00000
168	S	24	ZN	L	0.0	270000	0.00000

APPENDIX B

RESULTS OF STATISTICAL ANALYSIS BY PROCEDURE MEANS

```
                              TABLE B-I
                    MEANS GROUPED BY CONCENTRATION
                                CONC=H

VARIABLE       N          MEAN     STANDARD       MINIMUM        MAXIMUM
                                   DEVIATION       VALUE          VALUE

R             72       0.32703        0.358    0.0000000          1.135
X             72     741.78194     1954.396    0.0000000       9500.000
Y             72   57583.09722   134366.059   74.0000000     520000.000

----------------------------- CONC=L -----------------------------

R             72       0.82665       0.5171    0.0674157          3.056
X             72    4571.80556    7928.0570   47.0000000      31000.000
Y             72   13056.04167   30281.1573   59.0000000     111000.000
```

```
                                TABLE B-II
                          MEANS GROUPED BY TIME
                                 TIME=6

VARIABLE                N        MEAN     STANDARD       MINIMUM       MAXIMUM
                                          DEVIATION        VALUE         VALUE

R                      48    0.7435803     0.2846396     0.1538462      1.174603
X                      48   78.7500000    25.4462303    28.0000000    127.000000
Y                      48  119.5416667    51.7140083    59.0000000    260.000000

------------------------------ TIME=24 -----------------------------------------

R                      48     0.549280       0.44388      0.000842       1.29545
X                      48   436.118750     274.47403      1.600000     920.00000
Y                      48  1664.166667    1478.16855    430.000000    5700.00000

------------------------------ TIME=48 -----------------------------------------

R                      48       0.4377         0.679       0.00000         3.056
X                      48    7455.5125      8748.608       0.00000     31000.000
Y                      48  104175.0000    151363.504    6600.00000    520000.000
```

```
                                   B-III
                           MEANS GROUPED BY METAL
                                METAL=CD

VARIABLE       N        MEAN        STANDARD        MINIMUM        MAXIMUM
                                    DEVIATION        VALUE          VALUE

R             48       0.27902        0.2738      0.000000         0.930
X             48    3929.71458     8718.6899      0.000000     31000.000
Y             48   28002.45833    39862.1171    105.000000    111000.000

-------------------------- METAL=CR --------------------------

R             48       0.73423         0.681     0.0000946         3.056
X             48    2204.83333      5002.091    34.0000000     22000.000
Y             48   72904.29167    161551.769    72.0000000    520000.000

-------------------------- METAL=PB --------------------------

R             48      0.717276       0.33768     0.0674157        1.2941
X             48   1835.833333    2892.68237    47.0000000     9500.0000
Y             48   5051.958333    7503.58257    59.0000000    28000.0000
```

```
                              TABLE B-IV
                       MEANS GROUPED BY METHOD
                              METHOD=M

VARIABLE        N          MEAN       STANDARD       MINIMUM        MAXIMUM
                                      DEVIATION       VALUE          VALUE

R              72       0.65386          0.588     0.0000206          3.056
X              72    3488.32500       7186.028     1.4000000      31000.000
Y              72   39486.25000     111973.319    72.0000000     520000.000

--------------------------- METHOD=S ---------------------------

R              72       0.49983         0.4046     0.0000000          1.313
X              72    1825.26250      4590.2326     0.0000000      21000.000
Y              72   31152.88889     86042.0965    59.0000000     430000.000
```

```
                                  TABLE B-V
                   MEANS GROUPED BY CONCENTRATION AND TIME
                               CONC=H    TIME=6

VARIABLE          N          MEAN       STANDARD        MINIMUM        MAXIMUM
                                        DEVIATION       VALUE          VALUE

R                24     0.5961134     0.3050081      0.1538462       1.135135
X                24    78.3333333    32.9896778     28.0000000     127.000000
Y                24   147.2083333    56.5600713     74.0000000     260.000000

----------------------- CONC=H    TIME=24 -----------------------

R                24      0.286588       0.37851       0.000842        0.92941
X                24    294.320833     305.43460       1.600000      920.00000
Y                24   2393.750000    1697.90360     560.000000     5700.00000

----------------------- CONC=H    TIME=48 -----------------------

R                24        0.0984         0.164         0.0000          0.528
X                24     1852.6917      3121.018         0.0000       9500.000
Y                24   170208.3333    189402.259     11000.0000     520000.000

----------------------- CONC=L    TIME=6 ------------------------

R                24    0.89104727     0.1647667      0.4638554       1.174603
X                24   79.16666667    15.3131562     47.0000000     106.000000
Y                24   91.87500000    25.8546312     59.0000000     166.000000

----------------------- CONC=L    TIME=24 -----------------------

R                24     0.8119725      0.339616       0.282143        1.29545
X                24   577.9166667    136.731383     290.000000      800.00000
Y                24   934.5833333    686.452628     430.000000     2800.00000

----------------------- CONC=L    TIME=48 -----------------------

R                24       0.77693        0.8221        0.06742          3.056
X                24   13058.33333     9008.3496      600.00000      31000.000
Y                24   38141.66667    42950.7191     6600.00000     111000.000
```

```
                              TABLE B-VI
                MEANS GROUPED BY CONCENTRATION AND METAL
                         CONC=H    METAL=CD

VARIABLE      N       MEAN      STANDARD     MINIMUM      MAXIMUM
                                DEVIATION     VALUE        VALUE

R            24     0.08619      0.1264      0.000000       0.3306
X            24    14.30417     17.4619      0.000000      47.0000
Y            24 23001.70833  31971.9198    115.000000   76000.0000

---------------------- CONC=H    METAL=CR -----------------------

R            24      0.2164       0.278      0.000095        0.775
X            24    152.1667     175.515     34.000000      920.000
Y            24 143257.0833  207346.477    140.000000   520000.000

---------------------- CONC=H    METAL=PB -----------------------

R            24    0.678520     0.31602     0.1380952       1.1351
X            24 2058.875000  3005.80663    67.0000000    9500.0000
Y            24 6490.500000  9322.00437    74.0000000   28000.0000

---------------------- CONC=L    METAL=CD -----------------------

R            24     0.47185      0.2441      0.174419        0.930
X            24  7845.12500  11105.9384     77.000000    31000.000
Y            24 33003.20833  46611.9083    105.000000   111000.000

---------------------- CONC=L    METAL=CR -----------------------

R            24    1.252068     0.55713     0.7564103       3.0556
X            24 4257.500000  6504.27764    61.0000000   22000.0000
Y            24 2551.500000  3271.28362    72.0000000    7800.0000

---------------------- CONC=L    METAL=PB -----------------------

R            24    0.756032     0.36057     0.0674157       1.2941
X            24 1612.791667  2821.40696    47.0000000    8300.0000
Y            24 3613.416667  4882.31241    59.0000000   12500.0000
```

```
                               TABLE B-VII
                  MEANS GROUPED BY CONCENTRATION AND METHOD
                          CONC=H     METHOD=M

VARIABLE        N          MEAN      STANDARD       MINIMUM       MAXIMUM
                                     DEVIATION       VALUE         VALUE

R              36       0.35105         0.382    0.0000206         1.031
X              36    1069.40000      2610.853    1.4000000      9500.000
Y              36   64807.91667    151763.769   92.0000000    520000.000

---------------------- CONC=H     METHOD=S -----------------------

R              36       0.30302         0.336    0.0000000         1.135
X              36     414.16389       843.272    0.0000000      2900.000
Y              36   50358.27778    116123.600   74.0000000    430000.000

---------------------- CONC=L     METHOD=M -----------------------

R              36       0.95667        0.6068    0.2190476         3.056
X              36    5907.25000     9268.2299   70.0000000     31000.000
Y              36   14164.58333    32909.3143   72.0000000    111000.000

---------------------- CONC=L     METHOD=S -----------------------

R              36       0.69663        0.3735    0.0674157        1.3134
X              36    3236.36111     6159.1549   47.0000000    21000.0000
Y              36   11947.50000    27830.6411   59.0000000    93000.0000
```

```
                              TABLE B-VIII
                    MEANS GROUPED BY TIME AND METAL
                          TIME=6    METAL=CD

VARIABLE       N        MEAN      STANDARD       MINIMUM       MAXIMUM
                                  DEVIATION       VALUE         VALUE

R             16    0.5063293    0.2787056     0.153846      0.929825
X             16   64.1250000   28.5257194    28.000000    106.000000
Y             16  138.6250000   40.5608596   105.000000    260.000000

---------------------- TIME=6    METAL=CR ----------------------

R             16    0.7737278    0.2285727    0.4400000      1.123288
X             16   95.8750000   20.4511613   61.0000000    127.000000
Y             16  139.7500000   63.9755162   72.0000000    250.000000

---------------------- TIME=6    METAL=PB ----------------------

R             16   0.95068391    0.1310649    0.7121212      1.174603
X             16  76.25000000   15.7881812   47.0000000     99.000000
Y             16  80.25000000   13.0664966   59.0000000    100.000000

---------------------- TIME=24    METAL=CD ---------------------

R             16     0.214599     0.233445      0.00084       0.59091
X             16   349.543750   360.154089      1.60000     800.00000
Y             16  1806.250000   485.068724   1100.00000    2800.00000

---------------------- TIME=24    METAL=CR ---------------------

R             16     0.570287      0.53849     0.023636       1.29545
X             16   408.812500    235.39618   104.000000     920.00000
Y             16  2541.875000   2152.66414   440.000000    5700.00000

---------------------- TIME=24    METAL=PB ---------------------

R             16    0.8629544     0.219076     0.527027      1.294118
X             16  550.0000000   168.245852   290.000000    790.000000
Y             16  644.3750000   156.374284   430.000000    870.000000

---------------------- TIME=48    METAL=CD ---------------------

R             16      0.11613       0.1218       0.0000         0.279
X             16  11375.47500   12219.0483       0.0000     31000.000
Y             16  82062.50000   17353.0737   57000.0000    111000.000
```

```
                          TABLE B-XIII
        MEANS GROUPED BY CONCENTRATION, TIME, AND METAL
                   CONC=H    TIME=48    METAL=CR

VARIABLE      N       MEAN       STANDARD      MINIMUM      MAXIMUM
                                 DEVIATION      VALUE        VALUE

R             8      0.0002       0.0001        0.000        0.000
X             8     69.6250      33.1358       34.000      117.000
Y             8 425000.0000   71912.6454   330000.000   520000.000

---------------- CONC=H    TIME=48    METAL=PB ------------------

R             8     0.29504      0.15036       0.1381       0.5278
X             8  5487.50000   3058.21493    2500.0000    9500.0000
Y             8 18625.00000   5730.55719   11000.0000   28000.0000

----------------- CONC=L    TIME=6    METAL=CD ------------------

R             8   0.7564069    0.1436031     0.463855     0.929825
X             8  90.3750000   11.3507394    77.000000   106.000000
Y             8 122.1250000   19.7732684   105.000000   166.000000

----------------- CONC=L    TIME=6    METAL=CR ------------------

R             8  0.96264303    0.1330780    0.7954545    1.1232877
X             8 80.00000000   13.0054933   61.0000000   99.0000000
Y             8 83.25000000    8.6972902   72.0000000   94.0000000

----------------- CONC=L    TIME=6    METAL=PB ------------------

R             8  0.95409183    0.1405376    0.7121212    1.1746032
X             8 67.12500000   12.7440462   47.0000000   80.0000000
Y             8 70.25000000    8.0311892   59.0000000   82.0000000

---------------- CONC=L    TIME=24    METAL=CD ------------------

R             8    0.426893     0.117314      0.28214      0.59091
X             8  695.000000    71.912645    600.00000    800.00000
Y             8 1762.500000   599.851172   1100.00000   2800.00000

---------------- CONC=L    TIME=24    METAL=CR ------------------

R             8   1.0763583    0.1740948     0.775862     1.295455
X             8 542.5000000   69.6419414   450.000000   680.000000
Y             8 508.7500000   49.1172068   440.000000   580.000000
```

```
                              TABLE B-VIII
                    MEANS GROUPED BY TIME AND METAL
                         TIME=48    METAL=CR

VARIABLE       N         MEAN        STANDARD         MINIMUM         MAXIMUM
                                     DEVIATION         VALUE           VALUE

R             16        0.8587          1.031        0.00009           3.056
X             16     6109.8125       7341.160       34.00000       22000.000
Y             16   216031.2500     221342.602     6600.00000      520000.000

---------------------- TIME=48    METAL=PB ----------------------

R             16       0.33819        0.24098        0.06742          0.7308
X             16    4881.25000     3351.26220      600.00000       9500.0000
Y             16   14431.25000     5955.69405     7600.00000      28000.0000
```

```
                              TABLE B-IX
                 MEANS GROUPED BY TIME AND METHOD
                        TIME=6     METHOD=M

VARIABLE      N        MEAN       STANDARD      MINIMUM       MAXIMUM
                                  DEVIATION      VALUE         VALUE

R            24     0.7675739    0.2995122    0.1538462      1.087912
X            24    86.3333333   24.8712627   35.0000000    124.000000
Y            24   130.4166667   58.0381559   72.0000000    260.000000

--------------------- TIME=6     METHOD=S ----------------------

R            24     0.7195867    0.2732270    0.2335766      1.174603
X            24    71.1666667   24.1780833   28.0000000    127.000000
Y            24   108.6666667   43.0082567   59.0000000    210.000000

--------------------- TIME=24     METHOD=M ---------------------

R            24      0.580734      0.48745     0.002040       1.29412
X            24    482.625000    284.44361     5.100000     800.00000
Y            24   1832.500000   1632.85360   430.000000    5700.00000

--------------------- TIME=24     METHOD=S ---------------------

R            24      0.517826      0.40368     0.000842       1.29545
X            24    389.612500    261.77389     1.600000     920.00000
Y            24   1495.833333   1318.94529   440.000000    4400.00000

--------------------- TIME=48     METHOD=M ---------------------

R            24        0.6133        0.849      0.00002         3.056
X            24     9896.0167     9745.685      1.40000     31000.000
Y            24   116495.8333   171515.801   6800.00000    520000.000

--------------------- TIME=48     METHOD=S ---------------------

R            24       0.26206        0.397      0.00000         1.313
X            24    5015.00833     6999.686      0.00000     21000.000
Y            24   91854.16667   130703.754   6600.00000    430000.000
```

```
                              TABLE B-X
                   MEANS GROUPED BY METAL AND METHOD
                         METAL=CD     METHOD=M

VARIABLE      N        MEAN       STANDARD      MINIMUM      MAXIMUM
                                  DEVIATION      VALUE        VALUE

R            24      0.27846        0.2891     0.000021        0.930
X            24   4560.85000    10101.4428     1.400000    31000.000
Y            24  30138.54167    43227.5756   109.000000   111000.000

-------------------- METAL=CD     METHOD=S --------------------

R            24      0.27958        0.2638     0.000000       0.8571
X            24   3298.57917     7243.3199     0.000000   21000.0000
Y            24  25866.37500    36998.8844   105.000000   93000.0000

-------------------- METAL=CR     METHOD=M --------------------

R            24      0.83362         0.836    0.0001840        3.056
X            24   2973.95833      6468.487   70.0000000    22000.000
Y            24  82578.87500    183154.083   88.0000000   520000.000

-------------------- METAL=CR     METHOD=S --------------------

R            24      0.63484         0.477    0.0000946        1.313
X            24   1435.70833      2837.928   34.0000000     8800.000
Y            24  63229.70833    139971.189   72.0000000   430000.000

-------------------- METAL=PB     METHOD=M --------------------

R            24     0.849502       0.25702    0.3035714       1.2941
X            24  2930.166667    3704.77809   73.0000000    9500.0000
Y            24  5741.333333    8554.16126   72.0000000   28000.0000

-------------------- METAL=PB     METHOD=S --------------------

R            24     0.585050       0.36120    0.0674157       1.1746
X            24   741.500000     935.08507   47.0000000    2900.0000
Y            24  4362.583333    6394.53019   59.0000000   21000.0000
```

```
                              TABLE B-XI
               MEANS GROUPED BY CONCENTRATION, TIME, AND METHOD
                        CONC=H     TIME=6     METHOD=M

VARIABLE        N         MEAN         STANDARD        MINIMUM        MAXIMUM
                                       DEVIATION        VALUE          VALUE

R              12     0.6033826      0.3416495      0.1538462       1.031250
X              12    85.0833333     33.6167121     35.0000000     124.000000
Y              12   165.4166667     63.2001990     92.0000000     260.000000

----------------- CONC=H     TIME=6     METHOD=S -----------------

R              12     0.5888441      0.2787068      0.2335766       1.135135
X              12    71.5833333     32.3432115     28.0000000     127.000000
Y              12   129.0000000     44.3990172     74.0000000     210.000000

---------------- CONC=H     TIME=24    METHOD=M -----------------

R              12      0.311751        0.42518       0.002040        0.92941
X              12    331.083333      328.19604       5.100000      790.00000
Y              12   2591.666667     1903.57956     840.000000     5700.00000

---------------- CONC=H     TIME=24    METHOD=S -----------------

R              12      0.261425        0.34265       0.000842        0.92857
X              12    257.558333      290.51712       1.600000      920.00000
Y              12   2195.833333     1522.74548     560.000000     4400.00000

---------------- CONC=H     TIME=48    METHOD=M -----------------

R              12        0.1380          0.213         0.0000          0.528
X              12     2792.0333       4085.502         1.4000       9500.000
Y              12   191666.6667     216664.103     14000.0000     520000.000

---------------- CONC=H     TIME=48    METHOD=S -----------------

R              12        0.0588          0.089         0.0000          0.227
X              12      913.3500       1323.080         0.0000       2900.000
Y              12   148750.0000     164498.204     11000.0000     430000.000

----------------- CONC=L     TIME=6     METHOD=M -----------------

R              12    0.93176521      0.1096671      0.7629630       1.087912
X              12   87.58333333     12.6452171     70.0000000     106.000000
Y              12   95.41666667     19.3928681     72.0000000     135.000000
```

```
                               TABLE B-XI
              MEANS GROUPED BY CONCENTRATION, TIME, AND METHOD
                         CONC=L    TIME=6    METHOD=S

VARIABLE       N         MEAN        STANDARD        MINIMUM        MAXIMUM
                                     DEVIATION        VALUE          VALUE

R             12     0.85032933    0.2027805     0.4638554       1.174603
X             12    70.7500000    13.2605018    47.0000000      90.000000
Y             12    88.33333333   31.5316099    59.0000000     166.000000

---------------- CONC=L    TIME=24    METHOD=M -----------------

R             12       0.849717     0.397711      0.282143        1.29412
X             12     634.166667   106.554155    450.000000      800.00000
Y             12    1073.333333   832.906557    430.000000     2800.00000

---------------- CONC=L    TIME=24    METHOD=S -----------------

R             12      0.7742276     0.282631      0.365000        1.29545
X             12    521.6666667   144.337567    290.000000      730.00000
Y             12    795.8333333   499.535390    440.000000     2000.00000

---------------- CONC=L    TIME=48    METHOD=M -----------------

R             12        1.08852       0.9844       0.21905          3.056
X             12    17000.00000    8472.9516    7400.00000      31000.000
Y             12    41325.00000   47303.7020    6800.00000     111000.000

---------------- CONC=L    TIME=48    METHOD=S -----------------

R             12        0.46534       0.4818       0.06742         1.3134
X             12     9116.66667    7999.2992     600.00000     21000.0000
Y             12    34958.33333   39968.5435    6600.00000     93000.0000
```

```
                              TABLE B-XII
                 MEANS GROUPED BY TIME, METAL, AND METHOD
                      TIME=6     METAL=CD     METHOD=M

VARIABLE       N        MEAN       STANDARD       MINIMUM        MAXIMUM
                                   DEVIATION       VALUE          VALUE

R              8     0.5332185    0.3272101     0.153846       0.929825
X              8    70.1250000   31.3297554    35.000000     106.000000
Y              8   153.1250000   51.7036542   109.000000     260.000000

---------------- TIME=6     METAL=CD     METHOD=S ----------------

R              8     0.4794400    0.2402733     0.233577       0.857143
X              8    58.1250000   26.0737279    28.000000      90.000000
Y              8   124.1250000   19.2757249   105.000000     166.000000

---------------- TIME=6     METAL=CR     METHOD=M ----------------

R              8     0.7665186    0.2472057    0.4400000       1.087912
X              8   102.1250000   18.3881755   70.0000000     124.000000
Y              8   151.6250000   70.4474221   88.0000000     250.000000

---------------- TIME=6     METAL=CR     METHOD=S ----------------

R              8     0.7809369    0.2252224    0.5222222       1.123288
X              8    89.6250000   21.6526970   61.0000000     127.000000
Y              8   127.8750000   59.0361572   72.0000000     210.000000

---------------- TIME=6     METAL=PB     METHOD=M ----------------

R              8    1.00298458    0.0217145    0.9756098       1.031250
X              8   86.75000000   11.2852622   73.0000000      99.000000
Y              8   86.50000000   11.1355287   72.0000000     100.000000

---------------- TIME=6     METAL=PB     METHOD=S ----------------

R              8    0.89838324    0.1734540    0.7121212      1.1746032
X              8   65.75000000   12.4412907   47.0000000     84.0000000
Y              8   74.00000000   12.3519808   59.0000000     91.0000000

--------------- TIME=24     METAL=CD     METHOD=M ----------------

R              8      0.176466     0.190946      0.00204        0.42105
X              8    361.625000   385.533403      5.10000      800.00000
Y              8   2012.500000   491.172068   1400.00000     2800.00000
```

```
                              TABLE B-XII
                 MEANS GROUPED BY TIME, METAL, AND METHOD
                     TIME=24    METAL=CD    METHOD=S

VARIABLE        N         MEAN        STANDARD      MINIMUM      MAXIMUM
                                      DEVIATION      VALUE        VALUE

R               8      0.252733      0.277478      0.00084      0.59091
X               8    337.462500    359.141273      1.60000    730.00000
Y               8   1600.000000    407.080196   1100.00000   2200.00000

---------------- TIME=24    METAL=CR    METHOD=M -----------------

R               8      0.562654       0.56959     0.040426      1.24444
X               8    395.000000     186.16429   180.000000    680.00000
Y               8   2772.500000    2451.48789   450.000000   5700.00000

---------------- TIME=24    METAL=CR    METHOD=S -----------------

R               8      0.577919       0.54479     0.023636      1.29545
X               8    422.625000     289.21516   104.000000    920.00000
Y               8   2311.250000    1948.98829   440.000000   4400.00000

---------------- TIME=24    METAL=PB    METHOD=M -----------------

R               8     1.0030825      0.169707     0.816092     1.294118
X               8   691.2500000     85.429587   530.000000   790.000000
Y               8   712.5000000    170.021007   430.000000   870.000000

---------------- TIME=24    METAL=PB    METHOD=S -----------------

R               8     0.7228264      0.170771     0.527027     1.020408
X               8   408.7500000     88.064506   290.000000   520.000000
Y               8   576.2500000    113.507394   440.000000   740.000000

---------------- TIME=48    METAL=CD    METHOD=M -----------------

R               8       0.12569        0.1354       0.0000        0.279
X               8   13250.80000    14339.4083       1.4000    31000.000
Y               8   88250.00000    18668.1547   66000.0000   111000.000

---------------- TIME=48    METAL=CD    METHOD=S -----------------

R               8       0.10657        0.1152       0.0000       0.2326
X               8    9500.15000    10309.3383       0.0000   21000.0000
Y               8   75875.00000    14466.0934   57000.0000   93000.0000
```

```
                              TABLE B-XII
                 MEANS GROUPED BY TIME, METAL, AND METHOD
                     TIME=48     METAL=CR     METHOD=M

VARIABLE         N         MEAN       STANDARD        MINIMUM         MAXIMUM
                                      DEVIATION        VALUE           VALUE

R                8        1.1717         1.300        0.00018           3.056
X                8     8424.7500      9298.454       92.00000       22000.000
Y                8   244812.5000    255129.673     6800.00000      520000.000

---------------- TIME=48     METAL=CR     METHOD=S -----------------

R                8        0.5457         0.604        0.00009           1.313
X                8     3794.8750      4095.605       34.00000        8800.000
Y                8   187250.0000    194934.568     6600.00000      430000.000

---------------- TIME=48     METAL=PB     METHOD=M -----------------

R                8       0.54244       0.16169         0.3036          0.7308
X                8    8012.50000     775.40312      7000.0000       9500.0000
Y                8   16425.00000    6671.20679     10400.0000      28000.0000

---------------- TIME=48     METAL=PB     METHOD=S -----------------

R                8       0.13394       0.05427        0.06742          0.2273
X                8    1750.00000    1026.78416      600.00000       2900.0000
Y                8   12437.50000    4734.65567     7600.00000      21000.0000
```

```
                              TABLE B-XIII
              MEANS GROUPED BY CONCENTRATION, TIME, AND METAL
                        CONC=H    TIME=6    METAL=CD

VARIABLE       N        MEAN        STANDARD       MINIMUM        MAXIMUM
                                    DEVIATION       VALUE          VALUE

R              8     0.2562516     0.0536894      0.153846       0.330645
X              8    37.8750000     6.3118370     28.000000      47.000000
Y              8   155.1250000    50.1211033    115.000000     260.000000

----------------- CONC=H    TIME=6    METAL=CR -----------------

R              8     0.5848126     0.1125613      0.440000       0.775000
X              8   111.7500000    12.2911118     94.000000     127.000000
Y              8   196.2500000    37.3927036    140.000000     250.000000

----------------- CONC=H    TIME=6    METAL=PB -----------------

R              8    0.94727599     0.1305093     0.7444444       1.135135
X              8   85.37500000    13.4688795    67.0000000      99.000000
Y              8   90.25000000     8.5314544    74.0000000     100.000000

---------------- CONC=H    TIME=24    METAL=CD -----------------

R              8      0.002305      0.001240       0.00084        0.00450
X              8      4.087500      1.858907       1.60000        6.30000
Y              8   1850.000000    374.165739    1400.00000     2500.00000

---------------- CONC=H    TIME=24    METAL=CR -----------------

R              8      0.064215      0.075258       0.02364        0.24865
X              8    275.125000    270.254609     104.00000      920.00000
Y              8   4575.000000    692.304640    3700.00000     5700.00000

---------------- CONC=H    TIME=24    METAL=PB -----------------

R              8     0.7932429      0.151136      0.527027       0.929412
X              8   603.7500000    165.437731    390.000000     790.000000
Y              8   756.2500000    114.509825    560.000000     870.000000

---------------- CONC=H    TIME=48    METAL=CD -----------------

R              8       0.00001       0.00001        0.0000         0.0000
X              8       0.95000       0.72309        0.0000         1.8000
Y              8   67000.00000    6187.54509    57000.0000     76000.0000
```

```
                                TABLE B-XIII
                MEANS GROUPED BY CONCENTRATION, TIME, AND METAL
                         CONC=L    TIME=24    METAL=PB

VARIABLE         N        MEAN       STANDARD       MINIMUM        MAXIMUM
                                     DEVIATION       VALUE          VALUE

R                8    0.9326659     0.262479      0.578947       1.294118
X                8  496.2500000   163.352161    290.000000     710.000000
Y                8  532.5000000   103.337174    430.000000     720.000000

----------------- CONC=L    TIME=48    METAL=CD -----------------

R                8      0.23225      0.03142        0.1744          0.279
X                8  22750.00000   4920.80133    15000.0000      31000.000
Y                8  97125.00000   9402.69718    86000.0000     111000.000

----------------- CONC=L    TIME=48    METAL=CR -----------------

R                8      1.71720      0.77044       0.75641         3.0556
X                8  12150.00000   5664.92971    5900.00000     22000.0000
Y                8   7062.50000    413.82363    6600.00000      7800.0000

----------------- CONC=L    TIME=48    METAL=PB -----------------

R                8      0.38134      0.31238       0.06742         0.7308
X                8   4275.00000   3724.72434     600.00000      8300.0000
Y                8  10237.50000   1722.90245    7600.00000     12500.0000
```

```
                               TABLE B-XIV
     MEANS GROUPED BY CONCENTRATION, TIME, METAL, AND METHOD
                 CONC=H    TIME=6    METAL=CD    METHOD=M

VARIABLE        N         MEAN       STANDARD        MINIMUM       MAXIMUM
                                     DEVIATION        VALUE         VALUE

R               4    0.2323172     0.0530705       0.153846      0.269231
X               4   41.5000000     5.1961524      35.000000     47.000000
Y               4  187.5000000    54.3905629     130.000000    260.000000

------------ CONC=H    TIME=6    METAL=CD    METHOD=S -------------

R               4    0.2801859     0.0488045       0.233577      0.330645
X               4   34.2500000     5.5602758      28.000000     41.000000
Y               4  122.7500000    10.4043260     115.000000    137.000000

------------ CONC=H    TIME=6    METAL=CR    METHOD=M -------------

R               4    0.5695833     0.1448586       0.440000      0.775000
X               4  116.7500000     7.2743843     110.000000    124.000000
Y               4  212.5000000    41.1298756     160.000000    250.000000

------------ CONC=H    TIME=6    METAL=CR    METHOD=S -------------

R               4    0.6000418     0.0892240       0.522222      0.685714
X               4  106.7500000    15.2616076      94.000000    127.000000
Y               4  180.0000000    29.4392029     140.000000    210.000000

------------ CONC=H    TIME=6    METAL=PB    METHOD=M -------------

R               4   1.00824728    0.02292801      0.9800000      1.031250
X               4  97.00000000    2.16024690     94.0000000     99.000000
Y               4  96.25000000    3.30403793     92.0000000    100.000000

------------ CONC=H    TIME=6    METAL=PB    METHOD=S -------------

R               4   0.88630470    0.17118378      0.7444444     1.1351351
X               4  73.75000000    7.63216876     67.0000000    84.0000000
Y               4  84.25000000    7.93200269     74.0000000    91.0000000

------------ CONC=H    TIME=24    METAL=CD    METHOD=M ------------

R               4     0.003220      0.001063        0.00204       0.00450
X               4     5.750000      0.550757        5.10000       6.30000
Y               4  1900.000000    469.041576     1400.00000    2500.00000
```

```
                                TABLE B-XIV
     MEANS GROUPED BY CONCENTRATION, TIME, METAL, AND METHOD
                CONC=H    TIME=24    METAL=CD    METHOD=S

VARIABLE       N         MEAN      STANDARD      MINIMUM      MAXIMUM
                                   DEVIATION      VALUE        VALUE

R              4      0.001390     0.000479      0.00084      0.00194
X              4      2.425000     0.623832      1.60000      3.10000
Y              4   1800.000000   316.227766   1500.00000   2200.00000

----------- CONC=H    TIME=24    METAL=CR    METHOD=M -----------

R              4      0.046251     0.005604      0.04043      0.05263
X              4    235.000000    59.160798    180.00000    300.00000
Y              4   5025.000000   699.404509   4200.00000   5700.00000

----------- CONC=H    TIME=24    METAL=CR    METHOD=S -----------

R              4      0.082178     0.111011      0.02364      0.24865
X              4    315.250000   403.271104    104.00000    920.00000
Y              4   4125.000000   298.607881   3700.00000   4400.00000

----------- CONC=H    TIME=24    METAL=PB    METHOD=M -----------

R              4     0.8857807    0.0507853     0.816092     0.929412
X              4   752.5000000   35.0000000   710.000000   790.000000
Y              4   850.0000000   14.1421356   840.000000   870.000000

----------- CONC=H    TIME=24    METAL=PB    METHOD=S -----------

R              4     0.7007052    0.1669848     0.527027     0.928571
X              4   455.0000000   60.2771377   390.000000   520.000000
Y              4   662.5000000   83.4166250   560.000000   740.000000

----------- CONC=H    TIME=48    METAL=CD    METHOD=M -----------

R              4       0.00002      0.00000       0.0000       0.0000
X              4       1.60000      0.18257       1.4000       1.8000
Y              4   71250.00000   4991.65971   66000.0000   76000.0000

----------- CONC=H    TIME=48    METAL=CD    METHOD=S -----------

R              4       0.00000      0.00000       0.0000       0.0000
X              4       0.30000      0.24495       0.0000       0.6000
Y              4   62750.00000   4031.12887   57000.0000   66000.0000
```

```
                          TABLE B-XIV
     MEANS GROUPED BY CONCENTRATION, TIME, METAL, AND METHOD
                 CONC=L     TIME=6    METAL=CR    METHOD=S

VARIABLE       N        MEAN       STANDARD       MINIMUM       MAXIMUM
                                   DEVIATION       VALUE         VALUE

R              4    0.96183212   0.15210594    0.8026316     1.1232877
X              4   72.50000000   8.88819442   61.0000000    82.0000000
Y              4   75.75000000   4.50000000   72.0000000    82.0000000

------------ CONC=L     TIME=6    METAL=PB    METHOD=M -------------

R              4    0.99772188   0.02237543    0.9756098     1.0277778
X              4   76.50000000   3.51188458   73.0000000    80.0000000
Y              4   76.75000000   4.99165971   72.0000000    82.0000000

------------ CONC=L     TIME=6    METAL=PB    METHOD=S -------------

R              4    0.91046177   0.2012669     0.7121212     1.1746032
X              4   57.75000000  11.5000000    47.0000000    74.0000000
Y              4   63.75000000   3.5939764    59.0000000    67.0000000

------------ CONC=L     TIME=24    METAL=CD    METHOD=M ------------

R              4      0.349712     0.070961      0.28214       0.42105
X              4    717.500000    95.350232    600.00000     800.00000
Y              4   2125.000000   556.027577   1500.00000    2800.00000

------------ CONC=L     TIME=24    METAL=CD    METHOD=S ------------

R              4      0.504075     0.105793      0.36500       0.59091
X              4    672.500000    40.311289    640.00000     730.00000
Y              4   1400.000000   424.264069   1100.00000    2000.00000

------------ CONC=L     TIME=24    METAL=CR    METHOD=M ------------

R              4     1.0790562    0.2141445     0.775862      1.244444
X              4   555.0000000   95.3939201   450.000000    680.000000
Y              4   520.0000000   60.5530071   450.000000    580.000000

------------ CONC=L     TIME=24    METAL=CR    METHOD=S ------------

R              4     1.0736604    0.1576188     0.960000      1.295455
X              4   530.0000000   42.4264069   480.000000    570.000000
Y              4   497.5000000   40.3112887   440.000000    530.000000
```

```
                              TABLE B-XIV
         MEANS GROUPED BY CONCENTRATION, TIME, METAL, AND METHOD
                  CONC=H     TIME=48     METAL=CR     METHOD=M

VARIABLE       N          MEAN         STANDARD         MINIMUM        MAXIMUM
                                       DEVIATION         VALUE          VALUE

R              4        0.0002          0.0000          0.000          0.000
X              4       99.5000         11.7898         92.000        117.000
Y              4   482500.0000      35000.0000     440000.000     520000.000

------------ CONC=H     TIME=48     METAL=CR     METHOD=S ------------

R              4        0.0001          0.0000          0.000          0.000
X              4       39.7500          6.5511         34.000         48.000
Y              4   367500.0000      45000.0000     330000.000     430000.000

------------ CONC=H     TIME=48     METAL=PB     METHOD=M ------------

R              4       0.41384         0.11638         0.3036         0.5278
X              4    8275.00000      1034.00516      7000.0000      9500.0000
Y              4   21250.00000      6396.61369     14000.0000     28000.0000

------------ CONC=H     TIME=48     METAL=PB     METHOD=S ------------

R              4       0.17624         0.03963         0.1381         0.2273
X              4    2700.00000       182.57419      2500.0000      2900.0000
Y              4   16000.00000      4163.33200     11000.0000     21000.0000

------------ CONC=L     TIME=6     METAL=CD     METHOD=M -------------

R              4     0.8341198       0.0745719       0.762963       0.929825
X              4    98.7500000       8.8459030      86.000000     106.000000
Y              4   118.7500000      11.3247517     109.000000     135.000000

------------ CONC=L     TIME=6     METAL=CD     METHOD=S -------------

R              4     0.6786941       0.1626405       0.463855       0.857143
X              4    82.0000000       5.9441848      77.000000      90.000000
Y              4   125.5000000      27.4529901     105.000000     166.000000

------------ CONC=L     TIME=6     METAL=CR     METHOD=M -------------

R              4    0.96345395       0.1348511      0.7954545      1.0879121
X              4   87.50000000      12.8711564     70.0000000     99.0000000
Y              4   90.75000000       2.5000000     88.0000000     94.0000000
```

```
                                TABLE B-XIV
      MEANS GROUPED BY CONCENTRATION, TIME, METAL, AND METHOD
                CONC=L    TIME=24    METAL=PB    METHOD=M

VARIABLE        N       MEAN      STANDARD      MINIMUM      MAXIMUM
                                  DEVIATION      VALUE        VALUE

R               4    1.1203842    0.167121     0.968750     1.294118
X               4  630.0000000   76.157731   530.000000   710.000000
Y               4  575.0000000  129.743336   430.000000   720.000000

------------ CONC=L    TIME=24    METAL=PB    METHOD=S ------------

R               4    0.7449476   0.1971237     0.578947     1.020408
X               4  362.5000000  93.5859676   290.000000   500.000000
Y               4  490.0000000  57.1547607   440.000000   570.000000

------------ CONC=L    TIME=48    METAL=CD    METHOD=M ------------

R               4       0.2514     0.02521        0.219        0.279
X               4   26500.0000  3415.65026    23000.000    31000.000
Y               4  105250.0000  4193.24854   101000.000   111000.000

------------ CONC=L    TIME=48    METAL=CD    METHOD=S ------------

R               4      0.21314     0.02634       0.1744       0.2326
X               4  19000.00000  2708.01280   15000.0000   21000.0000
Y               4  89000.00000  3559.02608   86000.0000   93000.0000

------------ CONC=L    TIME=48    METAL=CR    METHOD=M ------------

R               4      2.34316     0.53269       1.7647       3.0556
X               4  16750.00000  4112.98756   12000.0000   22000.0000
Y               4   7125.00000   298.60788    6800.0000    7500.0000

------------ CONC=L    TIME=48    METAL=CR    METHOD=S ------------

R               4     1.091242     0.23750      0.75641      1.31343
X               4  7550.000000  1239.62360   5900.00000   8800.00000
Y               4  7000.000000   547.72256   6600.00000   7800.00000

------------ CONC=L    TIME=48    METAL=PB    METHOD=M ------------

R               4      0.67104    0.057898       0.5920       0.7308
X               4   7750.00000  387.298335    7400.0000    8300.0000
Y               4  11600.00000  920.144916   10400.0000   12500.0000
```

TABLE B-XIV

```
    MEANS GROUPED BY CONCENTRATION, TIME, METAL, AND METHOD
              CONC=L    TIME=48    METAL=PB    METHOD=S

VARIABLE          N        MEAN        STANDARD        MINIMUM        MAXIMUM
                                       DEVIATION        VALUE          VALUE

R                 4      0.091635     0.02303        0.06742         0.1184
X                 4    800.000000   141.42136      600.00000       900.0000
Y                 4   8875.000000  1062.62254     7600.00000     10200.0000
```

APPENDIX C

RESULTS OF STATISTICAL ANALYSIS BY PROCEDURE ANOVA

```
                 TABLE C-I
      ANALYSIS OF VARIANCE PROCEDURE
         CLASS LEVEL INFORMATION
       CLASS      LEVELS     VALUES
       METHOD          2     M S
       TIME            3     6 24 48
       METAL           3     CD CR PB
       CONC            2     H L

NUMBER OF OBSERVATIONS IN DATA SET = 144
```

TABLE C-I

```
                        ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: R

SOURCE                        DF       SUM OF SQUARES            MEAN SQUARE

MODEL                         35          34.85330002             0.99580857

ERROR                        108           2.20601832             0.02042610

CORRECTED TOTAL              143          37.05931834


MODEL F =                  48.75                            PR > F = 0.0001


R-SQUARE                    C.V.              STD DEV                 R MEAN

0.940473                 24.7762           0.14291989             0.57684260

SOURCE                        DF              ANOVA SS    F VALUE    PR > F

METHOD                         1            0.85416209      41.82    0.0001
TIME                           2            2.30068253      56.32    0.0001
METHOD*TIME                    2            0.70113633      17.16    0.0001
METAL                          2            6.39314703     156.49    0.0001
METHOD*METAL                   2            0.45920780      11.24    0.0001
TIME*METAL                     4            3.23027885      39.54    0.0001
METHOD*TIME*METAL              4            0.61652212       7.55    0.0001
CONC(METAL)                    3           14.72832293     240.35    0.0001
METHOD*CONC(METAL)             3            0.67033170      10.94    0.0001
TIME*CONC(METAL)               6            3.77858636      30.83    0.0001
METH*TIME*CONC(META)           6            1.12092227       9.15    0.0001
```

```
               TABLE C-II
      THREE-WAY ANALYSIS OF VARIANCE
                METAL=CD

      ANALYSIS OF VARIANCE PROCEDURE

         CLASS LEVEL INFORMATION

       CLASS      LEVELS     VALUES

       METHOD         2      M S

       TIME           3      6 24 48

       CONC           2      H L

NUMBER OF OBSERVATIONS IN BY GROUP = 48
```

TABLE C-II
THREE-WAY ANALYSIS OF VARIANCE
METAL=CD

```
                      ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: R

SOURCE                      DF       SUM OF SQUARES          MEAN SQUARE

MODEL                       11           3.35857014           0.30532456

ERROR                       36           0.16430805           0.00456411

CORRECTED TOTAL             47           3.52287819

MODEL F =                66.90                          PR > F = 0.0001

R-SQUARE                  C.V.              STD DEV               R MEAN

0.953360               24.2126           0.06755821           0.27902071

SOURCE                      DF            ANOVA SS    F VALUE    PR > F

METHOD                       1          0.00001511       0.00    0.9544
TIME                         2          1.31762358     144.35    0.0001
METHOD*TIME                  2          0.03628244       3.97    0.0276
CONC                         1          1.78481549     391.05    0.0001
METHOD*CONC                  1          0.00242598       0.53    0.4707
TIME*CONC                    2          0.15264910      16.72    0.0001
METHOD*TIME*CONC             2          0.06475844       7.09    0.0025
```

```
                 TABLE C-II
   THREE-WAY ANALYSIS OF VARIANCE
                 METAL=PB

   ANALYSIS OF VARIANCE PROCEDURE

      CLASS LEVEL INFORMATION

    CLASS       LEVELS     VALUES

    METHOD          2      M S

    TIME            3      6 24 48

    CONC            2      H L

NUMBER OF OBSERVATIONS IN BY GROUP = 48
```

TABLE C-II
THREE-WAY ANALYSIS OF VARIANCE
METAL=PB

```
                         ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: R

SOURCE                     DF        SUM OF SQUARES          MEAN SQUARE

MODEL                      11            4.79796368           0.43617852

ERROR                      36            0.56126165           0.01559060

CORRECTED TOTAL            47            5.35922533


MODEL F =               27.98                            PR > F = 0.0001


R-SQUARE                 C.V.               STD DEV               R MEAN

0.895272              17.4079            0.12486233           0.71727578


SOURCE                     DF              ANOVA SS    F VALUE    PR > F

METHOD                      1            0.83921890      53.83    0.0001
TIME                        2            3.51053441     112.58    0.0001
METHOD*TIME                 2            0.18620598       5.97    0.0058
CONC                        1            0.07209705       4.62    0.0383
METHOD*CONC                 1            0.08249865       5.29    0.0273
TIME*CONC                   2            0.03563235       1.14    0.3302
METHOD*TIME*CONC            2            0.07177633       2.30    0.1146
```

```
                TABLE C-II
      THREE-WAY ANALYSIS OF VARIANCE
                 METAL=CR

      ANALYSIS OF VARIANCE PROCEDURE

         CLASS LEVEL INFORMATION

       CLASS       LEVELS     VALUES

       METHOD         2       M S

       TIME           3       6 24 48

       CONC           2       H L

 NUMBER OF OBSERVATIONS IN BY GROUP = 48
```

```
                         TABLE C-II
               THREE-WAY ANALYSIS OF VARIANCE
                         METAL=CR

               ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: R

SOURCE                  DF     SUM OF SQUARES        MEAN SQUARE

MODEL                   11        20.30361917         1.84578356

ERROR                   36         1.48044862         0.04112357

CORRECTED TOTAL         47        21.78406779


MODEL F =            44.88                      PR > F = 0.0001

R-SQUARE              C.V.            STD DEV             R MEAN

0.932040           27.6193         0.20278948         0.73423131

SOURCE                  DF          ANOVA SS  F VALUE    PR > F

METHOD                   1        0.47413588    11.53    0.0017
TIME                     2        0.70280339     8.55    0.0009
METHOD*TIME              2        1.09517003    13.32    0.0001
CONC                     1       12.87141039   312.99    0.0001
METHOD*CONC              1        0.58540707    14.24    0.0006
TIME*CONC                2        3.59030491    43.65    0.0001
METHOD*TIME*CONC         2        0.98438750    11.97    0.0001
```

VITA

Benjamin Perry Gayle was born in Franklin, Virginia on September 4, 1954. He received his secondary education at Southhampton High School in Courtland, Virginia, graduating in June, 1972. In September, 1972, he entered Virginia Polytechnic Institute and State University, receiving his Bachelor of Science degree in Biology in June, 1976. In September, 1976, he entered Graduate School in Environmental Science and Engineering at Virginia Polytechnic Institute and State University.

Benjamin P. Gayle

AN EVALUATION OF A MODIFIED MEMBRANE FILTER TECHNIQUE FOR THE RECOVERY OF FECAL COLIFORMS EXPOSED TO SELECTED HEAVY METALS

by

Benjamin P. Gayle

(ABSTRACT)

A bench study was conducted to compare two membrane filter techniques for their efficiency in recovering fecal coliforms exposed to selected heavy metals. The effects on recovery by increasing time and metal concentration were also examined.

The recovery methods employed included the standard membrane filter technique (S-MF) and a modified membrane filter technique (M-MF), which consisted of a lactose agar overlay and a five-hour preincubation at 35 C. The heavy metals Cd, Cr, Pb, and Zn were examined, each at two concentrations, to evaluate their effect on the recovery of the _E. coli_ test organism, after exposure for 6, 24, and 48 hours.

A statistical analysis of the data found the recoveries obtained by the M-MF to be significantly greater (.0001 level) than those of the S-MF, in all cases. Time was also found to significantly effect recovery, with recoveries decreasing as time increased. A significant difference was also found between the effects of the heavy metals tested and the concentrations of metals was likewise found to

significantly effect recovery, with decreased recoveries being obtained at the high concentration of each metal.