METAL DISTRIBUTION IN A lysimeter - Experimental Methods

by

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Abstract

North Americans have traditionally used landfills as a cheap method of refuse disposal. Over the past few years governments have started forming legislation to prevent further landfilling of some industrial wastes. This reflects the realization that metals and organics are not always retained by landfills. The lack of information with respect to metal mobility has caused many metal wastes to be barred from landfills. This has created a disposal problem for commercial and industrial refuse producers.

In 1978 a co-disposal project was initiated at the University of British Columbia to determine the potential for enhanced retention of metals in a landfill. Electroplating sludge and septic tank pumpings were added to six airtight lysimeters and the liquid effluent was monitored for Cd, Cr, Fe, Ni, Pb and Zn releases. The study did not determine specific metal retention mechanisms. Therefore, the study report herein was initiated to establish experimental procedures to determine: relative metal mobility, the role of bacteria as metal binding agents and the importance of natural ligands as metal binding agents.

Three separate experiments were conducted using samples which had been removed from one of the UBC co-disposal study lysimeters. A technique for removing the samples in a nitrogen atmosphere was developed to keep the samples anaerobic. By using anaerobic samples any changes caused by exposure to air
were avoided.

The first experiment used a progressive extraction technique to determine the relative mobility of Cd, Cr, Cu, Fe, Ni, Pb, and Zn. Three samples were tested using this technique. The samples were suspensions which had been made by adding deionized distilled water to lysimeter material. An untreated sample suspension was used as a control. Another was sonicated to rupture cells and fragment particles. The third sample was aerated to determine the effects of exposure to air. By comparing the sonicated sample data with the control sample data it may be possible to estimate the mass of metal bound by bacteria. A comparison of the aerated sample and control sample data should give an upper bound to the proportion of the metal mass that will remain fixed in a lysimeter. The extraction procedure separated the metals into five distinct groups: mobile, easily complexed, organically bound, strongly bound and stable metals.

The second experiment was developed to enumerate the bacteria in a sample. A quick and accurate enumeration technique was required so an estimate of the metals associated with bacteria could be made. For this purpose the fluorescent dye 4,6-diamidino-2-phenylindole was selected to enhance visual detection of bacteria. Enumeration was unreliable because of the relatively small quantity of bacteria that were detected in the complicated mixture of organic materials.

The third experiment compared the complexing strength of
natural ligands with that of EDTA, ethanoic acid, glycine, histidine, 8-hydroxyquinoline (oxine), NTA and oxalic acid. Stability constants for the expected metal-chelate complexes were used to form a gradient of complexing agents. It was not possible to determine the presence or absence of natural ligands from the data collected.

The technique may provide a method for determining metal species in complex samples. It was theoretically possible to determine the mass of each metal species by using chelating agents which vary in their ability to complex each metal species. The major limitation of the theory is the lack of an accurate method for determining the stability constants of complexes which are expected to form.

With further work the three experimental methods tested could be used to determine the metal mobility, the mass of metal associated with bacteria, the strength of natural ligands and the metal species in an anaerobic landfill sample. The progressive extraction technique should be modified to use dry oxidation of organics to ensure complete oxidation. The chelation experiment requires further testing before a chelate gradient can be used to determine metal speciation and ligand strength. The fluorescent dye technique was the least successful of the three experiments tested. It may be possible to improve the technique by using mithramycin instead of 4,6-diamidino-2-phenylindole, by experimenting with the concentration of ethanol required to fix bacteria or by using a
chemical other than sodium pyrophosphate to break down the cellulose fibers in the sample. Once the three techniques have been successfully used to test landfill samples it will be possible to develop more informed landfill management policies.
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I. INTRODUCTION

North Americans have traditionally used landfills for refuse disposal. When refuse is defined as an arbitrary mixture of household, garden, food, commercial and some industrial wastes it is clear that large volumes of waste have been landfilled. The growing realization that landfills do not retain some metals and organics has caused governments to form legislation restricting the types of waste that can be landfilled. This has caused a disposal problem for industrial and commercial refuse generators.

When researchers first started studying landfills there was no effort directed towards metal studies because metals were not considered to be a problem. When leachates were analyzed for metals it was found that metal contaminants could be found in some leachates. Although leachate cannot be chemically defined it has a few distinguishing characteristics. It is a dark coloured, unpleasant smelling, liquid which drains from a landfill when the liquid holding capacity of a site is exceeded. Leachates have been found to vary from acidic to basic solutions depending upon the conditions and constituents of the landfill. The volume of leachate which forms is a function of the annual precipitation and infiltration rate at the site. An acidic leachate can be caused by organic acids which are produced as by-products of the organic degradation of refuse. The organic acids mix with the liquid percolating through a landfill leaching metals and acid soluble compounds from the refuse. The
net result being the generation of an obnoxious liquid suspension containing dissolved and particulate matter. This liquid mixture drains from landfills and is called leachate. The metals in leachate can cause environmental contamination and leachate treatment problems.

In 1978, to address the problem of metal contaminants in landfills, researchers at the University of British Columbia started to study the co-disposal of electroplating sludge with septic tank pumpings. The study was initiated to test the hypothesis that septic tank pumpings enhance metal retention in a landfill. Instead of using a landfill to test the retention hypothesis, the UBC group used six laboratory models of refuse leaching, called lysimeters. The liquid which drained from the lysimeters ("leachate") was analyzed for its metals content using an atomic absorption spectrometer. The metal concentrations in the lysimeter leachate and the volume of leachate collected were used to estimate the mass of metal which had escaped from the lysimeters.

The lysimeters used in the co-disposal study were constructed by packing 300 mm. diameter PVC (polyvinyl chloride) pipes with soil and refuse. A layer of pea gravel was placed at the bottom of the PVC pipes. After placing the gravel 15 cm. layers of refuse were added to the pipes. The final layer was a layer of soil. Electroplating sludges and septic

1. Most of the metals were in precipitate form but some soluble forms of cadmium were added from a cadmium plating bath to boost cadmium levels.
tank pumpings were added to the lysimeters during packing. Each lysimeter was sealed from air and watered an average of 96 ml./day to simulate a shallow landfill receiving roughly 1.0 m. of precipitation per year. The masses of electroplating sludges and septic tank pumpings were varied to evaluate the enhancement effects of septic tank pumpings.

Data were collected from the co-disposal lysimeters over a three year period. The concentrations of Cd, Cr, Fe, Ni, Pb, and Zn in the lysimeter leachates were determined and plotted. (An example plot is shown in Figure 1.) The rate of metal release from the lysimeters stabilized after roughly two years. Mass balance calculations proved that varying proportions of the electroplating metals were retained in the lysimeters after three years of operation. The study brought to light the following questions about metal retention in lysimeters:

a) Were the metal precipitates in the electroplating sludge assimilated by the lysimeter contents or had they remained unaltered in the lysimeters?

b) How long would the remaining metals stay in the lysimeters?

c) What proportion of the remaining metals were inert? Mobile?

d) Are any of the metals remaining in the lysimeters associated with bacteria?

e) Are organic chelating agents important metal retention mechanisms in a lysimeter?

No attempt was made to solve question 'b' above because an acceptable technique for projecting the stability of the residual metals would require a time study over a period of
Figure 1 - Plot Of Metal Release Vs Time

From Atwater et al. 1981
Note: The rest of the plots are in Appendix A.

years. Question 'a' above could be resolved by using an electron microscope to compare the crystal structure of metal precipitates in the electroplating waste and the lysimeter samples. This possibility was not checked. Questions 'c', 'd' and 'e' cannot be resolved until new experimental techniques are developed. These questions provided the stimulus for the research contained in this report.

A. OBJECTIVES AND RATIONAL

Methods for analyzing landfill samples are required for informed management and policy formation. This study was devised to provide a method for determining the mobility of metals, the importance of bacteria in retaining metals, and the strength of natural ligands in a landfill leachate. Metal retention in landfills is an important topic because of the cost and pollution implications. With this in mind the objectives of this study were:
a) To develop an experimental method which categorizes metals in a landfill sample according to their mobility.

b) To develop a test to determine the mass of metals associated with bacteria in a landfill sample.

c) To develop a technique for measuring the strength of natural ligands in a landfill.

B. METHODOLOGY

All three objectives are related to the distribution of metals in a landfill. After an initial review of the literature it was evident that the samples used for testing should be anaerobic. This would avoid any changes in metal distribution which would be caused by changes in pH and oxidation-reduction potential. It is difficult to obtain anaerobic samples from a landfill so a lysimeter was used for a sample source. A lysimeter which was used in the UBC co-disposal study was selected for this purpose because it was sealed from air and was packed with metal spiked refuse. The lysimeter contents were similar enough to landfill refuse that they could be used to develop experimental methods for testing landfill samples. The refuse was spiked with electroplating wastes so there should be no difficulty detecting metals in the samples. Also, leaching data was available to indicate the mass of metal which had migrated out of the lysimeter.

A literature survey was conducted to determine the findings of other lysimeter studies and to identify possible experimental techniques. It was apparent that little has been done to determine which mechanisms cause metal retention in landfills or
lysimeters. Most lysimeter studies attempt to characterize the leachate produced when various external factors (e.g., precipitation) or internal factors (e.g., shredding) are altered. The literature search was broadened to include aerobic or anaerobic sludge studies and soil studies which attempted to identify metal retention mechanisms.

The following factors were identified by this researcher and the literature as potential metal retention mechanisms in a landfill or lysimeter:

a) Precipitation by hydroxyl, carbonate, and sulphide anions.

b) Insoluble complex formation.

c) Conversion of metal species.

d) Sorption to extracellular polysaccharides.

e) Sorption to cell walls.

f) Intracellular uptake.

g) Sorption to solids.

After compiling a list of possible metal retention mechanisms, the literature was reviewed for experimental procedures which would isolate metals associated with one or more of the mechanisms. The procedures which required less time in the laboratory and which were simple to perform were favoured. Techniques for sampling and testing lysimeter contents were sought. An anaerobic sampling and testing procedure was devised to avoid sample changes caused by exposure to air. A lysimeter from the UBC co-disposal study which had a moderate addition of septic tank pumpings and a large addition
of electroplating wastes was selected as a sample source. (This lysimeter was labelled lysimeter 'F' in the UBC co-disposal study.) This study concentrated upon the development of a procedure which estimated metal mobility because the mobility indicates the type of retention mechanisms.

Three experimental procedures were chosen for this study. One experiment used a series of progressive extractions to determine the relative mobility of metals in a lysimeter. A second experiment used a fluorescent dye (4,6-diamidino-2-phenylindole) to count bacteria, so the importance of bacteria as metal retention mechanisms, could be evaluated. The third experiment used a gradient of chelating agents to estimate the strength of natural chelating agents.

The progressive extraction technique was first developed by Engler (1977) to extract metals from river bottom sediments. The technique categorized metals according to their relative mobility and allowed a determination of the metal mass in each category. Samples were obtained from a suspension of lysimeter material which was made by blending a mixture of deionized distilled water and lysimeter contents. Three samples of lysimeter suspension were used. A sample which was not pretreated was used as a control sample. The other two samples were pretreated prior to the progressive extractions. The first pretreatment bubbled air through a sample to oxidize the sample and change the pH. A comparison of the control sample and the aerated sample data should give an estimate of the long term
metal stability. The second pretreatment sonicated a sample to rupture cells and break up particles. A comparison of the control sample and the sonication sample data could be used to estimate the intracellular metal uptake. The extraction procedure should separate the mobile, cation exchanged, complexed and precipitated metals. It will not differentiate between metals sorbed to cells, extracellular polysaccharides and particles. Stable inorganic complexes may not be separated from precipitates but organically bound metals are separated from inorganically bound metals.

The second experiment was a technique for enumerating bacteria. Whole cell counts could be made before and after sample sonication to allow a quantitative determination of the metals associated with bacteria. A literature search was undertaken to determine the most recent methods for counting bacteria. The dye 4,6-diamidino-2-phenylindole (DAPI) was selected for this study. It is specific for bacterial DNA and fluoresces bright blue when irradiated with ultraviolet light. Normally bacteria cannot be observed using an optical microscope but the dye enhances visualization greatly, to allow optical detection and photography. A fluorescent staining technique using DAPI was developed, with the help of researchers at Simon Fraser University, to enumerate the bacteria in lysimeter samples. Some method of enhancement was necessary because of the complex nature of the samples and the relatively small number of bacteria that were detected.
The third experiment was designed to compare the complexation strength of natural ligands with chelates of known complexation strength. Stability constants of the expected metal-chelate complexes were used to compare the strength of ethylene-diamine-tetra-acetic acid (EDTA), ethanoic acid, glycine, histidine, 8-hydroxyquinoline (oxine), nitrilo-tri-acetic acid (NTA) and oxalic acid with natural ligands. The chelating agents were selected to form a gradient of complexation strengths ranging from weak agents (eg. acetic acid) to strong ones (eg. EDTA).

Examples of anaerobic sampling from lysimeters were not found in the literature. This study was assumed to be one of the first to try anaerobic sample removal from a lysimeter. Anaerobic samples were used for testing to more accurately reflect conditions in a landfill. Hopefully this will help to gain a better understanding of the metal retention mechanisms in landfills without the difficulty of removing anaerobic samples from them. This study was possible because a three year old, airtight lysimeter with metal additions was available for testing and sampling.
II. POTENTIAL METAL REMOVAL MECHANISMS IN LANDFILLS

A. GENERAL

The complexity of a landfill becomes apparent when one starts to review landfill studies. Many facets of landfills have been studied in an attempt to understand what happens to refuse when it is interred. Some studies have tried to modify the form of the refuse and others have varied external factors in an attempt to affect refuse degradation. Most landfill studies analyze the products of a landfill (leachate or gas) to monitor the internal workings of a landfill. To date no one understands the internal workings of landfills well enough to predict the type of leachate which will be produced. With these facts in mind, studies which considered metals in leachate, sludge or soil were reviewed.

1. LEACHATE STUDIES

Many landfill studies only analyze the leachate which is generated. A few simple variables are known to have an effect upon the volume and type of leachate that escapes a landfill. Generally the concentration of inorganic and organic compounds in leachate declines over time after a peak concentration has been reached. Rainfall affects the leaching rate and the volume of leachate generated. The hydraulic gradient and soil conditions surrounding a site affect the leachate after it has been generated. In order to study leachates some researchers have constructed refuse leaching models called lysimeters.

Lysimeters are generally quite small compared to a landfill.
and there is some controversy over their validity as models of refuse leaching in landfills. They are constructed by adding alternating layers of soil and refuse with soil as the first and last layer. The bottom and sides must be sealed from air to simulate ground conditions. Most researchers apply water to simulate precipitation in their area and then study the leachate generated by the lysimeters. By building lysimeters, the contents can be categorized and variables such as compaction or shredding can be controlled to determine their effects upon leachate generation.

A lysimeter study done in Israel (Raveh and Avnimelech 1979) characterized the local wastes which go into landfills. They noted that the average percent food waste in Israel (50%) was much higher than the average reported for typical U.S. refuse (14%). They estimated the average percentage of food waste, paper plus cloth, plastics, glass, metal cans and leaves plus wood in Israeli refuse. Then they used those figures to construct their lysimeters. Raveh and Avnimelech measured the carbon content of leachate samples to characterize each lysimeter. They found that an exponential curve could be fitted to their data.

\[ C = a \cdot b^t \]

\( C = \text{mg. carbon/ml.} \)
\( t = \text{time in seasons (Two seasons/year).} \)
\( a, b = \text{constants} \)

The constants varied between lysimeters due to the different refuse densities and rainfall rates. They felt that the high correlation between the data and the exponential function could be used to describe the organic decay in the lysimeter. Raveh
and Avnimelech (1979) found that increasing the density and shredding the refuse tended to: slow the rate of organic decay, increase the dissolved metals, decrease the pH, and increase the volume of water retained. The decay of iron and manganese concentration in their leachate correlated with an increase in pH and a decrease in organic acids. When they analyzed the iron data they found that the concentrations were higher than solubility data would predict ($K_{sp} = 1.1 \times 10^{-10}$). Raveh and Avnimelech believed that the discrepancy was due to chelating agents in the leachate. They further postulated that the chelating agents caused the dissolution and stabilization of other heavy metals and may even extract heavy metals from the soil surrounding a landfill.

In another lysimeter study, Kinman and Walsh (1980) varied pH, prewetting, infiltration, sewage sludge loading and industrial waste load. They measured metal concentrations in each lysimeter by sampling leachate from several sample ports, over a forty-six month period. They compared the metal releases of lysimeters with industrial sludge and sewage sludge loads to control lysimeters which had no sludge loads. Cadmium, nickel, chromium and zinc were released, from lysimeters with industrial sludges, at the same rate or less than the control lysimeters. Iron releases were less than control in all cases except the sewage sludge lysimeters. The authors were not specific when reporting the lead releases. Some of the lysimeters with sludge showed an increased lead release with time but it is not known if all the sludge lysimeters responded similarly. Copper
releases were prevented by sewage sludge additions. In all other lysimeters copper concentrations were a function of sample location.

A study by Atwater et al. (1981) considered changes in leachate composition as a function of time, septic tank pumpings load and electroplating waste load. They used lysimeters to simulate a shallow landfill on an impervious soil layer. During three years of lysimeter operation, cadmium, chromium, and especially lead attenuation were affected by septic tank pumpings while iron, nickel and zinc were relatively unaffected. Atwater et al. stated that the initial solubility of metals in the electroplating sludge could mask the retention effect of septic tank pumpings. They went further postulating three possible mechanisms for metal attenuation: intracellular uptake, the formation of insoluble sulphides and complexation with organic chelating agents.

Pohland and Gould (1980) attempted to understand reactions in a landfill by studying lysimeters and equilibria equations. Their study was initiated so control strategies for management and regulation of landfills could be developed. They studied sulphide, hydroxyl and carbonate reactions to determine the fate of heavy metals in a site containing municipal and industrial wastes. Sulphides should dominate the equilibrium reactions because of precipitate formation with Zn$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Cu$^{2+}$ and to a lesser degree Fe$^{2+}$. Chromium should form partially soluble hydroxyl compounds. At low sulphide levels, carbonate precipitates of cadmium and lead should form while copper will
probably form a hydroxy-carbonate compound.

Some efforts have been made to construct a chemical analogue of leachate to help form theoretical models of landfill processes. In order to form an analogue, the complexing ability, ionic strength, pH and reduction-oxidation potential are usually modelled. Stanforth and Stegmann (1979) attempted to specify a synthetic leachate but they were unable to model biological aspects and sorption characteristics of the particulate matter in leachate. However, synthetic leachates can supply very general comments about some of the chemistry of leachates. Stanforth and Stegman (1979) assumed that amine proteins and the hydroxyl groups associated with humic and fulvic acids were the major complexing agents in leachates. They concluded that the solubility of metals increased as the activity and pH of the solution decreased.

Theis and Richter (1979) used a computer program to calculate equilibrium and complexation states in ash pond leachates. They acid-digested ash pond leachates to determine total metals, then assumed that Fe, Mn and Si oxides were major sorbing sinks. Their computer calculations showed that Cd, Ni and Zn solubilities were controlled by the adsorption capacity of Fe and Mn oxides while Cr, Cu and Pb concentrations were controlled by precipitate solubilities. When they tested leachate which had passed through soil, they found that Zn, Cd and Ni were strongly adsorbed by iron oxides and to a lesser degree by manganese oxides. Copper precipitated out of the leachate as copper carbonate (CuCO₃) while lead formed the
partially soluble precipitate lead carbonate (PbCO$_3$). Chromium formed hydroxyl complexes which are soluble when the pH $> 7.5$ (Cr(OH)$_n^-$ forms). All the metals that they tested formed weak sulphate complexes.

Theis and Richter's models were most strongly influenced by pH, sulphate and carbonate concentrations. Also, the reactions with chromium were strongly dependent upon the oxidation state. For example, Cr(VI) reacts with dissolved sulphides and sulphydryl groups but Cr(III) does not. Also, Cr(III) sorbs to solids and is oxidized by Mn oxides (Schroeder and Lee 1975). The pH is critical as Cr(VI) is reduced by Fe(II) while Fe(OH)$_3$, sorbs Cr(III).

When Sposito (1981) was constructing a computer model of heavy metal interactions in solution, he was unable to obtain stability constants for fulvic acids. The model he used considered complexation and solubility equations to predict metal species. As he was more interested in fresh water interactions, the reduction-oxidation potentials were not considered. Co-ordination chemistry indicated that the toxicity of metals increases as the oxidation state, electronegativity, ionic size, polarizability, and/or affinity of metal ions for sulfide ions and organic sulphur increase. Sposito (1981) was unable to make many conclusions due to the lack of reliability in computer models. Until a reliable method for determining the stability constants of heterogenous ligands can be found, models will only serve as guesses.

Many leachate studies have been undertaken but so far none
have been able to predict metal releases. Some researches have proposed mechanisms which may be regulating metal release but the large number of variables which affect leachate composition have obfuscated the understanding of metal attenuation. Variations in results have caused the lysimeter studies to be useful only for the specific case that they model. So far generalizations between studies appear controversial.

2. METALS IN BIOLOGICAL TREATMENT SYSTEMS

There is very little information regarding bacteria in a landfill. Methane generating bacteria, perhaps some sulphur reducing bacteria and an unspecified bacterial population which generates organic acids are present in a landfill. The anaerobic nature of a landfill suggests that some similarities may exist between anaerobic sludge bacteria and landfill bacteria. Anaerobic sludges are relatively unstudied due to the technical problems associated with anaerobic microbiology. This lack of information led to a search for bacteria metal interactions in aerobic sludges. Studies of aerobic sludge systems have identified a number of metal retention mechanisms which may also exist in landfills. So the literature was reviewed for bacteria-metal interactions in anaerobic and aerobic sludges.

Most of the interest in heavy metals associated with sewage has been stimulated by a need to ensure safe disposal mechanisms. Cameron and Koch (1980) found that heavy metals were being removed and stabilized by mixed aerobic treatment of leachate. They could not identify the metal species that were being removed and thus could not identify specific metal removal
Hayes and Theis (1978) separated sludge metals into four categories: soluble, precipitate, extracellular and intracellular metals. They used a bench scale anaerobic digester to determine the distribution of Cd, Cr(III), Cu, Ni, Pb and Zn (as nitrate salts) and Cr(IV) (as dichromate) in the biofloc. Biofloc was collected for analysis by removing settling biofloc from the digestors. They used the findings of Cheng et al. (1975) to separate extracellular metals. Precipitate metals were collected by acid rinsing the biomass with pH=4.0 nitric acid. The intracellular metals were collected by acid digesting the biomass.

They found a lack of Cd, Cr, Cu, Ni, Pb and Zn in the extracellular category and concluded that this was due to extracellular metal complexes being transported into the cell. These findings disagree with those of Brown and Lester (1979) who reported that approximately 30% of the metal uptake was due to extracellular polymers in activated sludge. Hayes and Theis reported significant metal uptake in the intracellular and insoluble precipitate categories. Thirty to sixty percent of the total metals in the biofloc were associated with the intracellular category. Metals removed by bacteria are not subject to solubility equilibria and may be more stable as intracellular metals than precipitates in the long term.

DeWalle et al. (1979) found metal precipitated as

2. Cheng et al. (1975) found that EDTA had a stronger affinity for heavy metals than extracellular polysaccharides of the cell wall.
sulphides, carbonates and hydroxides in a mixed anaerobic filter. An earlier study showed that humic and fulvic like acids are present in an anaerobic filter and could chelate heavy metals. The presence of natural chelators helps to explain why the total metal concentrations exceed estimates based on sulphide and carbonate solubilities. Also, the data of DeWalle et al. indicated that solubility mechanisms were more important than adsorption mechanisms for determining suspended and soluble metal distributions.

Oliver and Cosgrove (1974) reported that precipitation and adsorption to biofloc were the dominant removal mechanisms for Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Ni and Zn in an activated sludge system. The removal efficiency was related to the ratio of dissolved metal to total metals. Ghosh (1971) reported that sulphide and carbonate ions are important for precipitation of heavy metals. The sulphide and carbonate ions may protect digestor organisms from toxic levels of metals. Another factor may be the interactions of the metals present. Synergism and antagonism could have important effects upon toxicity and speciation of metals. Fannin et al. (1980) reported that the addition of hydroxyl groups to anaerobic digestors will reduce toxicity due to metals. They were more specific about the effects of sulphides and reported that Ni and Hg were absorbed. Carbonate ions affect the solubility of Zn and to a lesser degree Cd and Pb. Copper solubility was strongly affected by hydroxide ions. The carbonate, hydroxide and sulphide ion levels have an effect upon the competition between bacterial
ligands and precipitates for metals. Table I shows some of the precipitates that are expected to exist in an anaerobic digester.

Table I - Likely Precipitates

<table>
<thead>
<tr>
<th>Metal</th>
<th>Precipitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>CdS</td>
</tr>
<tr>
<td>Cr</td>
<td>Cr(OH)₃</td>
</tr>
<tr>
<td>Cu</td>
<td>Cu₂S</td>
</tr>
<tr>
<td>Ni</td>
<td>NiS</td>
</tr>
<tr>
<td>Pb</td>
<td>PbS</td>
</tr>
<tr>
<td>Zn</td>
<td>ZnS</td>
</tr>
</tbody>
</table>

After Hayes and Theis (1978).

If metals are present at high concentrations they can have inhibitory effects which may reduce the uptake of metals by bacteria. An early study by Barth et al. (1965) reported that bacteria in activated sludge can acclimatize to Cu and Zn but acclimatization did not protect the bacteria from slug doses of metals. The treatment efficiency of activated sludge decreased as the concentration of Cu, Cr, Ni and Zn increased. Barth et al. found a correlation between digestor failure and soluble metals. Poon and Bhayani (1971) determined that different micro-organisms are affected differently by different metals. They compared a pure culture of sewage bacteria to a pure culture of fungus and used an enzyme inhibition model (Michaelis-Menten model) to test the effect of one species of metal ions at a time. Their technique used a linearized equation (Lineweaver-Burk) and is limited to pure cultures and simple substrates.
Lester et al. (1979) isolated three activated sludge bacterial strains and tested the effects of Cr, Cu, Pb and Cd on the activity. In all cases, the bacteria showed an initial decline followed by a recovery after prolonged exposure to metals. A number of possibilities for acclimatization were cited: the metal species could be altered to a form which precipitates, soluble ammonia-metal complexes could form, and organic ligands may be released upon cell lysis. So the acclimatization of bacteria could cause an increase in metal uptake or an increase in metal solubility (if organic ligands formed soluble metal complexes).

Biological treatment systems remove heavy metals from sewage. The proportion of metal which is removed varies with the metal species. Metal speciation also affects bacterial growth as high concentrations of some metals will slow down or stop sludge degradation due to bacteria. Anaerobic bacteria can use intracellular metal storage to retain 30% to 60% of the metals measured in sludge. Aerobic bacteria use extracellular polysaccharides to bind metals and store small quantities of metal within the cells.

Precipitation occurs in both anaerobic and aerobic systems. The mass of metal which precipitates depends upon the carbonate, sulphide and hydroxyl anions which are available. Also the competition for anions, pH and metal species affect precipitate formation. A large proportion of the influent metal which is

3. Moore et al. (1961) showed that Cr(VI) could be reduced to Cr(III) causing Cr(OH), to precipitate.
removed from sewage precipitates out of solution.

Removal mechanisms which were reported to occur in anerobic systems include:

a) Precipitation by hydroxyl, carbonate and sulphide anions.

b) Intracellular Uptake.

c) Complex formation.

d) Changing the oxidation state of a metal to cause precipitation.

e) Exocellular Uptake.

Some metal adsorption to cell walls may occur but the literature was not specific.

3. METALS IN SOIL

Soil is a component of a landfill. As it is used to surround and cover refuse, studies on the mobility of metals in landfills should consider metal mobility in soils. Alesii et al. (1980) studied the flow rate of leachate through soil. The particle size (or clay distribution) critically affects attenuation of leachate. Retention of Cd, Cr, Ni and Zn was a function of influent concentration. Kirkham (1977) experimented with different soil types to observe the effect on metal uptake. Generally, the organic materials in soil were found to have a varying effect. Kirkham ranked metals according to the rate of metal uptake by the soil. The uptakes of Fe(III) and Cr(III) were the highest. The order of metal uptake reported by Kirkam was: Fe(III) and Cr(III) > Cu(II) > Ni(II) > Co(II) > Fe(II) > Mn(II) and Zn(II) > Cd(II). Nickel, dichromate and chromium(III) were bound strongly to serpentine soil, while only
chromium uptake increased in alkaline soils. Kirkham indicated that the predominant mechanism for cadmium uptake was via cation exchange. Fuller and Alesii (1979) outlined a list of chemical and physical parameters usually associated with the migration of heavy metals in soil. Some of these parameters are listed in Table II.

Table II - Chemical And Physical Soil Leaching Parameters

<table>
<thead>
<tr>
<th>Chemical Parameters</th>
<th>Physical Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area of Soil particles</td>
<td>Clay content</td>
</tr>
<tr>
<td>pH</td>
<td>Physical shape</td>
</tr>
<tr>
<td>Lime content</td>
<td>Depth of soil</td>
</tr>
<tr>
<td>Quantity of hydrous oxides of Fe, Mn, and Al</td>
<td>Stratification of soil</td>
</tr>
<tr>
<td>Soluble salts content</td>
<td>Compaction of soil</td>
</tr>
<tr>
<td>Organic matter content</td>
<td>Water infiltration</td>
</tr>
<tr>
<td>Cation exchange capacity</td>
<td>Frequency of cementations and accretions</td>
</tr>
<tr>
<td></td>
<td>Hydraulic conductivity</td>
</tr>
</tbody>
</table>

The clay content and pH effect the mobility of all metals. A drop in pH will increase the concentration of metals in solution and an increase in clay content decreases the rate of flow through the soil. The metals Cu, Fe, Mn and Zn are the most sensitive to clay content, free iron oxides and the total soil content of Co, Cr, Mn, Ni and Zn. There is a complex relationship between adsorption sites, ligands and competing
ions in the soil. When Griffin et al. (1976) were studying metal attenuation due to clay, they found that Fe and Mn were eluted from the soil. The elution could have been due to cation exchange or reduction of the Fe and Mn in clay to a more soluble species. They reported that Cd, Hg, Pb and Zn were attenuated more than the other metals studied. The attenuation of metals by clay was a function of cation exchange capacity, initial cations in the system, leachate composition and leachate pH. Above pH=6, precipitation is the dominant metal removal mechanism (Frost and Griffin 1977). Another study by Griffin et al. (1977) found that the cationic species of Cd, Cu, Cr(III), Hg, Pb and Zn were increasingly retained with rising pH while the anionic species Cr(VI), As and Se were decreasingly retained. Table III lists some further comments by Griffin et al. with respect to specific metal ions.

Tirch et al. (1979) used ammonium acetate to estimate the cation exchange capacity of soil. The degree of precipitation and the pH had a significant effect on the cation exchange capacity. They ranked three ions based on their success at competing for ammonium acetate sites [Cu > Cd > Ca]. Wollan and Beckett (1979) tried to characterize sludge-soil interactions by the ratio of extractable metal to total metal. Twelve months after the sludge had been added, Cu, Ni and Zn ratios were independent of the sludge type (for the two sludges that they tested). Their EDTA extractions (for Cu and Zn) and acetic acid extractions (for Ni and Zn) were sensitive to experimental conditions. They found that added heavy metals were more
Table III - Metal Ions In Soil

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>Not effective in competition with iron for cation exchange sites. When the pH &gt; 6 hydroxyl complexes are adsorbed by clay and lead carbonate precipitates form.</td>
</tr>
<tr>
<td>Cu, Cd, Zn</td>
<td>Form hydrolyzed ions increasing the total metals in solution as the pH rises.</td>
</tr>
<tr>
<td>Cu</td>
<td>Competes successfully for cation exchange sites.</td>
</tr>
<tr>
<td>Cr^6</td>
<td>Very competitive for anionic exchange sites.</td>
</tr>
<tr>
<td>HCrO₄⁻</td>
<td>Is adsorbed by soil.</td>
</tr>
<tr>
<td>Cr^3</td>
<td>Forms hydrolyzed ions in acid and forms a precipitate when the pH &gt; 5. Competes for cation exchange sites.</td>
</tr>
</tbody>
</table>

readily extracted than native heavy metals.

Metals are retained by different soils to varying degrees. Some of the metals are bound, but still available to plants, and some are very mobile. The cation exchange capacity, clay content, pH and rate of water application appear to be the most important variables affecting leachate mobility. Table IV summarizes the effects on metals of various soil properties. There are many other variables which affect metal interactions in soil but until the metal species in leachate can be determined, prediction of leachate properties will be hampered.
Table IV - Summary Of Soil Effects

<table>
<thead>
<tr>
<th>Property</th>
<th>Metals Most Effected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent concentration</td>
<td>Cd, Cr, Ni and Zn</td>
</tr>
<tr>
<td>Cation exchange capacity</td>
<td>Cu&gt; Cd&gt; Ca</td>
</tr>
<tr>
<td>Bound by Soil</td>
<td>Cr, Ni</td>
</tr>
<tr>
<td>Adsorbed by Clay</td>
<td>Cu, Fe, Mn and Zn</td>
</tr>
<tr>
<td>Adsorbed by Iron Oxides</td>
<td>Cu, Fe, Mn and Zn</td>
</tr>
<tr>
<td>Effected by total content of Co, Cr, Mn, Ni and Zn</td>
<td>Cu, Fe, Mn and Zn</td>
</tr>
<tr>
<td>Attenuated by Clay</td>
<td>Cd, Hg, Pb and Zn</td>
</tr>
</tbody>
</table>

4. NATURAL CHELATORS

Evidence presented by Knox and Jones (1979) and Raveh and Avnimelech (1979) indicated the existence of natural chelating agents in lysimeter leachates. Other studies have shown the existence of natural chelating agents in soil, peat and lakewater. Estimates of the stability and capacity of natural ligands would help to estimate metal mobility in a landfill or lysimeter. Soil, sludge and leachate studies which consider natural ligands have been reviewed in an attempt to determine the variables which affect complex formation in the natural environment. (Details of co-ordination chemistry are contained in Appendix B.)

Knox and Jones (1979) investigated the complexation of Cd in lysimeter leachate. They tried to evaluate the effects of pH and metal competition but were unable to maintain anaerobic
conditions. Their results indicate that one to one ligand-metal complexes were predominant. By studying the amount of metal complexed at varying pH, they concluded that carboxylic, phenolic, hydroxyl, sulfhydryl and nitrogen groups were the most likely ligand types. As they were unable to identify specific ligands they did not obtain stability constants. Precipitated iron flocs absorbed Cd and other metal cations but the reactions were pH dependent.

Cheng et al. avoided the problem of identifying ligands in sludge by determining an apparent stability constant. They separated free ions from complexed ions and found that complex formation tended to increase with pH until hydroxyl complexes started to form. EDTA, NTA, oxalate and glycine (in order of decreasing strength) were stronger chelating agents than sewage sludge. Their ranking for sludge uptake of Pb > Cu > Cd > Ni does not agree with the general order of stabilities determined by Irving and Williams (Cu > Ni > Cd). Cheng et al. found that the Irving-Williams order depended upon the ligands available and the pH.

Mantoura and Riley (1975) used a gel that achieved 92% and 72% recoveries for humic acid and fulvic acid respectively. The gel was assumed to separate free ions from complexed ions. Mantoura and Riley used a Scatchard plot to calculate stability constants for complexes of Cu(II), Ni(II) and Zn(II) with humic and fulvic acids extracted from soil, peat, and lakewater. Table V shows stability constants for a few humic-metal complexes.
MacCarthy (1977) developed a theoretical method for calculating stability constants for soil samples assuming Schubert conditions ([ligand] >> [metal]). MacCarthy's method estimates an overall average stability constant for a solution under given temperature, pH, and ionic strength. He estimated the concentration of free and soil complexed metals by using a gel to complex free ions and by varying concentrations of a known ligand.

In a qualitative study Truitt and Weber (1979) found that fulvic acid enhanced the removal of Cu(II), Cd(II), and Zn(II) in wastewater during alum coagulation. They ranked five metals according to the relative strength of the fulvic acid-metal complex. (Pb, Hg > Cu > Cd, Zn). In a further study by Mantoura et al. (1978), it was reported that the traditional assumptions used for speciation modelling are not valid for high ionic strength solutions. They assumed that the humic acid-metal complexes were roughly one to one. Also, mixed ligand complexes, polynuclear complexes and interphase reactions were not significant. Although they used the Davies equation they concluded that humic-metal complexes were very significant in fresh water. Copper, calcium, and magnesium and to a lesser degree mercuric chlorides, complexed the humics in sea water.
The model used by Mantoura et al. was very sensitive to pH and the assumed stability constants. The stability constants that they were able to determine for humic-metal complexes are shown in Table VI.

<table>
<thead>
<tr>
<th></th>
<th>Ca$^{2+}$</th>
<th>Ni$^{2+}$</th>
<th>Cu$^{2+}$</th>
<th>Zn$^{2+}$</th>
<th>Cd$^{2+}$</th>
<th>Mg$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>3.65</td>
<td>4.98</td>
<td>7.85</td>
<td>4.83</td>
<td>4.57</td>
<td>3.81</td>
</tr>
<tr>
<td>Lakes</td>
<td>3.95</td>
<td>5.14</td>
<td>9.83</td>
<td>5.14</td>
<td>4.57</td>
<td>4.00</td>
</tr>
<tr>
<td>Seawater</td>
<td>4.12</td>
<td>5.51</td>
<td>9.71</td>
<td>5.31</td>
<td>4.69</td>
<td>3.98</td>
</tr>
</tbody>
</table>

Table VI - Log10 Stability Constants For Humics - II

After Mantoura et al. (1978).

Stoveland et al. (1979) found that NTA in sewage formed soluble metal complexes. They also added sodium tripolyphosphate to sewage and found that it formed metal complexes which precipitated. This indicates that soluble metal ions or complexes are available and that the soluble complexes are relatively weak. Jellinek and Sangal (1971) used a number of natural polyelectrolytes to determine their effectiveness at metal complexation in polluted water. None of the polyelectrolytes (polymethacrylic acid - from bovine albumin and gelatin; alginic acid - from gelatin; pectin; oxidized starch - from gelatin; and polygalacturonic acid (PGA)) complexed all of the metals tested (Cd, Cu, Ni, Zn and Cr(VI)). They found that PGA was the most effective chelating agent as it formed complexes with all the metals except Cr(VI). Estimates of the
PGA-metal complex stabilities are shown in Table VII. Their results indicate that more than one ligand is responsible for natural complexation.

Table VII - Log10 Stability Constants - PGA

<table>
<thead>
<tr>
<th>Metal</th>
<th>Log10</th>
<th>Removal Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu$^{2+}$</td>
<td>1.7</td>
<td>98%</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>0.59</td>
<td>90%</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>0.21</td>
<td>70%</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>0.34</td>
<td>78%</td>
</tr>
<tr>
<td>Cr$^{3+}$</td>
<td>---</td>
<td>75%</td>
</tr>
</tbody>
</table>

Note: Data was collected at 20°C by Jellinek and Sangal (1972).

In a study of chelation by NTA and EDTA from anthropogenic sources, Zn, Cd, and Cu chelation were favoured (Raspor et al. 1981). As long as Ca and Mg concentrations were not four orders of magnitude greater than the metal concentrations, the metals were chelated. The presence of humic materials only affected Cu(II) and Hg(II) concentrations.

Measurement of chelating capacity has been most successful in soils because a gel has been discovered which isolates the humic and fulvic acids. A major element in the determination of the chelating capacity in leachates, soils and water is the experimental technique being employed. To date researchers have been unable to measure chelating capacity directly but a number of observations can be made regarding the factors which influence chelate capacity. The following factors have been
shown to be significant in the competition of metals for complexing ligands.

a) Metal species.
b) pH.
c) Temperature.
d) Exposure to air.
e) Available ligands (e.g., fulvic acids, OH radical).
f) Available radicals for precipitate formation (e.g., sulphides).
g) Available sorbing sinks (e.g., iron and manganese oxides).
h) Clay content of soil in landfill.
i) Ionic strength of leachate.

The stability constants reported by Mantoura et al. (1978) are the most comprehensive available for humic-metal complexes in soils and water. Their findings and those of others indicate that Pb²⁺, Hg²⁺, Cu²⁺, Ni²⁺, Zn²⁺ and then Cd²⁺ are most likely to form natural complexes in soils and water.

5. BACTERIA AND METALS

Most studies of bacteria in sludge, leachate or sewage fall into two categories. They are either studies of a few specific bacteria known to exist in the environment or studies of unspecified bacterial masses. Bacteria definitely play some role in landfills but the extent of the role with respect to metal retention is difficult to determine. There is evidence suggesting that bacteria can bind heavy metals in extracellular or extracellular polysaccarides. Considerable speculation
exists regarding the sorbing capabilities of bacteria.

Guthrie et al. (1977) isolated naturally occurring bacteria and used pure strains to determine metal uptake. Hatch and Menawat (1979) isolated a pure bacteria strain and a pure fungus strain. They isolated the extracellular polysaccharides associated with a bacteria (Sphaerotilus natans) and a fungus (Leptonitus). Both micro-organisms occur in sludges and polluted waters and were found to bind metal salts. When the protein-polysaccharide-lipid complex that forms the extracellular polysaccharides was analyzed by a scanning electron microscope and X-ray crystallography, a uniform deposit of iron was found. The iron was distributed throughout the cell mass as a fine extracellular precipitate. Hatch and Menawat were able to grow S. natans in sulphates of Fe, Cu, Co, Cd and Mg. Their data showed that the bacteria reached a metals saturation point. An extensive literature review by Brown and Lester (1979) reported that the biofloc, which forms in an activated sludge process, sorbs a large quantity of metals. Also, more metal was sorbed by the viable sludge than the non-active sludge. They outlined four possible mechanisms for metal removal by biofloc and four types of metal transformation by bacteria that could cause an increase in metal sorption. Table VIII lists the removal and transformation mechanisms presented by Lester and Brown.

The least likely mechanism is the volatilization of metals to the atmosphere as there are no mass balance studies to support this theory. All of the other mechanisms have been
Table VIII - Biofloc - Metal Interactions

<table>
<thead>
<tr>
<th>Removal Mechanisms</th>
<th>Metal Transformations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical trapping of precipitates</td>
<td>Formation of organo-metallic complexes in floc</td>
</tr>
<tr>
<td>Binding of soluble metal to exocellular polysaccharides</td>
<td>Metal substitution</td>
</tr>
<tr>
<td>Accumulation of soluble metals in cells</td>
<td>Biomethylation</td>
</tr>
<tr>
<td>Volatilization of metal to atmosphere</td>
<td>Changing the metal valency</td>
</tr>
</tbody>
</table>

shown to occur to some degree. Extracellular polysaccharides could bind soluble metals by sorption and/or cation exchange. It has been shown by many researchers that extracellular polysaccharides retain metals. Further details of extracellular polysaccharides metal uptake mechanisms are shown in Appendix C. Generally extracellular polysaccharides retain metals by adsorption, chemical bonding, absorption, complexation or by providing sites for precipitation. Both anaerobes and aerobes are capable of manufacturing extracellular polysaccharides. Also it may be possible for some bacteria to oxidize extracellular polysaccharides to form stable end products.

B. SUMMARY

Leachate studies show that metals are retained to varying degrees in lysimeters. Very little information is available to explain why some metals are retained longer than others. Also, little has been done to estimate the long term stability of metals in a lysimeter. The metal species seems to be an
important factor for metal retention and stability but there are few studies of the metal species in landfills or lysimeters. It is most likely that multiple factors are responsible for the retention of each metal in a lysimeter or landfill.

The degree of metal fixation by bacteria in a landfill is not documented. Studies of aerobic and anaerobic sewage treatment systems show that bacteria can sorb and perhaps complex metals. Bacteria probably immobilize metals in their surrounding environment by one or more of the following methods:

a) Intracellular uptake.
b) Sorption to cell wall.
c) Bonding with extracellular polysaccharides.
d) Entrapment in extracellular polysaccharides.
e) Conversion of metal to a species which complexes or precipitates.

Three other metal removal mechanisms could occur in a lysimeter or landfill: sorption, precipitation and complexation. Precipitates of sulphides, carbonates and hydroxides are expected to form. (A list of expected precipitates is shown in Table I.) Complexation occurs between organic components of soil and metals. Organic components other than humic and fulvic acids may complex metals. Both precipitation and complexation are affected by pH, metal species and competition for reactive radicals.

Sorption by soils may also be a significant metal removal mechanism. The most important variables affecting sorption by soils are the clay content, cation exchange capacity, pH and
rate of water application. If iron or manganese oxides are present, they can adsorb some metal species.
III. EXPERIMENTAL PROCEDURES

A chelation experiment, an extraction procedure and a fluorescent dye technique were used to determine the metal mobility, bacterial numbers and the relative strength of natural ligands in a lysimeter. The first experiment was a progressive extraction technique which categorized metals according to their mobility. Three samples were tested with this procedure: a sonicated sample, an aerated sample and an untreated sample used as a control. The mass of metals extracted in each category was divided by the dry weight of each sample for comparison of the three sample types. Sonication should rupture cells releasing intracellular metals and aeration should cause all metals, which are not stable when exposed to air, (i.e. Fe(II) will be converted to Fe(III)) to be released. By using a second experiment to determine bacterial numbers it may be possible to correlate some metal releases with the destruction of viable bacteria. For these purposes the fluorescent dye 4,6-diamidino-2-phenylindole was tested. The third experiment used a gradient of chelating agents to compare the strength of natural ligands with ligands of known complexing ability. Thus, an estimate of the stability constants for metal-natural ligand complexes could be made. All three experiments required suspended samples which had not been exposed to air. To achieve this, samples were removed in a nitrogen atmosphere from one of the lysimeters used in the UBC co-disposal study. After removal, the samples were blended with deionized distilled water to form a slurry. A short discussion of the anaerobic sampling method is included in
this chapter and further details are provided in Appendix F.

A. SAMPLING

1. SAMPLE SOURCE

A lysimeter which had electroplating wastes added to it was selected for sampling. The electroplating wastes boost metal concentrations in the lysimeter simplifying metal detection requirements. One lysimeter from the co-disposal study conducted by Atwater et al. (1981), was used as the sample source. Plots of metal release vs time for the lysimeter indicate that the rate of metal release had stabilized. (Plots of metal release vs time for the lysimeter are reproduced in Appendix A.) The age of the lysimeter may have helped to reduce complications which could arise in data interpretation.

Figure 2 is a cross-section view of the lysimeter. It shows the internal layers and the locations of the sampling holes. The first hole was 15 cm. from the bottom of the lysimeter. This sample site was estimated to coincide with the top of the first lift. All but the top lift were composed of a 15 cm. layer of refuse compacted at 220 psi. The final lift was a 15 cm. layer of soil. A second hole was made 45 cm. from the bottom of the lysimeter and should be near the top of the third lift. These locations were chosen because electroplating wastes were added to the top of the first and third lifts.
Figure 2 - Cross-section Of Lysimeter

Where:

- Native Clay Loam Soil
- Refuse
- Pea Gravel Nominal (0.6)
- Electroplating Waste
- Septic Tank Pumpings

Note: All refuse was hydraulically packed at a maximum pressure of 220 psi.
All dimensions are in cm.
After Atwater et al., 1981

2. LIQUID SAMPLE COLLECTION

Before drilling a hole in the lysimeter a leachate sample was collected from the bottom of the lysimeter. This sample was taken to ensure that no changes had occurred in the lysimeter since the last metal analysis of the co-disposal study. A nitrogen atmosphere was maintained in the leachate collection flask to prevent precipitate formation. All of the samples
collected were analyzed using a Jarrel Ash 810 atomic absorption spectrophotometer. An estimate of the liquid volume required for analysis was based on data from the UBC co-disposal study. The data and calculations are shown in Appendix I.

The leachate was allowed to drain by gravity into a flask filled with nitrogen gas. The nitrogen gas pressure was kept slightly above atmospheric pressure by submerging the gas exit tube 5 mm. below the surface of a beaker of water. This prevented an air leak in the flask if the gas flow failed.

3. SOLID SAMPLE COLLECTION

Solid samples were collected in a nitrogen atmosphere to avoid pH or metal speciation changes. A pressure of 4 cm. of water above atmospheric was used to prevent contamination by air. Also, a nitrogen atmosphere may help to keep true anaerobic bacteria viable until they are fixed. A sampling chamber, made of lucite, was constructed to fit against the side of the lysimeter. (A detailed diagram of the chamber is shown in Appendix F.) The chamber was designed to allow sampling to occur in a nitrogen atmosphere. Nitrogen gas was supplied from a 100 lb. cylinder with a regulator to maintain a steady nitrogen pressure. Details of the sampling procedure are contained in a step by step procedure listed in Appendix F.

After a hole was drilled into the side of the lysimeter, two drill bits were used to remove samples. One was a steel tube with a band saw blade welded onto the end. The other was a bar with two tungsten carbide teeth and a high speed twist drill welded to it (Sketches of the drill bits are shown in Appendix
F). Both bits tended to tear pieces of sample from the lysimeter resulting in a shredded sample.

A stainless steel tube or blade was used to pick refuse pieces from the lysimeter. The pieces were piled on a paper towel which was used to funnel the refuse into a 4 litre nalgene container. When sufficient sample had been collected, the nalgene container was sealed using a thin film of plastic ('Parafilm').

To calculate the approximate volume of sample that was required, it was assumed that the metals in the lysimeter were uniformly distributed. The masses of metals in the lysimeter were estimated by subtracting the masses of metals leached from the masses applied, to the lysimeter. This should give a low estimate of the metals contained in the lysimeter as the masses of metals in the refuse are not included. The calculations are shown in Appendix J. Roughly 1200 ml. of solid sample are required for the three experiments.

4. **SAMPLE STORAGE**

All the containers used to store samples were acid washed with a 30% nitric acid solution then rinsed with deionized distilled water. Each pipette, funnel, spatula or other implement that contacted the sample was acid washed and rinsed with deionized distilled water. Samples which were not in use were sealed, using parafilm, and stored at 4°C, away from light.

4. The manufacturers of parafilm claim that it prevents gas diffusion.
Large containers were sealed using parafilm which was taped to make sure it stayed in place. The small 50 ml. Erlenmyer flasks used for bacterial samples were sealed with a plastic stopper. Bacterial samples were fixed with ethanol or 5% formaldehyde solutions to prevent bacterial growth.

5. SAMPLE TREATMENT

Before the solid samples were tested, the solids were suspended in deionized distilled water. A Waring blender was used to homogenize the solids. Large pieces of metal, porcelain and wood were removed from the sample to avoid damaging the blender. Solids and deionized distilled water were added to the blender to make a total volume of 600 ml. in the blender. Initially all the blending was conducted outside the anaerobic chamber. So a lucite top with a neoprene seal and elastics, to hold the top down, was used to keep samples anaerobic (A diagram is shown in Appendix F). When solids from the second hole were blended, all the blending was conducted inside the chamber using the lucite top to retain splashes.

The first solids sample was roughly 1.2 L. and the second was 0.9 L. before dilution. The first sample was blended and diluted to 10 L. The second sample was divided into three samples. Each third was diluted to 0.9 L. and blended just prior to use. This was more convenient as smaller volumes were manipulated. A mass balance was used to determine the dry mass of lysimeter solids being tested in each sample.
6. SAMPLE ANALYSIS

Gas samples were taken from the bench top anaerobic chamber, the anaerobic sampling box and the liquid collection flask. A Fisher Hamilton gas partitioner was used with a Hewlett Packard strip chart recorder to determine oxygen levels. In all cases five minutes of flushing with nitrogen reduced oxygen to less than 0.5\% of the atmosphere. So the various anaerobic chambers were flushed for five minutes before a sample was exposed to the nitrogen atmosphere.

A Jarrel Ash 810 atomic absorption spectrophotometer linked to a Hewlett Packard 7127A strip-chart recorder was used for metals analysis. Other analysis methods were considered and they are discussed in Appendix H. The advantages of the atomic absorption spectrometer over the other methods were: operating simplicity, minimal sample preparation, availability and repeatability. The desired accuracy was obtained using the parameters listed in Table IX. Prior to analysis with the Jarrel Ash 810 atomic absorption spectrophotometer, all samples were acid digested using concentrated nitric acid as recommended by S. Liptak of the UBC Environmental Engineering Laboratory. Each sample was evaporated to dryness then the residue was redissolved using concentrated nitric acid. The mixture was diluted with deionized distilled water and filtered through a Whatman number 541 filter before diluting up to a final volume of 100 ml. Stock solutions supplied by Fisher Chemicals were used to calibrate the atomic absorption spectrometer readings.
To avoid inaccuracies due to old solutions, all the stock solutions were compared to freshly made test solutions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamp Current in ma.</td>
<td>6</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Flame Type</td>
<td>Oxid</td>
<td>Redu</td>
<td>Oxid</td>
<td>Redu</td>
<td>Oxid</td>
<td>Oxid</td>
<td>Oxid</td>
</tr>
<tr>
<td>Absorbing Wavelength (A)</td>
<td>2289</td>
<td>3579</td>
<td>3247</td>
<td>2484</td>
<td>2320</td>
<td>2833</td>
<td>2138</td>
</tr>
<tr>
<td>Wavelength (A)</td>
<td>2268</td>
<td>3520</td>
<td>2961</td>
<td>2316</td>
<td>2825</td>
<td>2127</td>
<td></td>
</tr>
<tr>
<td>Non-absorbing Wavelength (A)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

An attempt to measure pH and oxidation-reduction potential (orp) was made to characterize the insitu conditions of lysimeter F. Unfortunately the orp probe started to respond erratically during warmup. Another orp probe was not available at that time so an orp measurement was not made. The pH measurements were obtained by surrounding the tip of the probe with the lysimeter contents. This was done to ensure contact with the probe tip because there was no free liquid in the lysimeter. The pH probe was placed three times to ensure consistency. All the pH readings and calibrations were conducted in a nitrogen atmosphere to avoid exposing the lysimeter contents to air.
B. CHELATION PROCEDURE

A chelation experiment was devised to test the existence and strength of natural ligands in a lysimeter. The experiment compared the metal masses complexed by seven chelating agents (EDTA, ethanoic acid, glycine, histidine, 8-hydroxyquinoline (oxine), NTA and oxalic acid) to the masses that they should theoretically complex. This is accomplished by plotting the mass of metal complexed vs the ligand used for each complex. A separate curve is generated for each metal considered. A theoretical curve can be generated by plotting the mass of metal that would be expected to complex with each ligand knowing the initial metal concentration, the initial ligand concentration and the stability constant for each complex. If the ligands are arranged in order from weakest to strongest a chelate gradient is established. By correcting the measured mass of metal for chelation efficiency the measured data can be compared to the theoretical data. Any differences between the theoretical and measured curves should indicate the presence of natural ligands. The point where the theoretical and measured curves diverge represents the upper limit of natural ligand complex strength. It is possible to further refine the experiment by selecting ligands to provide a series of smaller increments for the chelate gradient. The stability constants, that were available, for the metals tested are shown in Table X.

Two different experimental techniques were used to determine the mass of metal complexed. The first was called the
### Table X - Log10 Stability Constants

<table>
<thead>
<tr>
<th>Ion</th>
<th>EDTA</th>
<th>Etha</th>
<th>Glyc</th>
<th>Hist</th>
<th>8-Hy</th>
<th>NTA</th>
<th>Oxal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu²⁺</td>
<td>18.92</td>
<td>2.23</td>
<td>8.6</td>
<td>10.21</td>
<td>13.49</td>
<td>11.5</td>
<td>4.85</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>18.36</td>
<td>1.43</td>
<td>6.4</td>
<td>8.5</td>
<td>11.44</td>
<td>11.54</td>
<td>5.16</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>16.26</td>
<td>1.57</td>
<td>5.9</td>
<td>6.4</td>
<td>9.96</td>
<td>10.44</td>
<td>3.44</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td>18.32</td>
<td>2.68</td>
<td>5.11</td>
<td>5.96</td>
<td>10.61</td>
<td>11.47</td>
<td>3.32</td>
</tr>
<tr>
<td>Cd²⁺</td>
<td>16.9</td>
<td>1.93</td>
<td>6.0</td>
<td>5.65</td>
<td>9.43</td>
<td>10.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Fe³⁺</td>
<td>25.1</td>
<td>2.63</td>
<td>10.0</td>
<td>4.0</td>
<td>13.69</td>
<td>15.91</td>
<td>7.39</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>14.2</td>
<td>1.82</td>
<td>4.3</td>
<td>5.85</td>
<td>8.77</td>
<td>8.84</td>
<td>3.05</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>14.5</td>
<td>1.4</td>
<td>3.9</td>
<td>3.24*</td>
<td>7.3</td>
<td>8.6</td>
<td>3.97</td>
</tr>
<tr>
<td>Cr³⁺</td>
<td>23.4</td>
<td>---</td>
<td>8.62</td>
<td>---</td>
<td>13.3</td>
<td>13.5</td>
<td>5.34</td>
</tr>
<tr>
<td>Cr²⁺</td>
<td>13.61</td>
<td>1.8</td>
<td>10*</td>
<td>---</td>
<td>13.29</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>11.0</td>
<td>1.28</td>
<td>---</td>
<td>---</td>
<td>4.35*</td>
<td>5.46</td>
<td>2.39*</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>11.0</td>
<td>1.12</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>6.46</td>
<td>1.66</td>
</tr>
<tr>
<td>Al³⁺</td>
<td>16.7</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>9.5</td>
<td>---</td>
</tr>
</tbody>
</table>

Note: * - indicates a stability constant for two Ligands per metal.

After Sillen 1971.

The method of standard comparisons and the second was called the method of standard additions. Both techniques required the use of spike solutions or standard solutions. The standards were made up by mixing atomic absorption stock solutions in molar proportions that were similar to those expected in the samples. Table XI lists the metal concentrations that were used to estimate the moles of chelating agents that would be required. Table XII lists the concentrations of each metal in the spike solutions.

The method of standard comparisons assumes that the extraction efficiency of each chelating agent is the same for the sample and the standards. Also, the metal species in the standards and sample are assumed to be the same. This last assumption was found to be invalid after the first set of chelation data was collected. It was discovered that a precipitate was forming in the standards rendering them useless.
Table XI - Applied And Measured Metals In Lysimeter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm/mole</td>
<td>112.4</td>
<td>52.0</td>
<td>63.5</td>
<td>55.9</td>
<td>58.7</td>
<td>207.2</td>
<td>65.4</td>
</tr>
<tr>
<td>Net input In mM</td>
<td>4.78</td>
<td>153</td>
<td>14000</td>
<td>13.8</td>
<td>180</td>
<td>2400</td>
<td></td>
</tr>
<tr>
<td>uM/ml of contents</td>
<td>0.096</td>
<td>3.06</td>
<td>279</td>
<td>0.276</td>
<td>3.6</td>
<td>48.0</td>
<td></td>
</tr>
</tbody>
</table>

Measured Values in uM metal/gm dry wt

<table>
<thead>
<tr>
<th>Sample</th>
<th>A-raw</th>
<th>B-blended</th>
<th>C-blended</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4</td>
<td>0.35</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>0.535</td>
<td>0.205</td>
</tr>
<tr>
<td></td>
<td>0.59</td>
<td>0.178</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>24.6</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>0.032</td>
<td>0.175</td>
<td>0.143</td>
</tr>
<tr>
<td></td>
<td>1.37</td>
<td>2.11</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>4.73</td>
<td>5.15</td>
<td>9.83</td>
</tr>
</tbody>
</table>

Notes: 1. Net tank values from Atwater et al. (1981).
2. A--14.2 gms. of lysimeter material digested in acid.
   Sample was taken near a layer of electroplating waste.
   14.2x0.4 = 5.68 gms. dry weight.
3. B--258.4 gms. of blended sample. Sample was taken near a layer of electroplating waste.
   258.4x0.013 = 3.4 gms. dry weight.
4. C--70.6 gms. of blended sample. Sample was taken near a layer of septic tank waste.
   70.6x0.069 = 4.87 gms. dry weight.
5. Six different moisture content determinations were taken for three lysimeter samples:
   0.394, 0.364, 0.439 giving an average = 0.40
6. Tank Volume = 50 l.
   Diameter = 30 cm.
   Height = 70 cm.
   Volume = \( \pi \times 70(30)^{2}/4 \)
   = 50000 cm\(^3\) = 50 l.
   The gravel layer (15 cm.) and the compressed soil cover (10 cm.) were subtracted from the height of the lysimeter contents (95 cm.) for the height estimate.

Table XIII lists the solubility of various chromium compounds which could be precipitating in the standards. After some testing it was determined that lead chromate (PbCrO\(_4\)) had
Table XII - Concentration Of Metals In Spike Solutions

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS1 uM/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 ml.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix 1</td>
<td>0.44</td>
<td>4.81</td>
<td>1.34</td>
<td>206</td>
<td>1.45</td>
<td>4.83</td>
<td>36.7</td>
</tr>
<tr>
<td>Mix 2</td>
<td>0.04</td>
<td>0.48</td>
<td>0.13</td>
<td>20.6</td>
<td>0.14</td>
<td>0.48</td>
<td>3.8</td>
</tr>
<tr>
<td>Spike 3</td>
<td>0.44</td>
<td>3.85</td>
<td>--</td>
<td>35.8</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Mix 4</td>
<td>0.89</td>
<td>9.62</td>
<td>3.15</td>
<td>358</td>
<td>3.41</td>
<td>9.65</td>
<td>76.5</td>
</tr>
<tr>
<td>Mix 5</td>
<td>0.18</td>
<td>1.92</td>
<td>0.63</td>
<td>71.6</td>
<td>0.68</td>
<td>1.93</td>
<td>15.3</td>
</tr>
<tr>
<td>DS2 uM/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spike</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>2.5</td>
<td>16.9</td>
<td>5.67</td>
<td>358</td>
<td>27.3</td>
<td>8.88</td>
<td>67.3</td>
</tr>
<tr>
<td>50</td>
<td>1.25</td>
<td>8.4</td>
<td>2.83</td>
<td>179</td>
<td>13.6</td>
<td>4.44</td>
<td>33.6</td>
</tr>
<tr>
<td>10</td>
<td>0.25</td>
<td>1.69</td>
<td>0.57</td>
<td>35.8</td>
<td>2.7</td>
<td>0.89</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Notes:
1. DS1 = Chelation data set one
2. DS2 = Chelation data set two
3. Mix 1, 2, 3, 4 or 5 = Standards which were chelated.
4. Spike 3 = This is a spike which was added to a sample.
5. Spike 10, 50 or 100 = These are spike solutions which were added to samples for data set two.

Table XIII - Solubility Of Chromium Compounds

<table>
<thead>
<tr>
<th>Metal</th>
<th>M(Cr$_2$O$_4$)</th>
<th>M(Cr$_2$O$_7$)</th>
<th>M(CrO$_4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>i</td>
<td></td>
<td>i</td>
</tr>
<tr>
<td>Cu</td>
<td>i</td>
<td>vs</td>
<td>i</td>
</tr>
<tr>
<td>Fe</td>
<td>i</td>
<td>s</td>
<td>5.8x10^-6</td>
</tr>
<tr>
<td>Pb</td>
<td>i</td>
<td>d</td>
<td>i</td>
</tr>
<tr>
<td>Zn</td>
<td>i</td>
<td>vs</td>
<td>i</td>
</tr>
</tbody>
</table>

After Weast (1971).
Note: CrO$_4^-$ and Cr$_2$O$_7^-$ should be in equilibrium in the spike solution, with a majority of the chromium in the CrO$_4^-$ form.
Where: i=insoluble
d=decomposes
s=soluble
vs=very soluble
precipitated in the spike solutions. Instead of using the atomic absorption spectroscopy stock solution for chromium spikes, a chromium chloride (CrCl$_3$) solution was used.

The second technique (method of standard additions) was used to avoid the problems of choosing standards in the same concentration range as the samples. Spike solutions were added to three samples and a fourth sample had no spike addition. The atomic absorption spectrometer readings were plotted vs the percent spike added to the sample (The plots are shown in Appendix 0). The concentration of each metal in the spike solution was known (Values are listed in Table XII) so it is theoretically possible to determine the concentration of complexed metal in a sample. There is an underlying assumption with this technique, that the complex formation and extraction efficiency is the same for the spike solutions and the samples.

The organic solvent methyl-iso-butyl ketone$^5$ (MIBK) was used to extract all the complexes. MIBK has a lower density than water so it forms a layer above water. The MIBK and sample mixture were well mixed by shaking and then left to sit overnight. That allows the MIBK enough time to dissolve a portion of the metal complexes and to form a layer above the water-based sample. In this study it was necessary to centrifuge samples to remove the solids because they tended to float at the MIBK-water interface making separation of the two

5. Care should be taken when using MIBK because it is very volatile. It is also a skin and inhalation irritant.
layers impossible. The resulting supernatant contained MIBK and a water solution which was readily separated.

Each chelate was allowed to react independently so there was one sample per chelate and seven samples per sample set. The number of moles of each chelate was determined after considering the strength of the chelate, the number of binding sites per molecule of chelate and the total moles of metal expected in a sample. The ligands were added so that they would be a factor of five in excess of the expected metals. This should allow other potential complexes (eg. calcium complexes) to form without causing a ligand shortage. Table XIV lists the moles of chelating agent added to the samples.

All the chelation reactions were contained in extraction funnels as they are specifically designed for chelation extractions. Figure 3 shows a diagram of an extraction funnel. The valve at the bottom was used to gravity drain the water layer from the funnel. Plastic stoppers were used to help keep the samples anaerobic until the chelating agents were added. Unfortunately there was insufficient clearance in the anaerobic chamber to allow pipetting of the chelating agents.

After the chelating agents were added, the extraction funnels were shaken and allowed to sit overnight. MIBK was added the next day and the samples were mixed and left overnight again. Then the whole sample was transferred to light polyethylene bottles for centrifuging (Nalagene centrifuge bottles were attacked by MIBK). After centrifuging, the
Table XIV - Moles Of Chelating Agent

<table>
<thead>
<tr>
<th>Binding Sites/Ligand</th>
<th>EDTA</th>
<th>Eth</th>
<th>Gly</th>
<th>Hist</th>
<th>Hyd</th>
<th>NTA</th>
<th>Oxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ligand] mM/l</td>
<td>95.4</td>
<td>1715</td>
<td>857</td>
<td>171.2</td>
<td>85.42</td>
<td>425.5</td>
<td>856.3</td>
</tr>
<tr>
<td>mM Bond Sites/ml</td>
<td>0.38</td>
<td>1.7</td>
<td>0.86</td>
<td>0.17</td>
<td>0.085</td>
<td>1.28</td>
<td>1.7</td>
</tr>
<tr>
<td>mM Added to DS1</td>
<td>7.6</td>
<td>11.9</td>
<td>6.0</td>
<td>3.0</td>
<td>6.0</td>
<td>9.0</td>
<td>11.9</td>
</tr>
<tr>
<td>mM Added to DS2</td>
<td>4.77</td>
<td>17.15</td>
<td>8.57</td>
<td>6.85</td>
<td>5.98</td>
<td>6.38</td>
<td>8.56</td>
</tr>
</tbody>
</table>

Note:
1. DS1 used 120 ml. samples with a 0.03 solids content = 3.6 gm. dry weight.
2. DS2 used 50 ml. samples with a 0.0363 solids content for Blk and 50% samples = 1.8 gms dry weight. Samples 10% and 100% had a 0.0307 solids content = 1.5 gms dry weight.
3. DS1 required 2.5 mM. of chelating agent.
   DS2 required 1.5 mM. of chelating agent.

Supernatant was decanted into the extraction funnels. Then the water layer was removed. The MIBK layer was drained through a Whatman number 541 filter and collected for analysis with the atomic absorption spectrometer. A flow chart of the chelation procedure is shown in Figure 4.

C. METAL EXTRACTION PROCEDURE

A successive extraction technique was used to determine the mobility of metals. Samples were extracted using a slightly modified version of a procedure developed by Engler (1977). The metals were grouped into six categories: free, readily extracted, cation exchangeable, organically bound, moderately extractable and residual. Engler's procedure was modified by
using a centrifuge to separate supernatants instead of a filtration system. This modification helped to maintain anaerobic samples.

The extraction procedure used three sample types, all of which had been suspended and homogenized to allow wet chemistry manipulations. One sample was aerated before extraction so an estimate of the "stable" metal mass could be made. Another sample was sonicated before extraction to determine the metal mass retained by organisms. A third sample was not aerated or sonicated before extraction. This sample was used as a control for comparison with the aerated and sonicated samples. The samples were labelled by using the sample type and extraction phase to form sample numbers. There were six extraction phases numbered 1 through 6 which were prefixed by FOX, SON or BLK.
Dilute 300 ml. of blended sample and dilute with deionized distilled water to 900 ml.

Put 50 ml. of sample into each extraction flask.

Add spike solution.

Add chelating agent.

Shake well

Add methyl-iso-butyl ketone.

Centrifuge and pour supernatant into extraction funnels.

Then allow the residue to drain into funnels.

Shake the extraction funnels well and leave overnight.

Drain off the lower layer of liquid and discard. (This is the water layer.)

Drain the remainder of the liquid through a filter into the sample containers.

(This is the MIBK layer.)

Analyze.

which referred to aerated, sonicated or control samples respectively. An initial sample of the blended lysimeter mixture was taken before commencing with the extraction procedure. These samples were labelled AERKO, SON0 and FOX0.

Each phase of the extraction procedure used the residue from the previous phase for an extraction sample. The samples were diluted using deionized distilled water and then allowed to react with the extracting agent. The only exceptions occurred in phases four and six where a sample digestion proceeded the addition of an extracting agent. After adding the extraction agents the samples were centrifuged and the supernatant was
collected. The residue was kept for the next phase of extraction and the supernatant was kept as the sample for that phase. The phases are described in Table XV and a flow chart of the extraction procedure is shown in Figure 5.

Table XV - Extraction Procedure

<table>
<thead>
<tr>
<th>Phase</th>
<th>Treatment</th>
<th>Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial Water</td>
<td>Dilute and Centrifuge Pour off supernatant</td>
<td>Free Water Soluble metals</td>
</tr>
<tr>
<td>Exchangeable</td>
<td>Add Ammonium Acetate</td>
<td>Easily Extracted Metals</td>
</tr>
<tr>
<td>Easily Reducible</td>
<td>Add Hydroxylamine Hydrochloride</td>
<td>Manganese Oxides Metals with Manganese Oxides</td>
</tr>
<tr>
<td>Organic</td>
<td>Digest with Hydrogen Peroxide*** and Extract with Ammonium Acetate</td>
<td>Digests Organics Metals released by destruction of organics</td>
</tr>
<tr>
<td>Moderately Reducible</td>
<td>Add Sodium citrate Sodium dithionite</td>
<td>Extracts Iron Oxides Metals with Iron Oxides</td>
</tr>
<tr>
<td>Residual</td>
<td>Acid Digest</td>
<td>Extracts metals from residue 'Fixed' metals</td>
</tr>
</tbody>
</table>

*** See the discussion in the section titled 'Procedure Modifications' on page 68.

The supernatant from each phase and the residue from the last phase were acid digested using concentrated nitric acid. After digestion, the samples were filtered through a Whatman number 541 filter and made up to volume using deionized distilled water. Phase 4, 5 and 6 samples were made up to 250
Figure 5 - Flow Chart Of The Extraction Procedure

Split roughly 900 ml. of sample into
3-300 ml. groups

Dilute all 3 samples up to 900 ml.
with deionized distilled water

Put one sample into a 4000 watt sonication bath for 30 min.
Bubble air through another for 30 hr.
Do not treat the third sample.

Remove a subsample from each sample for:
 i) A bacterial sample.
 ii) moisture content sample.
 iii) An initial metals sample.
     (Subsamples BLK0, SON0, FOX0)

Remove supernatant and dilute sample to
900 ml. with deionized distilled water.
     (Subsamples BLK1, SON1, FOX1)

Add 19.3 gm. ammonium acetate to each
sample and shake well.
Centrifuge each sample.
Remove supernatant
and dilute sample to
900 ml. with deionized distilled water.
     (Subsamples BLK2, SON2, FOX2)

Add 2.61 gms. of hydroxylamine hydrochloride to each sample
and Centrifuge

Remove supernatant and dilute sample to
1000 ml. with deionized distilled water.
     (Subsamples BLK3, SON3, FOX3)

Add 150 ml. of hydrogen peroxide to 1000 ml. of
sample.

Add 9.65 gms. ammonium acetate to 500 ml. of sample
and Centrifuge

Remove supernatant and dilute sample to
300 ml. with deionized distilled water.
     (Subsamples BLK4, SON4, FOX4)

Add 10 gm. sodium citrate and
20 gms. sodium dithionite to
each sample.
Centrifuge.

Remove supernatant (Subsamples BLK5, SON5, FOX5)

The residue forms
Subsamples BLK6, SON6, FOX6

* Note that this step should be replaced by dry oxidation in an
oven at 500°C.
ml. and the filters were rinsed with ammonium acetate to improve metal recovery. Samples from all other phases were made up to 100 ml. and the filters were only rinsed with deionized distilled water.

D. BACTERIAL COUNTS

To determine the mass of metals associated with bacteria, techniques for counting bacterial numbers were investigated. A fluorescent dye staining technique which employed the dye 4,6-diamidino-2-phenylindole was selected for this study. Many researchers reported their success at counting small numbers of bacteria with an optical microscope (Hobbie 1977, Daley and Hobbie 1979, Kapuscinski and Skoczylas 1977, Salari and Ward 1979, Cowell and Franks 1980, Porter and Feig 1980, Alan and Miller 1980, Coleman 1980, Coleman et al. 1981). (Further details of the information available in the literature is contained in Appendix L). None of the articles reviewed reported the use of 4,6-diamidino-2-phenylindole for counting bacteria in complex organic samples like leachate. After some experimentation and personal communications with Iqubal Velgi (SFU), a procedure was formulated. A detailed list of the experimental steps is contained in Appendix G.

Bacterial samples were taken from the sonicated aerated and blank samples. Each sample was weighed and the moisture content was determined. All the bacterial samples were placed in acid washed, deionized distilled water rinsed, 50 ml. Erlenmyer flasks and diluted to 40 ml. with deionized distilled water. After dilution 5 ml. of formaldehyde solution was added to fix
the bacteria. A later modification replaced formaldehyde with 5 ml. of 70% ethanol solution. The samples were stored in a dark room at 4°C until the counts were to be taken.

To obtain counts, sodium pyrophosphate was added to the fixed bacterial samples, before sonication, to break up the substrate. After sonication, the sample was filtered through a 25 mm. diameter, 0.02 um. Nucleopore filter. The filters were stained with irgalan black before use, to reduce background luminescence under the microscope. When 6 ml. of prepared sample had been filtered, the filters were placed on a microscope slide. One drop of cargyle B oil was added before a cover slip was placed over the filter.

The slides were observed using a Xenon lamp with a 350 - 380 nm. excitation filter. A drop of cargyle B oil was used for immersion oil and a 100x magnification was used. Six fields of view were observed on each slide and ten slides were made of each sample. According to Trolldenier (1973), that should give a 5% probability that the real value was more than 10% different from the counts obtained. A flow chart of the dye procedure is shown in Figure 6.
Figure 6 - Flow Chart Of The Fluorescent Dye Technique

Add 5 ml. of 70% ethanol to 5 ml. of solids in a 50 ml. sterilized erlenmeyer flask.

| Dilute mixture with filtered deionized distilled water to 50 ml. |
| Dilute 1 ml. of mixture to 10 ml. with filtered deionized distilled water and mix well. |
| Add 5 ml. of 0.01% filtered sodium pyrophosphate to 2 ml. of diluted mixture. |
| Incubate mixture at room temperature for 30 min. and mix every five min. |
| Sonicate pyrophosphate mixture for 5 min. |
| Add 0.2 ml. of 0.1 M DAPI solution and mix well. |

After 15 min. filter through a 25 mm. diameter, 0.02 µm. Nucleopore filter stained with irgalan black. (Use 8 mm. Hg vacuum to filter.)

| Put filter on a microscope slide. |
| Add a drop of cargyle B immersion oil and cover with a cover slip. |
| Observe slide at 100X using a 340-370 nm. excitation beam. |

Count 4-6 fields of view on 10 or more slides.
IV. DATA RESULTS AND DISCUSSION

Three separate experiments were conducted for this study: a chelation experiment; an extraction experiment; and a bacterial staining experiment. The extraction and bacterial staining experiments were to be used together to determine the mass of metals retained by bacteria. They were tested separately to evaluate their feasibility. None of the experiments had been previously used to test lysimeter samples. So a preliminary sample, taken from the first sampling hole, was used to test the safety and workability of the experimental procedures.

A. CHELATE DATA

The chelation experiment was designed to determine the presence and strength of natural ligands in lysimeter or landfill samples. The experiment used seven complexing agents (ligands) to form complexes with metals in lysimeter sample suspensions. One ligand was added to each sample and the complexes were extracted in methyl-iso-butyl ketone (MIBK). The MIBK extracts were analyzed to determine the number of moles of complex that had formed. These measured values were then to be compared with calculated values.

The complex concentrations (ML) were calculated knowing the initial ligand concentration (LO), the stability constant (Ks) of each complex and the approximate number of moles of metal initially in the samples (MO). Any differences between measured and calculated complex molarity that could not be accounted for by the recovery efficiency were attributed to the effects of
natural ligands and unavailable metals.\textsuperscript{6} Metals which were not complexed by the ligands with the highest stability constants were considered unavailable for complexation.

To calculate the expected molarity of ligands in the MIBK extracts the following assumptions were made:

a) The metals reacted in 1:1 proportions with the ligands.
b) An excess quantity of ligand was added.
c) The metal concentrations measured in the extraction experiment could be used to estimate the initial metal concentrations of the samples.
d) All the metal extracted in the MIBK had formed a complex.

These assumptions resulted in the formula:

\[ K_s (M_0 - ML)(L_0 - ML) = ML \]

This formula is derived in Appendix P. It has only one unknown so the complex concentration can be determined. Table XVI shows the complex concentrations expected for a 1.8 gm. sample (dry weight at 104°C). (This corresponded to roughly 50 gms. of the wet chelation samples used for the second data set.)

A ligand gradient was established to facilitate data interpretation. Each ligand was put in order according to its ability to form complexes with the metals tested. The ordering

6. This study defined the recovery efficiency (Re) as the moles of metal that were complexed, divided by the moles of metal initially present. The recovery efficiency combines the extraction efficiency of MIBK with the complexation efficiency of a ligand.
Table XVI - Expected Complex Concentrations

uM of Metal Complexed/ 1.8 gm of Dry Lysimeter Sample

<table>
<thead>
<tr>
<th></th>
<th>EDTA</th>
<th>Eth</th>
<th>Gly</th>
<th>Hist</th>
<th>Hyd</th>
<th>NTA</th>
<th>Oxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd$^{+2}$</td>
<td>0.72</td>
<td>0.43</td>
<td>0.72</td>
<td>0.72</td>
<td>0.72</td>
<td>0.72</td>
<td>0.67</td>
</tr>
<tr>
<td>Cu$^{+2}$</td>
<td>2.34</td>
<td>1.74</td>
<td>2.34</td>
<td>2.34</td>
<td>2.34</td>
<td>2.34</td>
<td>2.34</td>
</tr>
<tr>
<td>Fe$^{+3}$</td>
<td>148</td>
<td>130</td>
<td>148</td>
<td>146</td>
<td>148</td>
<td>148</td>
<td>148</td>
</tr>
<tr>
<td>Fe$^{+2}$</td>
<td>148</td>
<td>78</td>
<td>147</td>
<td>148</td>
<td>148</td>
<td>148</td>
<td>134</td>
</tr>
<tr>
<td>Ni$^{+2}$</td>
<td>0.32</td>
<td>0.1</td>
<td>0.32</td>
<td>0.32</td>
<td>0.32</td>
<td>0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>Pb$^{+2}$</td>
<td>5.37</td>
<td>4.79</td>
<td>5.36</td>
<td>5.37</td>
<td>5.37</td>
<td>5.37</td>
<td>5.09</td>
</tr>
<tr>
<td>Zn$^{+2}$</td>
<td>17.8</td>
<td>6.9</td>
<td>17.8</td>
<td>17.8</td>
<td>17.8</td>
<td>17.8</td>
<td>17.1</td>
</tr>
</tbody>
</table>

The data is calculated for chelation data set two. The formulae are shown in Appendix P.

used was (from smallest to largest stability constant): ethanoic acid, oxalic acid, glycine, histidine, NTA, EDTA and 8-hydroxyquinoline. This ordering used maximum stability constants because some ligands (eg. 8-hydroxyquinoline) form much stronger complexes when two ligands or more are associated with a coordinating metal.

When the measured and calculated data are compared using a ligand gradient it should be possible to determine the effects of natural ligands. The differences between the measured and calculated data at the high stability constant end of the gradient should be due to unavailable metals. If this is established as a base value a new calculated metals concentration can be determined by subtracting the base concentration from each of the calculated concentrations. Then any differences between the measured and newly calculated
concentrations should be due to natural ligands alone. A sharp decrease in metal complexed by natural ligands would indicate that the bulk stability constant of the natural ligands is less than the stability constant of the added ligand. In this way an upper bound to the bulk stability constant of natural ligands can be established for each metal.

1. CHELATION DATA SET ONE

Initially a method of standard comparisons was used to determine complex concentrations and extraction efficiencies. This technique did not work well because the metal species in the standards were not the same as those in the samples and the chromium standard formed a precipitate when mixed with the lead standard. So the mixed metal standards were useless. The problems with the standard comparison technique gave rise to two changes: the use of CrCl$_3$ dissolved in 0.05% nitric acid for a chromium standard and the use of the method of standard additions. The quantity of ligand was also increased for the second data set because only Cr, Cu, Fe and Zn were detected in the MIBK extracts. The raw data is shown in Appendix M.

Metal concentrations could not be determined for the standard comparison data because a precipitate formed in the standard solutions and the precipitate was not observed until the standards were prepared for the second data set. The lack of concentration data prevented the determination of recovery efficiencies. This study was not of sufficient duration to permit a repeat of the standard comparison data.
A pseudo mass ratio (Rx) value was calculated for each metal to provide a clear example of the calculations that would be required to compare chelates. It may be possible at a future date to use the Rx values for a comparison with other studies.

To calculate the pseudo mass ratios the absorbancy values registered by the atomic absorption spectrometer were assumed to be directly proportional to the metal concentrations. This assumption could not be verified without repeating the chelation procedure. The pseudo mass ratio is the ratio of absorbancy to corrected sample mass. The corrected sample mass represented the dry weight of sample divided by the volume of MIBK that was recovered. To translate the Rx values to mass ratios the recovery efficiency (Re), and the concentration conversion factor must be known. More specific details for calculating the Rx values, shown in Table XVII, are contained in Appendix Q.

Table XVII - Pseudo Mass Ratios For Data Set One

<table>
<thead>
<tr>
<th></th>
<th>EDTA</th>
<th>Eth</th>
<th>Gly</th>
<th>Hist*</th>
<th>Hyd</th>
<th>NTA</th>
<th>Oxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>2.65</td>
<td>2.35</td>
<td>2.60</td>
<td>1.63</td>
<td>4.03</td>
<td>6.42</td>
<td>1.43</td>
</tr>
<tr>
<td>Cu</td>
<td>0.81</td>
<td>0.68</td>
<td>0.59</td>
<td>0.61</td>
<td>&gt;6.3</td>
<td>0.34</td>
<td>2.1</td>
</tr>
<tr>
<td>Fe</td>
<td>0.75</td>
<td>1.07</td>
<td>4.10</td>
<td>1.37</td>
<td>&gt;8</td>
<td>1.14</td>
<td>&lt;0.53</td>
</tr>
<tr>
<td>Zn</td>
<td>&gt;8</td>
<td>0.15</td>
<td>0.35</td>
<td>0.14</td>
<td>1.48</td>
<td>0.19</td>
<td>0.65</td>
</tr>
</tbody>
</table>

* Spilt roughly 7 ml. of sample.
See Appendix Q for pseudo mass ratio calculations.

7. The concentration conversion factor is the factor used to convert absorbancy values to concentrations.
2. CHELATION DATA SET TWO

The second chelation data set was collected using the method of standard additions. Absorbancy values, from the analysis of MIBK extracts, were plotted on absorbancy vs percent spike graphs. The data was expected to form a smooth curve so the metal concentrations and recovery efficiencies could be determined. Unfortunately this was not the case. As the plots in Appendix 0 show, none of the data formed a smooth curve. This may be due to variations in the samples or to problems with the standards.

To demonstrate the required calculations and to filter out the effects of different sample sizes a pseudo mass ratio (Xm) was calculated for the second data set. The method of calculation for the Xm values differed from the Rx values in two ways but the assumptions were the same. The Xm value did not correct for fluctuations in the volume of MIBK that was recovered and the Xm values were normalized by dividing the wet weights of the samples by fifty. The Xm values represented pseudo mass ratios for a 1.8 gm. sample (after drying at 104°C). An example Xm calculation is shown in Figure 7 and further details of the calculations are contained in Appendix Q. Table XVIII shows the calculated Xm values. The 10, 50 and 100 numbers correspond to samples with a 10%, 50% and 100% spike addition. The Xm values for a given metal and chelator complex can be compared to the other complexes of the same metal if the recovery efficiencies are assumed to be similar.
Figure 7 - Calculations Of Pseudo Mass Ratios For Data Set Two

\[
RR = \frac{Mm}{WP} \\
RR = \frac{Cm}{Vi} \frac{Vi}{WP} \\
RR/Vi = \frac{Cm}{WP} \\
Cm = xAm \\
RR/Vi = xAm/50gms \\
x = \text{a constant which is different for each metal.} \\
\text{Multiply both sides of the equation by 50 gms. to calibrate the samples to a 50 gms. wet mass sample.} \\
RR50/5Vi = Am50/5WP = Xm
\]

Let:
\[f2 = 50/WP\] \\
\[Xm = Am(f2)\]

Note: Appendix W contains a list of symbols.

| Table XVIII - Pseudo Mass Ratios For Data Set Two |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                | EDTA            | Eth            | Gly            | Hist           | Hyd            | NTA            | Oxa            |
| Cr Blk         |                 |                |                |                |                |                |                |
| 10             | 7.21            | 4.42           | 2.9            | 1.7            | 8.0            | 5.1            | 0.6            |
| 50             | 10.9            | 17.1           | 7.3            | 7.7            | 22             | 8.1            | 3.2            |
| 100            | 6.0             | 6.5            | 4.8            | 5.0            | 7.7            | 5.5            | 1.2            |
| Cu Blk         |                 |                |                |                |                |                |                |
| 10             | 2.3             | 0.8            | 1.3            | 1.5            | 49             | 1.3            | 1.5            |
| 50             | 2.0             | 3.0            | 1.2            | 1.3            | 84             | 1.5            | 2.0            |
| 100            | 2.3             | 1.8            | 1.5            | 0.9            | 5.1            | 2.5            | 1.8            |
| Fe Blk         |                 |                |                |                |                |                |                |
| 10             | 20              | 0.17           | 5.8            | 6.6            | 310            | 1.0            | 13             |
| 50             | 21              | 9.9            | 3.5            | 4.8            | 210            | 1.4            | 6.4            |
| 100            | 29              | 1.3            | 2.4            | 9.8            | 92             | 49             | 25             |
| Zn Blk         |                 |                |                |                |                |                |                |
| 10             | 17              | ---            | 3.0            | 8.6            | 11             | 0.3            | 0.9            |
| 50             | 17              | 2.4            | 0.7            | 1.8            | 2.6            | 1.4            | 1.2            |
| 100            | 20              | 0.29           | 1.1            | 2.1            | 0.45           | 33             | 5.0            |
|                | 18              | 0.84           | 0.27           | 2.0            | 0.76           | 14             | 6.2            |

Note: Zn has a non-linear response if the Measured concentration exceeds 12 mg/l. Similarly for Fe > 60 mg/l. See Appendix Q for pseudo mass ratio calculations.
Again, only Cr, Cu, Fe and Zn were detected in MIBK extracts. Either Cd, Ni and Pb were unavailable for complexation or the chelation extraction efficiency was very poor for these metals. It is unlikely that a ligand shortage occurred to prevent Cd, Ni or Pb complexation. Iron was the most abundant metal in the lysimeter and there should have been enough ligand to chelate over six times the available iron mass. Also, the stability constants of Cd, Ni and Pb are comparable with those of Zn so even an abundant metal like Ca would not cause a ligand shortage. Calcium would need to be available at concentrations four orders of magnitude greater than nickel to successfully compete for ligands. (Note: \( \log(\text{Stability constant}) = 6.5 \) for Ca-NTA and 11.5 for Ni-NTA).

The \( X_m \) data cannot be used to prove the existence of natural ligands because the metal concentrations are unknown. This also prevented the calculation of the sample recovery efficiencies. Instead the data was used to demonstrate how the predominant metal species in a sample could be identified. For this purpose the recovery efficiencies were assumed to be similar for all the complexes of a given metal. Then the \( X_m \) data were plotted to form histograms. These histograms were for comparison with the stability constant vs ligand histogram shown in Figure 8. The ligands were ordered so the stability constant increased toward the right for most metals. The same ligand ordering was used for the plot shown in Figure 9. If the pattern of relative magnitudes is the same for a given metal in the \( X_m \) histogram and the \( K_s \) histogram then the metals were
assumed to be the same species. In this manner the histograms could be used to determine metal species.

Figure 8 - Log10(Stability Constant) Vs Ligand

Log10(Ks) vs Ligand

The relative ordering of the Xm magnitudes changed as more spike was added to the samples. The Xm histogram indicates that Cr(III), Fe(II) and Zn(II) are present in the blank. Copper is probably present as Cu(I) in the blank, which would explain why the order differs from the stability constant order for Cu(II). As more spike was added the ordering altered to indicate Cr(III), Cu(II), Fe(III) and Zn(II). (Only the blank and 100% spike data were shown in the histograms as they represented the extremes.) This corresponded with the species known to predominate in the spike solutions. Table XIX lists the metal species in the spike solutions and those expected in the
Before the chelation experiment can be used to determine the strength of natural ligands or metal speciation, some improvements are required. First, the spike solutions must be reduced. This could be accomplished by bubbling nitrogen gas through the spike and by using the reduced forms of iron and copper for the spike solution. Secondly, the extraction efficiency of MIBK must be determined (method of standard comparisons) for all the metals under consideration. Thirdly, an excess supply of ligands is essential if the effects of reaction
Table XIX - Species In Spike Solutions

<table>
<thead>
<tr>
<th>Species in Spike Solutions</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data Set 1 Diluted in</td>
<td>metal</td>
<td>KCrO₄⁺</td>
<td>CuO</td>
<td>FeCl₃⁺</td>
<td>metal</td>
<td>metal</td>
<td>ZnO⁺²</td>
</tr>
<tr>
<td>Species</td>
<td>DN</td>
<td>Cr⁺³</td>
<td>Cu²⁺</td>
<td>DN</td>
<td>Ni²⁺</td>
<td>DN</td>
<td>Zn⁺²</td>
</tr>
<tr>
<td>Data Set 2 Diluted in</td>
<td>metal</td>
<td>CrCl₃⁻</td>
<td>CuO</td>
<td>FeCl₃⁺</td>
<td>metal</td>
<td>metal</td>
<td>ZnO⁺²</td>
</tr>
<tr>
<td>Species</td>
<td>DN</td>
<td>Cr⁺³</td>
<td>Cu²⁺</td>
<td>DN</td>
<td>Ni²⁺</td>
<td>DN</td>
<td>Zn⁺²</td>
</tr>
<tr>
<td>Species in</td>
<td>Cd⁺²</td>
<td>Cr⁺³</td>
<td>Cu⁺</td>
<td>Fe⁺²</td>
<td>Ni⁺²</td>
<td>Zn⁺²</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DN = Dilute Nitric Acid (0.05%)
W = Water

time and solution activity, upon chelation efficiency, are to be avoided. The formulae required for determining metal speciation are shown in Appendix R. Values for the stability constants, initial ligand molarity, initial metal molarity and complex molarity must be determined to solve the equations for the molarity of each metal species.

B. EXTRACTION DATA

Four sets of extraction data were collected. The first data set was a preliminary run to see how well the procedure worked. All the other sets were run simultaneously. One set was sonicated, another was oxidized with air, and the third was left untreated as a blank for comparison. Major improvements after the first data set were the increased number of moisture content samples and a better mass balance.

Two different sample pretreatments were selected to help differentiate between bacterial effects and chemical reactions.
The first pretreatment method sonicated a sample for 1/2 hr. By using a fluorescent dye to enumerate the bacteria before and after sonication the actual percentage of bacteria which were solubilized could be determined. Then a comparison between the metals extracted from the sonicated and blank samples may indicate the mass of metals released by ruptured bacteria. Unfortunately the bacterial staining method did not work well so the bacteria were not enumerated. Without bacterial counts a correlation between metal releases and sonication cannot be made because the particle fragmentation caused by sonication may release metals.

The bacteria should be enumerated before and after aeration to determine if the number of whole cells changes after the sample has been aerated. A reduction in the number of cells could indicate the presence of anaerobic bacteria. If a correlation can be established between metal release and a drop in whole cell numbers it would be possible to estimate the mass of metal held by anaerobes.

The second pretreatment method aerated a sample for 36 hours. Aeration should destroy anaerobes, increase the pH and increase the oxidation-reduction potential. It was hoped that 36 hours would provide enough time for complete mixing of the

8. Slade and Vetter (1956) reported that after 1/2 hr. Of sonication only 10% of the bacteria were viable and that 50% of the cells were solubilized.

9. Prolonged exposure to air may cause a population of aerobes to grow.
sample and air without allowing new populations of aerobes to propagate. The bacteria should be enumerated before and after aeration so metal releases can be correlated to changes in bacteria numbers. Aeration will probably oxidize organic and inorganic materials in the samples. Only relatively stable compounds and newly formed precipitates should remain after aeration. The stable compounds left in the residual phase of the extraction procedure should provide an upper limit to the mass of metals which will remain in the lysimeter. Only gradual leaching of low solubility compounds should release more metals.

The extraction procedure was a slightly modified version of the procedure developed by Engler (1977). It produced six subsamples and a seventh subsample was taken for a total metals analysis. Each step of the procedure used the residue of the previous step for extraction. After removing the liquid phase by centrifuging, the residue was ready for the next step. Other methods were considered and a summary of the literature reviewed is contained in Appendix H. Table XV shows the order and categories of the extraction procedure developed by Engler.

1. **PROCEDURE MODIFICATIONS**

a. **Hydrogen Peroxide**

There were two major problems encountered in the procedure used. One was the hydrogen peroxide digestion and the other was acid-digestion of samples with a high concentration of suspended solids. The hydrogen peroxide digest recommended by Engler et al. (1977) requires heating of a sample until all the hydrogen
peroxide is used up. This is extremely dangerous because the hydrogen peroxide can become concentrated. When hydrogen peroxide is concentrated beyond 90%, it is flammable and explosive (See Appendix K for details of health hazards). A hydrogen peroxide digest can be accomplished by adding deionized distilled water during heating to maintain the liquid volume and keep the hydrogen peroxide dilute. With this modification a visual check does not indicate when the hydrogen peroxide is used up. To ensure that the reaction was complete the solution was heated for at least one hour after the last visible reaction of hydrogen peroxide with the organics (ie. After the foaming stopped).

Another modification was the slow addition of hydrogen peroxide. All the samples had high levels of organics which resulted in the formation of foam when hydrogen peroxide was added. It was necessary to use a mechanical stirrer to keep the foam mixed and to add the hydrogen peroxide 10 ml. at a time. After all the hydrogen peroxide had been added and allowed to react, the sample was slowly heated. The whole hydrogen peroxide digest took four to five hours to perform and it is unknown how complete the digestion was. (Note: the sample did not foam when the last 10 ml. of hydrogen peroxide was added which may indicate that all the organics were oxidized.) In the future, it would be safer to use dry-ashing for oxidizing samples with such high concentrations of organic materials. This would ensure complete oxidization of organics but would cause the release of strongly sorbed water associated with
mineral colloids and the release of hydroxyl groups.

b. Acid-digestion

Acid-digestion of samples with high suspended solids can only be achieved by using more dilute samples and larger digestion containers. If the suspended solids are too high, the sample tends to 'bump'. (The whole sample heaves instead of small bubbles rising to the surface of the sample.) The bumping can cause the beaker to vibrate. Also, bumping tends to spit sample out of the digestion container.

Another difficulty encountered when acid-digesting samples with high suspended solids was dissolving the residue left after digesting samples. All of the sub-samples numbered 0, 4, 5, and 6 did not redissolve properly. Sub-samples 4, 5, and 6 were diluted to 250 ml. instead of 100 ml. and the filter cakes were washed with ammonium acetate to minimize metal losses. An analysis of the filter cake to determine the mass of metals lost was not made. A comparison of the total metals extracted and the sum of the metals extracted from each subsample indicated incomplete recovery of metals. Subsample four did not contain large concentrations of metals so the digested sample cannot be diluted very much. The other three subsamples, zero, five and six could all be diluted by a factor of 100 for analysis, which circumvented the redissolution problem.

10. The '0' represented the initial samples labelled BLK0, SON0 and FOX0. The numbers 1, 2, 3, 4, 5 or 6 indicate the number of extractions performed on the sample.
c. Centrifuging

A major difference between the procedure used and the one reported by Engler et al. was the use of a centrifuge. When the initial data set was collected, the first phase was filtered. To avoid losing metals adsorbed to the filter, the filter cakes were rinsed with acidified deionized distilled water. The filters were very quickly clogged so vacuum was applied. All the filtering had to be done in a nitrogen atmosphere to keep the system anaerobic. When all the difficulties with filtering were compared to the simplicity of centrifuging, all steps in the procedure which required filtration, except those following acid-digestion, were replaced with centrifuging. Centrifuging and filtration recover free water so the solutions collected should be comparable.

Centrifuging does not collect fine suspended solids as efficiently as filtration. The fine suspended solids will be in the supernatant of the first extraction phase but very little metal was detected in the supernatant of the first phase. So centrifuging was considered adequate for the extraction experiment.

2. DATA

Each sub-sample was analyzed on a Jarrel Ash 810 atomic absorption spectrophotometer for Cd, Cr, Cu, Fe, Ni, Pb, and Zn. Every sub-sample was compared to a minimum of three single species standard solutions. Then a graph of concentration vs absorbancy was generated. The concentration of metal in the
sub-sample was interpolated from the concentration vs absorbancy graphs contained in Appendix S. After determining the concentration in each sub-sample, the moisture content of each sub-sample was found. Then a mass ratio (RR= the mass of metal /the dry weight of lysimeter contents), was calculated so the data could be compared. The mass ratio values were plotted vs the extraction phase to show any trends which may occur. An example calculation of a mass ratio is shown in Figure 10.

**Figure 10 - Mass Ratio Calculation**

\[
\begin{align*}
Ci & = \text{metal concentration in sample} \\
Vi & = \text{volume of sample} \\
Mm & = \text{metal mass in sample} \\
Mm & = CiVi \\
W & = \text{weight of sample} \\
P & = \text{solids fraction} \\
RR & = Mm/WP
\end{align*}
\]

So for sample BLK0 (An initial control sample):

\[
\begin{align*}
Ci & = 1.75 \text{ mg/l of Cd} \\
Vi & = 100 \text{ ml.} = 0.1L. \\
Mm & = 0.175 \text{ mg. of Cd} \\
W & = 70.6 \\
P & = 0.069 \\
AW & = \text{atomic weight of the metal} \\
& = 112.4 \text{ gm/mole} = 112.4 \text{ ug/umole for Cd} \\
RR & = 0.175/70.6x0.069 \\
& = 0.36 \text{ ug/dry gram sample} \\
OR \\
RR & = 36/112.4 \\
& = 0.32 \text{ uM/ gms. of dry sample}
\end{align*}
\]

Note: Appendix W contains a list of symbols.

Initial metal concentrations were determined for each sample set. The sum of the metal mass ratios extracted in each extraction phase should correspond to the total mass ratio of a
raw sample. Only the sums of cadmium and copper extracted from the control sample were within five percent of the total of each metal extracted. There are two reasons for the finding. The first reason was the small size of the control sample which was readily dissolved after acid-digestion. A second reason was the probable loss of metal on the filter cake coupled with some precipitate formation after acid digestion. This was especially true for samples from phases 0, 4, 5 and 6. The three initial samples (BLK0, SON0, and FOX0) should have roughly the same metals levels because each sample was removed from the same bulk sample. Appendix T contains the figures used to calculate the mass ratio at each phase of extraction and the calculated mass ratio values. Figure 11 shows histograms of the log(mass ratio) vs the extraction phase for each metal. In all seven histograms the metal concentrations are expressed as a ratio of the metal mass over the dry weight of sample.

a. Mass Ratio Plots

The sonicated, aerated and blank data, for each metal, are shown in the histograms in Figure 11. All seven histograms have the extraction phase plotted on the x-axis. Phase one corresponds to metals which were washed from the sample when deionized distilled water was added. This will include any metals released by cells which ruptured due to the osmotic pressure created by adding salt free water. The metals released in this phase are soluble and mobile. Ammonium acetate extracted phase two metals which are easily complexed metals. These metals are weakly held in the lysimeter and correspond to
Figure 11 - Extracted Metal Vs Extraction Phase

**Extraction Phase vs. Mass Ratio**

- **Blank**
- **Sonicated**
- **Aerated**

Cadmium

**Extraction Phase vs. Mass Ratio**

- **Blank**
- **Sonicated**
- **Aerated**

Chromium
**Extraction Phase vs. Mass Ratio**

_Nickel_

**Extraction Phase vs. Mass Ratio**

_Lead_
metals which would be held by soil in a cation exchange reaction. Hydroxylamine hydrochloride was used to extract phase three metals. Metals in this phase are easily exchanged so they would be bound by soil if they escaped a landfill. Phase four used hydrogen peroxide to oxidize organic materials. Oxidation could result in any combination of the following circumstances:

a) Cell lysis releasing metal complexing agents.
b) Cell lysis providing new sites for metal sorption.
c) Cell lysis releasing bound, complexed, precipitated or soluble metals.
d) Release of precipitates trapped by organics.
e) Stabilization of organo-metallic complexes.
f) Formation of new metal precipitates with stable oxidation products.

The strong oxidizing capability of hydrogen peroxide should prevent the formation of any organo-metallic combinations but new precipitates or the release of precipitates could result in metals which will not be extracted in phase four. Phase five
used sodium citrate and sodium dithionite to extract iron oxides. Sodium citrate and sodium dithionite are very strong iron complexing agents so only very stable compounds should remain in the residual phase (phase six).

Metals associated with phases one, two or three will probably leach from a lysimeter. Most of the other metals should remain fairly stable as long as:

a) The reducing conditions are maintained.
b) The bacterial population remains the same.
c) Anaerobic conditions are maintained.

An increase in the pH will cause many of the metals to form precipitates rendering them immobile. If a pH change alters the bacterial population, metals may be released. It is not known if the metals associated with organics are held by bacteria or humic compounds. By comparing the sonicated samples to the control samples, one could infer what proportion of the metals are held by bacteria. This assumes that sonication only destroyed bacteria and did not break up anything else which may hold metals. Metals associated with bacteria can be held by a number of methods.

a) Adsorbed inside the cell.
b) Precipitated inside the cell.
c) Sorbed to the cell wall.
d) Bound to extracellular polysaccharides.
e) Precipitated in extracellular polysaccharides.

Some general comments can be made with respect to the
histograms. In the iron and zinc histograms the three samples responded similarly. This indicates that very similar retention mechanisms are operating for both metals or that some of the applied precipitates never dissolved in the lysimeter. If these metals were retained then their predominance in the phase five extracts indicates that co-precipitation is the probable removal mechanism. Cadmium was the most mobile of all the metals in all three circumstances and was the only metal which was added to the lysimeter in a soluble form. Thus, any cadmium retention is entirely due to the contents of the lysimeter. Chromium was predominant in the phase four extracts while lead consistently had high masses in the residual phase. The shape of the copper curve was the same for all three sample types. It showed a peak at phases four and six. Very little copper was associated with phase five indicating different retention mechanisms from all the other metals.

It is important to realize that the lack of mobile metals is probably due to the age of the lysimeter which was sampled. Any natural complexes which remain would be in precipitate form. Metals held by organic ligands should be released by aeration or hydrogen peroxide oxidation. Complexes which are stabilized by aeration or oxidation should be extracted in phase five or six. The mobile metals in the samples indicate that some Cd, Cu and Ni retention mechanisms form moderately soluble compounds.

11. Extra cadmium was added as liquid from a cadmium plating bath to boost cadmium levels. This was done because there was very little cadmium in the electroplating wastes.
Otherwise the highly mobile metals that were extracted should have been leached from the lysimeter already. So copper, nickel and cadmium were retained in mildly exchangeable and soluble forms. These metals were more stable after sonication. This effect could be due to sorption or the formation of compounds after cell lysis. The large mass of cadmium which is mobile after aeration indicates that cadmium compounds are very susceptible to oxygen and/or pH increases. Cadmium and nickel are probably sorbed to organics as shown by the increase in phase four metals after sonication.

Sonication causes the disruption of cells and the fragmentation of solid particles. Cell lysis could release organic compounds which will complex metals and/or cell fragments could sorb metals. Particle fragmentation just increases the exposed surface area. This should increase the availability of metals for cation exchange and increase the sites for metal sorption. If cell lysis causes metals to be complexed, the insoluble compounds will show up in phase four while the soluble complexes should be recovered in phase one. The fragmentation of particles should cause: more metals to be recovered in phases two and three if metals are weakly sorbed or increased recovery in phases five and six if metals are strongly sorbed.

Sonication data indicates that organic complexes of cadmium, chromium and copper form. All the metals except copper are strongly sorbed by iron compounds extracted in phase five.
All the metals are strongly sorbed by minerals present in the residue (especially copper and lead) or they form stable precipitates.

The residual phase of the aerated sample indicates the mass of metal which is stable after: exposure to air and a pH increase. These metals will probably be retained in a lysimeter for many years. Some leaching will occur but it may not be measurable. If they are slightly soluble they would leach out of the lysimeter eventually. Roughly 40% of the lead, 20% of the iron, 15% of the chromium, 15% of the copper, 15% of the nickel and 10% of the zinc is fixed (phase six aerated metals). It is possible that aeration and the corresponding pH increase caused some of the metals to form precipitates. So the estimates provide an upper limit for the unleachable metal mass. The large release of cadmium in phase one indicates that cadmium is readily complexed by organics and/or aeration destroys cadmium compounds in a lysimeter.

Metals associated with organics other than bacteria fall into two categories: mobile or fixed compounds. All the fixed compounds should remain stable until the pH, oxidation-reduction potential or anaerobic conditions are changed. If they are in precipitate form, they will maintain an equilibrium with the surrounding water film, so they will slowly leach from the system. If the metals are fixed to non-bacterial organics by sorption, bonding or entrapment, they will remain fixed until the organics are degraded. In either case, metals are probably
released slowly over a very long time interval.

Generally, the extraction procedure quantifies the metals in a landfill or lysimeter. It is also possible to determine the relative mobility of various metals and hence predict the metals that would be expected to leach from a lysimeter or landfill. A major problem associated with extending the procedure to analysis of landfill material is the removal of an anaerobic sample. There may also be some problems when acid digesting the extract from phases one, two and three because of the bumping effect during volume reduction. (Dry oxidation at 500°C may be necessary.)

b. Bacterial Counts

To further refine the extraction procedure, an experiment was devised to determine the effects of bacteria upon metal mobility. The experiment required two samples, one with bacteria and another with bacteria which were disrupted by sonication. Each sample would be extracted using the extraction procedure. Differences in metal mobility could then be attributed to the effects of bacteria. To quantify the results, the number of bacteria/gram of sample must be known. This required a bacterial enumeration technique so a method using an epifluorescent dye was selected. The dye 4,6-diamidino-2-phenylindole (DAPI) was chosen for the experiment as it is very specific for bacteria. The staining technique used was developed by Iqubal Velgi at Simon Fraser University for a Masters thesis in Biology.
The samples were found to have at least two types of bacteria shape: rod and cocci. Initially only small areas of a slide preparation could be counted due to a blue haze which obscured most of the slides. After some experimentation, researchers at SFU found that most of the background material could be broken up by adding pyrophosphate to the sample. Pyrophosphate treatment coupled with sonication broke up all the background luminescence that was previously observed. One drawback with the pyrophosphate is that it forms a precipitate with potassium, calcium, and magnesium. These precipitates are probably what confuse counting as the slide preparations look like a field of gravel. It is very difficult to discern a few bright blue specs representing bacteria among the very many specs of dull blue to white 'gravel'. In fact, it was not possible to obtain consistent counts.

The low concentration of bacteria in the sample prevented further dilution to make counts easier. So it was not possible to use the technique as planned. Other methods are available for determining biological mass or even estimating bacterial numbers but they could not give an accurate and quick estimate of the number of whole bacterial cells. The most effective DAPI procedure tried is outlined in Appendix G. Figure 12 shows photographs of slides under a microscope at 100X power. The first photograph shows a slide of a lysimeter sample. No

A photograph of a slide of lysimeter suspension stained with 4,6-diamidino-2-phenylindole. Note the blue haze which obscures enumeration.

A photograph of a slide prepared by Iqubal Velgi to show bacteria (The bright blue dots are bacteria).
bacteria can be discerned due to the haze. The second slide is an example of Iqubal Velgi's showing bacteria. The very small bright blue dots are bacteria.

c. Liquid Sample

A lysimeter leachate sample was collected under anaerobic conditions. Data from the UBC co-disposal study (Atwater et al. 1981) was used with a total metals analysis of the lysimeter leachate to determine the mass of metal which had leached from the lysimeter before the first solids sample was removed. The mass released from the tank is shown in Table XX and a sample calculation is shown below the table.

Table XXI shows the results of the liquid analysis. The data corresponds with the trends established in the co-disposal report by Atwater et al. (1981). An analysis of the liquid was performed to ensure that none of the metals had suddenly leached out of the system. There was an iron release after the first hole was made in the lysimeter. The iron release cannot be readily explained and seems to be a temporary occurrence. There was an air leak due to problems with the plug that was used to seal the hole. The air leak would cause a shift in pH and oxidation-reduction potential which should cause iron to precipitate. The observed increase of iron in the leachate must be due to the release of complexed iron. The faulty plug was subsequently replaced by a layer of parafilm, allowing the system to go anaerobic again.
Table XX - Mass Released From The Lysimeter

<table>
<thead>
<tr>
<th>Metal</th>
<th>Mass Released (gms.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>0.033</td>
</tr>
<tr>
<td>Cr</td>
<td>0.347</td>
</tr>
<tr>
<td>Fe</td>
<td>62.6</td>
</tr>
<tr>
<td>Ni</td>
<td>0.59</td>
</tr>
<tr>
<td>Pb</td>
<td>0.009</td>
</tr>
<tr>
<td>Zn</td>
<td>10.0</td>
</tr>
<tr>
<td>Ca</td>
<td>36.9</td>
</tr>
<tr>
<td>Mg</td>
<td>7.38</td>
</tr>
</tbody>
</table>

Use leachate analysis to determine the concentration of metal in a sample = Ci
Measure the total volume of leachate collected from the lysimeter = Vi
So for a time interval T1 to T2:
At time T1:
\[ C_1 = \text{metal concentration in sample taken at } T_1 \]
\[ V_1 = \text{total volume of leachate generated} \]
\[ M_1 = \text{cumulative mass to time } T_1 \]
At time T2:
\[ C_2 = \text{metal concentration in sample taken at } T_2 \]
\[ V_2 = \text{total volume of leachate generated} \]
therefore
\[ M_2 = (C_2 + C_1/2) \times (V_2-V_1) + M_1 \]
\[ M_2 = \text{mass released over time } T_1 \text{ to } T_2. \]

Table XXI - Metal Concentrations In Liquid Sample

<table>
<thead>
<tr>
<th>Metal</th>
<th>ug/l</th>
<th>uM/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td>Cr</td>
<td>73</td>
<td>1.4</td>
</tr>
<tr>
<td>Cu</td>
<td>46</td>
<td>0.72</td>
</tr>
<tr>
<td>Fe</td>
<td>2290</td>
<td>41</td>
</tr>
<tr>
<td>Ni</td>
<td>990</td>
<td>17</td>
</tr>
<tr>
<td>Pb</td>
<td>10</td>
<td>0.05</td>
</tr>
<tr>
<td>Zn</td>
<td>2810</td>
<td>43</td>
</tr>
</tbody>
</table>

Note: Cd and Pb were at the limits of detection.
A total of 356 ml. were tested.
V. CONCLUSIONS AND RECOMMENDATIONS

The experimental methods developed in this study could be used to increase the understanding of metal distribution in a landfill. It is so difficult to remove anaerobic samples from a landfill that the preliminary tests conducted in this study used samples from a lysimeter. Three different experiments were conducted to determine metal mobility, natural ligand strength and bacterial numbers.

A. CHELATION

Two methods, standard comparisons and standard additions, were used to develop a test for the strength and presence of natural ligands. The experimentation gave rise to the following conclusions:

a) Both the standard comparison method and standard additions method should be used.

b) The standards additions used upset the metal equilibrium in the samples.

c) The volume of MIBK recovered should be measured for each sample although this increases MIBK losses.

d) Centrifuging the MIBK-sample suspension mixture does work but may be improved with higher centrifugal forces.

e) The use of CrCl$_3$ for a chromium standard did prevent visible precipitation in the standard solutions.

f) The lack of Cd, Ni and Pb in MIBK extracts was due to low interactions with other metals or a low recovery efficiency for these metals.

g) The similar responses of Fe and Zn indicated similar chelation behavior.

h) A gradient of complex strengths can be established to determine metal speciation.
i) A comparison of the pseudo mass ratios and stability constants of each metal indicated the presence of Cr(III), Cu(I), Fe(II) and Zn(II) in the sample. The comparison indicated Cr(III), Cu(II), Fe(III) and Zn(II) in the standards.

Both analysis methods should be used so the recovery efficiency can be determined for the sample. A method was not found for breaking the recovery efficiency into its two components, namely chelation efficiency and MIBK extraction efficiency. The method of standard additions can be used to determine the recovery efficiency for the sample+standard. The recovery efficiency for the standards can be determined using the method of standard comparisons. Any differences between the two methods can be attributed to a different recovery efficiency for metals in the sample.

The lack of Cd, Ni and Pb in the MIBK extracts may be due to a low recovery efficiency for these metals. There was insufficient time available to rerun the standard comparison experiment using CrCl₃ so the recovery efficiencies are unknown. It is also possible that a reaction between these metals and the non-reduced standards occurred which prevented their chelation.

A gradient of stability constants can be established for each metal species. A comparison of the order of stability constant gradients and pseudo mass ratios can then determine the species present in the sample if the following are true:

a) The stability constants of the added ligands exceed those of the natural ligands.

b) The recovery efficiencies can be determined for each metal in the sample.
c) The concentration of the complex that is recovered is dependent upon the magnitude of the stability constant of a complex.

d) If the recovery efficiencies are equivalent then a higher stability constant will result in a greater concentration of complex in the MIBK extract.

e) There is a different gradient ordering for each species of a metal.

The presence and strength of natural chelating agents can only be determined if the complex concentrations and recovery efficiencies are measured. A possible spinoff of the technique may be the ability to determine the metal species and their concentrations. The species determination will only be affected by natural ligands if they form very strong complexes. To determine the concentration of each species the formulae in Appendix R can be used. The complex concentrations used for the calculation should be those due to the strongest complexing agents. This will avoid errors caused by the presence of natural ligands.

B. EXTRACTION

A series of extractions were performed to determine the relative mobility of metals in four samples. The metals in the samples were separated according to their ease of extraction. One sample was sonicated to separate bacterial and non-bacterial metals. Another sample was aerated to discover the fraction of metals which would be mobilized in an oxidizing environment. The aerated and sonicated samples were compared to untreated samples (blanks or controls). The samples were collected near a
layer of electroplating waste.

The progressive extraction experiment was successfully employed to categorize the mobility of metals in a lysimeter sample. Sonication and aeration of two different samples did provide more information about metal retention. Unfortunately the mass of metal associated with bacteria could not be determined because the bacterial enumeration technique did not work well enough. All the extraction data was converted to a mass ratio value (moles of metal/ mass of lysimeter suspension after drying at 104°C) to facilitate comparison with other studies. After analyzing the data the following conclusions were made:

a) After three years of lysimeter operation most of the mobile metals had already been leached out of the lysimeter.

b) Over 10% of the cadmium and nickel was mobile because they formed partially soluble precipitates or a chemical was slowly released which formed soluble compounds with these two metals.

c) The large metal releases caused by aeration confirm the belief that metal retention mechanisms are sensitive to pH and oxidation-reduction potential changes.

d) The smaller proportion of metals in phases two and three extracts indicates that cation exchange is not a major retention mechanism in the lysimeter tested.

e) Sonication caused more Cd and Ni to be retained by organics indicating that cell chemicals may bind these metals.

f) Aeration mobilized all the metals except nickel indicating the presence of very stable nickel compounds.

g) Most of the chromium was extracted in phases 4, 5 and
6 indicating the presence of fairly stable Cr compounds.

h) A maximum of 40% of the lead, 20% of the iron, 15% of the chromium, 15% of the nickel, 10% of the copper and 1% of the cadmium is stable after aeration.

C. FUTURE RECOMMENDATIONS

The chelation experiment tested in this study could be developed further. Future attempts to measure the strength of natural ligands should incorporate the following:

a) Determine stability constants for all the species under consideration.

b) Use reduced spike solutions which have had nitrogen gas bubbled through them.

c) Extract a sample using only the solvent to check for metals which are soluble in the solvent.

d) Prove whether metal species can be quantified.

e) Try to determine stability constants for natural ligands by comparison and/or MacCarthy's method (1977).

f) Determine why Cd, Ni and Pb were not detected.

The chelation gradient did not produce good data in this study because of problems with the spike solutions but the technique offers an opportunity to measure species concentration and natural ligand strengths. Previously the species could not be determined in most cases and natural ligand strengths could only be estimated for a few metals. So further work should be conducted to test the feasibility of the technique for metal speciation measurements.

A standard without chelating agents should be extracted
using MIBK. If the mass of metal extracted is comparable to the complexed metal extracted another solvent should be used. This extraction was overlooked in this study.

Further efforts to determine bacterial numbers should be directed at:

a) Avoiding dilution of samples which may cause cell lysis due to osmotic pressures.
b) Testing the slightly more specific bacterial stain called mithramycin.
c) Experimenting with the ethanol fixation to determine the optimal concentration for fixing bacteria from a lysimeter.

The determination of bacterial numbers must be successful before metal retention due to bacteria can be evaluated.

The extraction procedure demonstrated the success of chelation without solvents. As the extraction data collected in this study was used to test the technique, further work should be done to:

a) Compare anaerobic landfill samples to lysimeter samples to determine the validity of using lysimeters as refuse leaching models.
b) Compare different landfills.
c) Compare different sample locations in a landfill.
d) Replace wet oxidation of organics with dry oxidation by oven drying samples at 500°C to ensure complete oxidation of the organics.
e) Conduct a time study of a landfill to determine the metal mobility changes over time.
f) Try different aeration contact times to check the sensitivity of samples and to determine the contact time required for maximum metal release.
Progressive extraction studies of anaerobic landfill samples will help to determine the validity of lysimeter studies. Also, time studies should be conducted so estimates of metal leaching can be made. Lastly, a comparison of the metal distribution at different landfill locations will help to understand the metal leaching process.
REFERENCES


GLOSSARY

-A-

Absorb - To hold within a volume.

Activity - Concentration x activity coefficient (fi) - the activity is a function of the number of ions in a solution.

\[ \log_{10}(fi) = -0.5 \frac{Z_i^2 (U)^{0.5}}{1 + (U)^{0.5}} \]

Where:
- \( C_i \) = molarity
- \( U \) = ionic strength
- \( Z_i \) = charge

Adsorb - To hold on the surface.

Antagonism - The ability of a cation to decrease the toxicity of another cation.

-B-

Biofloc - A zoogal mass of cells joined by slightly soluble extracellular polysaccharides which form polymer fibrils. The floc forms in activated sludge systems and settles out of solution in a clarifier.

Biota - Refers to all of the local biological ecosystems.

-C-

Cation Exchange Capacity - CEC. The number of moles of adsorption sites that are available in a soil for cation exchange. Cation exchange occurs when a free cation is more stably bound to the soil than the cation that is already bound by the soil.

Chelating agent - A compound which is coordinated by a chelator. A chelating agent provides a supply of ligands for a chelate to coordinate with.

Chelation - When a chelator bonds covalently with a ligand to form one or more rings chelation has occurred.

Chelator - Usually a metal which is joined by covalent bonds to two or more other atoms. The atoms can be supplied by one or more molecules called ligands.

Codisposal Report (UBC) - Atwater et al. (1981) studied the interaction of electroplating wastes with septic tank wastes in a lysimeter.

Colloidal Particles - Generally in the 2.5-12.0 nm particle size range. The actual range used by an author may vary according to the filters that were available.
Complex - A complex forms when a ligand molecule binds to a chelator.

-D-

Dentate - literally means tooth.
- bidentate - two teeth - in co-ordination chemistry it refers to a ligand which provides two locations for a chelator to bond to.

DAPI - 4', 6 diamidino-2-phenylindole. A strong base and a fluorescent dye.

Davies Equation - \( \log f_i = 0.51Z^2 \left( \frac{U^{0.5}}{1 + (U^{0.5} - 0.3U)} \right) \)

\( Z = \text{Charge on the ion} \)
\( U = \text{ Ionic strength} \)
\( f_i = \text{Activity} \)

Dialysis - The use of differential concentrations to drive molecules across a membrane and hence separate small molecules from large molecules. Small molecules diffuse across the membrane more rapidly.

-E-

Electrophoresis - The application of a potential difference to a solution to separate positive and negative species.

Elute - to wash out.

-F-

Fulvic acid - that fraction of the organic matter in soil that remains in solution after extraction with alkali and precipitation by acid.

-G-

Gram-negative - bacteria that fail the gram-staining test - indicates a two layer cell wall - more resistant to environmental stresses.

Gram-positive - gram-staining is successful with this type of bacteria - indicates a single layer cell wall - less resistant to environmental stresses.

Gram-staining test - uses crystal violet to stain bacteria - positive results stain purple - negative results do not stain with gram stain.
Humic acid - the portion of the organic matter in soil that precipitates when acid is added to an alkali extraction of the soil.

Humic substances - substances from the organic fraction of soil.

Humin - the fraction that is left in the soil after acid and base extraction.

Humus - dark organic fraction of soil formed by decaying substances.

Ion-exchange - separates positive, negative, and neutral species.

Ionic strength - \[ U = 0.5 \left( C_i \times Z_i^2 \right)^{-0.5} \]

Irving-Williams - established an order for the strength of complexes. Irving and Williams reported the following order:

\[ \text{Mg} < \text{Ca} < \text{Cd}, \text{Mn} < \text{Co} < \text{Zn}, \text{Ni} < \text{Cu} < \text{Hg}. \] [Nature Vol 162 p746 1948 and J of Chem Soc Vol 3 p3192 1953]

Landfill - generally a site where refuse from households and industry is interred. Materials which are brought to the site are dumped on the surface or in an excavated area which is predetermined. Then the refuse is covered with six inches or more soil. The site is built on until there is no more soil available to cover the refuse.

To be a sanitary landfill the site must be isolated from the surrounding soil. The refuse must be covered by soil within 24 hrs. of dumping and the site should be drained to collect any leachate which may be generated. The whole site should be above the local water table. Optimally, the materials being dumped at the site can be restricted.

Leachate - A dark coloured, unpleasant smelling, liquid which drains from a landfill when the liquid holding capacity of a site has been exceeded. The volume of leachate which forms is a function of the ground water infiltration and the annual precipitation at the site. Leachates can be acidic, basic or neutral. The acidic nature of leachate can be caused by organic acids
which are produced as by-products of the organic degradation of refuse. These acids mix with the water seeping through a landfill. They can leach metals and acid soluble compounds in the refuse. The net result is the generation of an obnoxious liquid suspension containing dissolved and particulate matter. This liquid is called leachate.

- The liquid drained from the lysimeter was referred to as lysimeter leachate because it was similar to landfill leachate.

Ligand - a compound or molecule which provides one or more bonding sites for a chelator. (Note: two sites = bidentate to six sites = hexadentate)

Lineweaver-Burk Equation \[ \frac{1}{V} = \frac{K_m}{V_m S} + \frac{1}{V_m} \]
Where:
- \( V \) = mg/l \( O_2 \) Uptake/gm. of biosolid culture/min.
- \( V_m \) = \( V \) for an unhindered substrate
- \( S \) = substrate concentration in gm. COD/gm. biological solid
- \( K_m \) = Michaelis-Menten constant

Lysimeter - laboratory size model of a sanitary landfill. In this case a 30 cm. diameter PVC tube with alternating layers of soil and refuse packed under a 220 psi pressure.

Lysis - the rupturing of the cell wall of a whole cell.

Michaelis-Menten Model - a model of enzyme inhibition.

Oxidation - Occurs when an ion donates electrons.

Phenols - a class of compounds with one or more hydroxy groups attached to a benzene ring.

Reduction - Occurs when an ion gains electrons reducing it's positive charge.

Recovery efficiency - Defined as the ratio of metal detected in MIBK extract / the initial concentration in the sample
to be chelated. Note that this is the combination of the MIBK extraction efficiency and the chelation extraction efficiency.

\[-S-\]

Scatchard plot - a plot of Vav/Mf vs Vav. The plot describes the equation
\[ \frac{Vav}{Mf} = K_i (n_i - Vav) \]
Where:
- \( Vav = \) Moles of metal bound / Moles of Humic acid
- \( Mf = \) concentration of free metal ion
- \( n_i = \) Number of binding sites / molecule of Humic acid (Note: This is the Vav axis intercept)
- \( K_i = \) Stability constant (Slope of plot).

Schubert conditions - occur in co-ordination chemistry when the concentration of ligands is very much greater than the concentration of the chelator. (eg \([\text{ligand}] >> [\text{metal}]\))

Sorb - to hold by adsorption or absorption.

Stability constant - when it refers to a chelation reaction, it is of the form:
\[ K = \frac{[MnLm]}{[M] \cdot [L]} \]
where \( M \) is generally the chelator metal, \( K \) is the stability constant and \( L \) is the ligand molecule and [] indicates concentration. The equilibrium equation would be:
\[ xM + yL = MmLn \]

Synergism - when two or more cations have a greater effect together than the addition of their independent effects.

\[-U-\]

Ultrafiltration - Using a very fine filter made of plastic to filter out molecules over a specified size. Usually requires an applied pressure to overcome the large head loss.
APPENDIX A — PLOTS OF METAL RELEASED VS TIME

Figure Chromium Conc. vs. Time - CDI, CDFHMJK

Figure Iron Conc. vs. Time - CDI, CDFHMJK
After Atwater et al. 1981.
APPENDIX B - CO-ORDINATION CHEMISTRY

When a metal bonds with a radical to form one or more covalent bonds, it has been complexed. Co-ordination chemistry is the study of complex formation. A radical which donates one or more electron pairs to form a complex is called a ligand. When the atoms of a ligand and a metal form a ring, the complex is called a chelate and the ligand is called a chelator or a chelating agent. All the ligands in this study are chelators except ethanoic acid. Each of the metals analyzed in this study (Cd, Cr, Cu, Fe, Ni, Pb, and Zn) can co-ordinate up to six covalent bonds.

In general a ligand that has 'x' donor atoms will form 'x-1' chelate rings (Bell 1977 and Dean 1979). The larger the value of 'x' the more stable the complex will tend to be. Some ligand-metal complexes will display enhanced stability due to a particularly good fit. Table XXII lists some basic and acidic ligand groups. The basic ligands interact with a metal by donating a lone pair of electrons to form a covalent bond. Acidic groups lose a proton (H+) before donating an electron pair to form a covalent bond. Figure 13 shows the bonding sites for the seven ligands used.

When a complex forms, the constituent molecules form a ring. The stability of a complex decreases as the number of members in the ring, the bulkyness or the asymmetry of the core ion increases. Core symmetry is a function of the orbitals which are occupied. Table XXIII illustrates the orbital
Table XXII - Ligand Groups

<table>
<thead>
<tr>
<th>Basic Ligands</th>
<th>Acidic Ligands</th>
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<tbody>
<tr>
<td>-NH₂</td>
<td>-COOH</td>
</tr>
<tr>
<td>-NH</td>
<td>-SO₃H</td>
</tr>
<tr>
<td>-N=</td>
<td>-PO(OH)</td>
</tr>
<tr>
<td>or Hetero-</td>
<td>-OH</td>
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<tr>
<td>cyclic N</td>
<td>Enolic</td>
</tr>
<tr>
<td>=O</td>
<td>Phenolic</td>
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<tr>
<td>=N-OH</td>
<td>Oxime</td>
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<tr>
<td>-OH</td>
<td>Thioenolic</td>
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<tr>
<td>-S-</td>
<td>Thiophenolic</td>
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<tr>
<td>Amino</td>
<td>Carboxylic</td>
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<tr>
<td>Imino</td>
<td>Sulphonic</td>
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<tr>
<td>Tertiary Acyclic</td>
<td>Phosphoric</td>
</tr>
<tr>
<td>or Hetero-cyclic</td>
<td>Enolic</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>Phenolic</td>
</tr>
<tr>
<td>Oxime</td>
<td>Oxime</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Thioenolic</td>
</tr>
<tr>
<td>Thioether</td>
<td>Thiophenolic</td>
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Table XXIII - Orbital Configurations

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<thead>
<tr>
<th>Ion</th>
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<th>2s</th>
<th>3s</th>
<th>3p</th>
<th>4s</th>
<th>3d</th>
<th>4p</th>
<th>5s</th>
<th>4d</th>
<th>5p</th>
<th>6s</th>
<th>4f</th>
<th>5d</th>
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<td>Fe</td>
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<td>Cd⁺²</td>
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<td>Cd⁺⁴</td>
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<td>Pb⁺²</td>
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<tr>
<td>Pb⁺⁴</td>
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<td>2</td>
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<td>10</td>
<td>14</td>
<td>10</td>
<td>2</td>
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</tr>
</tbody>
</table>

configurations for Cd, Cr, Cu, Fe, Ni, Pb, and Zn.

Stability constants are used to numerically indicate the strength or stability of a given complex. The more positive the stability constant the greater the stability and the greater the likelihood that the complex will form.

The stability constant 'Kn' is defined by the following
Figure 13 - Ligand Bonding Sites

Acidic Ligand =COOH₂

Basic Ligand =NH₂

Note: That carbon and nitrogen bonds are shown.

*- marks bonding locations

C = carbon; N = nitrogen; O = oxygen; H = hydrogen

LIGAND (Molecular Formulae)

EDTA
C₁₀H₁₆O₈N₂

Ethanic Acid
C₂H₂O₂

Glycine
C₂H₅O₂N

Histidine
C₆H₅N₃O₂

8-Hydroxyquinoline
C₉H₇ON

NTA
C₆H₅NO₆

Oxalic Acid
C₂H₂O₄

formula:

Kn = [MLn][M][MLn-1]⁻¹

Where: M=metal ion
L=ligand
n=number of ligands complexed

The bulk stability constant 'Bn' is the product of the Kn.

\[ B_n = [ML_n]^n ([M][L])^{-1} = \prod Ki \]
APPENDIX C - EXOCELLULAR POLYSACCHARIDES

Corpe (1975) separated the surface active polymers (extracellular polysaccharides) produced by primary film forming bacteria in salt water. He found substantial quantities of metals bound to cell envelopes and extracellular polysaccharides. The bacteria were capable of forming insoluble precipitates with salts of Fe, Cu, and Pb. Neutral polymer solutions formed non-diffusible complexes with Co, Ni, Zn, Mn, and Ca. Table XXIV lists the radicals responsible for the charge on extracellular polysaccharides. Jeanes (1972) observed

Table XXIV - Chemical Radicals Of Charged Exocellular Polymers

<table>
<thead>
<tr>
<th>Species</th>
<th>Radical Causing Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral Polysaccharides</td>
<td>Neutral or N-substituted amino sugars</td>
</tr>
<tr>
<td>Polyanionic Polysaccharides</td>
<td>Uronic acid constituents or acidic substitutes</td>
</tr>
<tr>
<td>Polycationic Polysaccharides</td>
<td>Unsubstituted amino sugars</td>
</tr>
</tbody>
</table>

that uncharacterized bacterial polysaccharides were responsible for the uptake of uranium and other ores.

Extracellular polysaccharides can be produced by some strains of waste water, plant and soil bacteria. Some aerobic and anaerobic bacteria can generate extracellular polysaccharides. Obayashi and Gaudy (1973) studied two waste water, two soil, and one plant bacteria. They found that bacteria can oxidize extracellular polysaccharides and form a
stable end product. Extracellular polysaccharides can be neutral, polyanionic or polycationic (Jeanes 1974). When extracellular polysaccharides contain metal ions they tend to be hydrophilic and form colloidal dispersions in solution.

Sandford (1979) suggested three categories of polymers associated with bacteria. They are intracellular, cell wall, and extracellular polymers. The extracellular polysaccharides are either loose or covalently bonded to cells. Klimek and Ollis (1980) reported that production of polysaccharides may be growth related, wholly non-growth related or mixed. They were able to use rate equations to model production of extracellular polysaccharides. Pace and Righelato (1980) studied the synthesis of extracellular polysaccharides. Some of the extracellular polysaccharides are synthesized outside the cell wall but most are synthesized in the cell wall. The molecular weight of extracellular polysaccharides appears to be highly variable. Powell (1979) theorized that the extracellular polysaccharides exist near the cell wall to allow cells to interact with their environment. It may be possible for the extracellular polysaccharides to adopt a configuration that would allow complexation to occur.

Many micro-organisms are capable of producing extracellular polysaccharides. It is not known if they can form complexes but they do retain metals. Analysis of some extracellular polysaccharides has shown that precipitates can form in them. There is also a possibility that they can form ionic bonds with metal ions. Usually extracellular polysaccharides are
polyanionically and neutrally charged. Most important is the discovery of a stable end product after they were oxidized by leachate bacteria. This may mean that metals bound by extracellular polysaccharides can be fixed.
APPENDIX D - CHELATION PROCEDURE

A. Acid wash seven extraction funnels (at least 250 ml. size), one 100 ml. graduated cylinder and one funnel. Then rinse them with deionized distilled water.

B. Weigh the funnels, a deionized distilled water wash bottle and the sample.

C. Place the funnels, a wash bottle, parafilm, scotch tape, the graduated cylinder, and the blended sample into the glove box. Flush the glove box with nitrogen and raise the pressure until it is 2 cm. of water above atmospheric. Note: the tops of the extraction funnels should be removed to allow the air inside to be flushed out.

D. Shake the sample container to mix the sample. Pour 50 ml. of sample into the graduated cylinder. Then pour contents of cylinder into an empty funnel. Repeat six times so all the funnels are full.

E. Replace the extraction funnel tops. Seal the sample container with parafilm and tape.

F. Turn off the nitrogen gas. Remove the sample container and extraction funnels.

G. Weigh the sample container and each extraction funnel. Put the sample container back in the cold room if sample removal is finished.

H. Using a volumetric pipette, add the calculated volume of spike solution to each funnel. Shake each funnel and leave them two hours to allow equilibrium to be approached.

I. Add the calculated volume of chelating agent to each sample. Shake each funnel and leave overnight.

J. Add 50 ml. of MIBK to each extraction funnel. Shake well. Note: a pressure build-up will occur so the pressure must be relieved. Invert the flask and open the stopcock to do this. Leave funnels overnight.

K. Pour the contents of each extraction funnel into a 250 ml. acid washed light polyethylene container. Centrifuge at a relative centrifugal force of 1600 g. for ten minutes.

L. Pour the supernatant into the corresponding funnel. Allow the light polyethylene bottles to drain for 15
minutes.

M. Shake each flask then leave them to sit overnight.

N. Drain off the water (the bottom layer).

O. Drain the MIBK layer into a Whatman number 541 filter and collect the filtrate for analysis with a Jarrel Ash 810 atomic absorption spectrometer.
APPENDIX E — EXTRACTION PROCEDURE

For Each Sample:

A. Weigh a one litre flask. Add roughly 300 ml. of blended sample and weigh the flask. Make the addition in the glove box under nitrogen atmosphere. Seal the flask using parafilm and tape.

B. Place the sample in the glove box and dilute with deionized distilled water to roughly 900 ml. Weigh the flask.

C. Under a nitrogen atmosphere, remove a moisture content sample, bacterial count sample, and a sample for an initial metals determination (BLK0, SON0, or FOX0).

D. Do one of the following to each sample:
   a) Place sample in a Mettler Electonics 5.5 gal. sonication bath for half an hour at 20 KHz and 4000 peak watts.
   b) Bubble air through sample for 30 hours. Before aerating the sample, bubble the air through an acid trap to remove particles, metals, and water.
   c) This sample receives no treatment as it is the control.

E. Take a bacterial count sample.

F. Inside the glove box, pour sample into four 250 ml. centrifuge bottles. Then weigh the centrifuge bottles.

G. Centrifuge at a relative centrifugal force of 1600 g. Remove the supernatant for analysis (BLK1, SON1, or FOX1).

H. Inside the glove box add 9.635 gms. of ammonium acetate to each centrifuge bottle. Then dilute the samples with deionized distilled water until each centrifuge bottle contains roughly 250 ml. Shake well and leave overnight.

I. Weigh the centrifuge bottles and balance the bottles in pairs by adding deionized distilled water.

J. Centrifuge at a relative centrifugal force of 1600 g. and remove supernatant for analysis. (BLK2, SON2, FOX2).

K. Inside the glove box add 0.435 gms. of hydroxylamine hydrochloride to each centrifuge bottle. Then dilute with deionized distilled water until each centrifuge
bottle contains roughly 250 ml. Shake well and leave overnight.

L. Weigh the centrifuge bottles and balance the bottles in pairs by adding deionized distilled water.

M. Centrifuge at a relative centrifugal force of 1600 g. and remove supernatant for analysis. (BLK3, SON3, FOX3).

N. Weigh out a moisture content sample and weigh the residue.

O. Put the residue in a two litre beaker and dilute with deionized distilled water to roughly one litre.

P. Add hydrogen peroxide 10 ml. at a time until 150 ml. of hydrogen peroxide has been added to the sample. Stir continuously with a mechanical stirrer. Only add more hydrogen peroxide when the foaming has subsided.****

Q. Pour some of the hydrogen peroxide digested sample into two centrifuge bottles and add 9.635 gms. of ammonium acetate to each bottle.

R. Centrifuge at a relative centrifugal force of 1600 g. and pour off the supernatant for analysis. (BLK4, SON4, FOX4).

S. Dilute the residue with deionized distilled water. Add 5.0 gms. of sodium dithionite and 12.5 gms. of sodium citrate to each of the two centrifuge bottles.

T. Centrifuge at a relative centrifugal force of 1600 g. and pour off the supernatant for analysis. (BLK5, SON5, FOX5).

U. Weigh the residue and take a moisture content sample.

V. Acid-digest the residue then filter through a Whatman number 541 filter. (BLK6, SON6, FOX6).

W. Rinse the filter cake with ammonium acetate.

X. Add acidified deionized distilled water (0.5% nitric acid) to make sample up to 250 ml.

NOTE: All the sub-samples for analysis were acid-digested and filtered through a Whatman number 541 filter. The filter cake was rinsed with ammonium acetate (Sub-samples 4, 5, and 6) or acidified deionized distilled water (Sub-samples 0, 1, 2, and 3). Then sub-samples 4, 5, and 6 were diluted up to
250 ml. and sub-samples 0, 1, 2, and 3 were diluted up to 100 ml. (Acidified deionized distilled water was used to dilute the sub-samples) Sub-samples 0, 1, 2, and 3 were made up to 100 ml. and the filter cakes were rinsed with acidified deionized distilled water.

*** An alternate method for oxidizing organics which avoids the use of hydrogen peroxide:

Oven-dry the sub-samples at 500°C, then dissolve the residue in deionized distilled water. Add ammonium acetate to extract the metals released by the oxidation of the organics.

Dry-ashing tends to vapourize the volatile organics and some lead. If volatile organics and lead form a significant portion of a sub-sample, the use of hydrogen peroxide may be advantageous. Otherwise dry-ashing is recommended as it is a safer method for oxidizing organics.
APPENDIX F - SAMPLE EXTRACTION

It is very clear that samples need to be kept at in-situ conditions if one wishes to measure metal speciation. The most difficult in-situ condition to maintain, is an anaerobic environment. In order to retrieve solid and liquid samples under anaerobic conditions, special designs were required. An anaerobic environment is required before the pH can be measured in-situ.

1. LIQUID SAMPLE

To ensure that enough sample was collected to be able to measure cadmium, 400 ml. of liquid (leachate) was collected under an anaerobic atmosphere, from the bottom of a lysimeter. An anaerobic atmosphere was created by allowing the leachate to drain from the lysimeter into a one litre erlenmeyer flask which had a nitrogen atmosphere. The whole flask was acid-washed to prevent contamination.

The nitrogen pressure was 2 cm. of water above atmosphere. A very small pressure was used to prevent air entering the flask but also to avoid flushing the lysimeter with nitrogen. (Nitrogen was supplied by a 100 lb. cylinder). The purity of the nitrogen atmosphere was checked by using a Fisher Hamilton gas partitioner and a Hewlett Packard Integrator Model 3380 A. Oxygen was less than 0.5% of the atmosphere after five minutes of flushing with nitrogen. It took over twenty-four hours to collect 400 ml. of sample. So a flow restriction was used, to avoid draining the nitrogen cylinder too quickly. Figure 14
show a side view of the liquid sample collection flask. The

Figure 14 - Liquid Sample Collection Flask

Note: The \( N_2 \) input tube is longer to minimize short-circuiting.

The \( N_2 \) input was maintained at a pressure 2 mm. above
local atmospheric.

cork in the top had three tubes, one for the leachate to enter,
one for the nitrogen gas to enter, and one for the gas to exit.
To prevent short circuiting of the nitrogen gas flow, the input
tube was located near the bottom. A water trap was put on the
nitrogen gas outlet to prevent oxygen diffusing into the system.

After the sample was collected it was concentrated to 100
ml. before preparation for analysis with a Jarrel Ash 810
atomic absorption spectrometer.
2. **SOLID SAMPLE COLLECTION**

Collection of a solid sample below the surface of a sealed lysimeter presented two problems. One was cutting a hole under anaerobic conditions and the other was removing the sample under anaerobic conditions. A sampling box was devised to meet these needs. Figure 15 shows a side view and an end view of the sampling box. The box was made of plexiglass. It had a removable end plate which had a hole in the centre. A hole was required to allow a steel rod or a wire into the box. There were three gas ports on the bottom of the box. One was to measure gas pressure, one was a nitrogen gas inlet, and the third was a nitrogen gas outlet. The outlet went to a water trap. At the front of the box there was a removable end piece with a curve to fit the curvature of the lysimeter. A strip of neoprene foam was glued to the edge of the curved piece to help form an airtight seal. Two tags, one on either side of the box, were used to attach a loop of rope to the box. The loop was passed around the lysimeter and tightened to help form a tight seal. A stand was made to support the weight of the box. There were two gloves, attached to a collar by hose clamps, on each side of the box. They were flexible giving an operator, outside the box, the ability to manipulate items inside the box.
Add foam to form a seal 16.5 cm.

To fasten box to lysimeter

All ports 16mm OD.

N₂ ports for:
• Input
• Output
• Pressure gauge

Support collar

Collar for glove

0.6 mm Plexiglass

Hole for drill shaft

Hole for wires

Hole for drills

PROFILE

BOTTOM VIEW

END VIEW
a. Drilling

To remove a sample from the box a 8.9 cm. wood drill bit was used to cut the wall of the lysimeter (The lysimeter wall was made of PVC). A variable speed, 1.9 cm. chuck, electric drill was used for all the drilling. The power cord entered the box via the hole in the back plate. A split cork was used to form a seal with the wire.

Once a hole was cut in the side of the lysimeter, a piece of parafilm was taped to the side of the lysimeter to form an airtight seal. (Initially a wooden cork was used but it did not form a good seal.)

After cutting the initial hole, the in-situ pH was measured. An attempt to measure the oxidation-reduction potential was made but the instrument did not function properly. The temperature was also measured before resealing the lysimeter. Once the in-situ measurements were made, the anaerobic box was set up for drilling.

To drill out the sample, two bit types were tested. The first bit was a 7.6 cm. diameter pipe with a band saw blade welded to the leading edge. It was hoped that the bit would remove a core sample. The mixture of materials in the lysimeter proved too much for a band saw blade. After cutting through one tin can, the bit was too dull for use. So, another bit was devised. Figure 16 shows the two bits.

The second bit used two tungsten carbide teeth to cut and a high speed twist drill to centre the bit. (The twist drill broke when it was a quarter of an inch longer than the tungsten
carbide teeth). With the second bit, a longer bit life and a higher drilling rate were achieved. One of the features of the second bit was that it mulched the sample.

In order to apply some pressure on the drill bit, a rod, three feet long, was used. A neoprene ring was inserted in a collar over the hole in the back plate of the anaerobic box. The ring helped provide a low friction bearing surface and formed a seal around the steel rod. It was possible to leave a four litre sample container in the sample box while drilling because the drill could be attached to the end of the rod. Also, the drill could be held properly outside the box. (A large torque could be generated by the bit jamming so it was
important to have a firm grip on the drill).

To fill the sample container, a bent piece of one inch pipe was used. The bend gave some prying capability. There was not much room to manipulate the pipe due to the large collar (3.8 cm. collar) used to attach the gloves to the side of the sample box. Once the sample was scooped into the sample box, the top was sealed using parafilm and freezer tape. The parafilm was stretched to help form a tight seal. Initially, the container top was screwed in place but it tended to break the seal formed by the parafilm. Once the sample was retrieved, the lysimeter and the sample container were sealed. A flow chart of the sample removal is shown in Figure 17.

b. Sample Manipulation

A glove box was used to manipulate samples after removal. The glove box had been purchased previously for another experiment. It was made of heavy plastic and supported by steel rods. A double lock plastic zipper was used to maintain an air-tight seal. The top of the box was translucent to allow manipulations. There was one gas inlet and one gas outlet port. Originally, the box had a side chamber but the zipper for this section was no longer air-tight. A tube from the gas outlet went to a water trap and a U-tube for measuring gas pressure.

When the sample was removed from the lysimeter, it was partially broken-up by the drill bit. For the experimental work, a Waring blender was used to break down the sample even further. A special top was made out of lucite to form an air-tight seal at the top of the blender jug. Figure 18 shows a
Figure 17 - Sample Removal Flow Chart

Attach sample box
Pressurize with $N_2$
Drill hole
seal and place pH probe and thermometer in box
Attach and pressurize box
Measure pH and temperature
Seal lysimeter and depressurize box
Attach drill bit to the end of the rod
Put a sample container and removal tool in box
Attach and pressurize box
Drill into lysimeter contents
Dig, pry and scrape lysimeter contents into sample container
Seal lysimeter and seal sample container
Depressurize and disconnect the box
Put the sample container into a dark, cold ($4^{\circ}$C) room

Figure 18 - Blender Lid

Slots for elastics
Use foam strips to form a seal
$N_2$ Input
$N_2$ Output
side view and bottom view of the lid. There were three gas ports on the lid. One gas port was an intake, another an outlet, and the other port was for measuring the pressure. A neoprene foam was used to form a seal between the jar top and the lucite lid. The lid was held in place by four pieces of rubber, one from each corner.

Initially, the blending was done outside the glove box. That method only allowed blending of 600 ml. of sample and dilution water. Blending outside the glove box required more time and nitrogen gas although it did allow more working room. There was a problem with the sample blocking the gas outlet, so most of the blending was done inside the glove box. To avoid shearing the bacteria, a two minute maximum blending time was used. After blending, the sample was placed in four litre nalgene sample containers. The containers were acid washed and sealed with parafilm and freezer tape. Large pieces of wood, porcelain, and cans were removed from the sample to avoid damaging the blender.

A list of the step by step sample extraction procedure is included below.

A. Place the drill, drill bit, parafilm, and scotch tape in the sampling box.

B. Move the sample box into position and cinch the curved end against the side of the lysimeter using a piece of rope.

C. Flush the sample box with nitrogen gas and pressurize to roughly 2 cm. of water above atmospheric pressure.
D. Drill a 8.9 cm. hole into the side of the lysimeter.
E. Seal the lysimeter and turn off the nitrogen gas.
F. Remove the drill, drill bit, and piece of lysimeter from the sample box. Replace them with: a piece of parafilm, scotch tape, a thermometer, a pH probe, buffer solutions, a wash bottle, and a oxidation-reduction potential probe.
G. Repeat steps 'B' and 'C'.
H. Remove the lysimeter seal to measure the temperature, pH, and oxidation-reduction potential. Place a new seal on the lysimeter.
I. Remove the contents of the sample box. Place in the box: parafilm, scotch tape, a digging tool, a sample container, and a drill bit. Note: it is easier to attach the bit to the drilling shaft prior to positioning the sampling box.
J. Repeat steps 'B' and 'C'.
K. Remove roughly 1.2 litre of sample and place in the sample container. Seal the lysimeter and sample container using parafilm. Use freezer tape to hold parafilm to the sample container.
L. Turn off the nitrogen gas and remove the sample container.
M. Put the sample container in a dark cold room (4°C.) until use.
APPENDIX G - RECOMMENDED DAPI PROCEDURE

1. Add roughly 5 ml. of solids to an acid washed flask.

2. Filter all solutions through a 25 mm. diameter, 0.02 um. Nucleopore filter.

3. Add roughly 5 ml. of 70% ethanol solution. Then dilute up to 50 ml. with deionized distilled water.

4. Store at 4°C until time of slide preparation.

5. Remove 1 ml. of mixture and dilute up to 10 ml. Then shake vigorously.

6. Remove 2 ml. of mixture and add 4 ml. of 0.03M sodium pyrophosphate (filtered).

7. Allow mixture to incubate at room temperature for 30 minutes shaking vigorously every 5 minutes.

8. Sonicate with an ultrasonic probe (120 W) for 60 sec.

9. Dilute up to 6 ml. with deionized distilled water and shake vigorously. Add 0.2 ml of 0.1 M DAPI solution. Leave 15 minutes shaking every 5 minutes.

10. Filter mix through a 0.22 um. nucleopore filter, which has been soaked in irgalan black for a minimum of 8 hrs. and rinsed in deionized distilled water, using 8 mm. of Hg vacuum until the filter is dry.

11. Place filter on a slide and add 1 drop of Cargyle B immersion oil.

12. Allow oil to spread before placing cover slip on top.

13. Place a drop of Cargille B immersion oil on the cover slip and observe the slide under a 340-370 nm. excitation beam at 100X power.
14. Count 4-6 fields of view in 10 or more preparations.

NOTE: It is very important to filter all solutions and sterilize the sample containers. Residual nitric acid, from acid washing containers, should be washed off by rinsing with deionized distilled water.
APPENDIX H - AVAILABLE METHODS

Many experiments have been devised which separated metals into groups according to their physical properties. So far no one has managed to determine which species are in those groupings. Cation exchange gels, resins, dialysis, ultrafiltration, electrophoresis and centrifuging are some of the methods used to separate metals from sludges, soil and leachates. Florence and Batley (1977) looked at five general methods for analysis of metals in fresh water. They stated that separation techniques could only segregate metals according to their chemical or physical characteristics. Examples of separation methods are dialysis, centrifuging, electrophoresis, ion exchange and ultrafiltration. All of these techniques probably alter the balance of the metal species in a sample. Separation methods typically have contamination problems especially from rubber o-rings which can add Zn and Cd to samples. A number of potential methods were considered. Specific electrodes and anodic strip voltametry have characteristically poor detection limits (except in the case of copper where 1 ppm. can be detected) and long warm-up times. The technique of polarographic half wave potential shifts is still in the experimental stages but has been used successfully for stability constant determinations in synthetic solutions. Florence and Batley reported that chelation extraction methods tend to underestimate the percent metal associated with the organic phase. So they developed a procedure which separated metals according to physical properties (Batley and Florence
Batley and Florence used anodic strip voltametry titrations for metal analysis of each category they had separated. They warned that inorganic colloids (ie. hydrated iron or manganese oxides, clay silica and sulphates) and organic colloids (ie. humics, animal debris and plant debris) in natural waters have a sorbing capacity which may give rise to a low estimate of the metal concentration. Their results for Cd, Cu, Pb and Zn speciation in natural water and sea water were unaffected by storage at 4°C. They were able to separate metal species into groups but were unable to identify the mass of a given species within the physically separated groups.

Bergman et al. (1979) used zonal centrifugation to separate density fractions. The technique does not separate metal species but the data obtained indicated at least two methods of metals uptake in sewage sludges. They found metals in the low and high density ranges. Most of the metals were found in a narrow density range which was probably associated with organic materials. Srivastava et al. (1980) used a gel (chromium ferrocyanide) for separating metals from solutions with a high acidity and salt content. They reported that the gel did not recover all metals but specific gels may one day be a good analysis tool.

Chen et al. (1974) used filtration and centrifugation to separate the particulate fraction from sewage effluent. A 0.22 um. filter removed 99% of the metals and a 0.8 um. filter removed 97% of the metals. The optimum metal and sludge...
separation was achieved by centrifuging at 225 g's. (relative centrifugal force) for ten minutes. In order to compare samples Chen et al. calculated the mass of metals per dry weight mass of particulate. Chian and DeWalle (1977) used membrane ultrafiltration, gel permeation, gas chromatography, and specific organic analysis to separate organic categories in leachate. By collecting samples under anaerobic conditions, they prevented colloidal iron hydroxide formation. They used the membranes to separate weight fractions and then analyzed the fractions. The largest organic fraction contained free volatile fatty acids and the next largest organic fraction contained 'fulvic-like' organics which were high in carboxyl and aromatic hydroxyl groups. The smallest organic fraction contained high molecular weight humic carbohydrate complexes with hydroxylzable amino acids. Their results were similar for different leachates. Hoffman et al. (1981) used an ultrafiltration cascade and a mass balance to characterize colloidal metal complexes in river water. They found that Cd, Fe, and Mn ions were associated with oxides, hydroxides or surface adsorbing particles. Their data indicated that Cu, Cd, and Pb would probably be complexed with organics.

Engler et al. (1977) developed an extraction procedure for characterizing metals in sediment. The procedure used ammonium acetate, hydroyxylamine hydrochloride, hydrogen peroxide, sodium citrate to form an extraction gradient. Engler used a combination of extraction methods which had been developed for extracting metals from soil. Chao (1972) reported
that hydroxylamine hydrochloride selectively dissolved manganese oxides, leaving iron oxides in the residue. Holmgren (1967) determined the optimum concentrations of sodium dithionite and sodium citrate for dissolving extractable iron. Jackson (1958) outlined an experimental procedure for using ammonium acetate to determine exchangeable metallic cation species. He also outlined a technique for oxidizing organic matter with hydrogen peroxide. The low temperature oxidation (less than 100°C) prevents the release of hydroxyl and strongly sorbed water associated with mineral colloids.
APPENDIX I - DATA AND CALCULATIONS FOR ESTIMATE OF LIQUID SAMPLE VOLUME

Samples were prepared for atomic absorption spectrometer analysis by acid digesting a bulk sample and evaporating the sample to dryness. Then the residue was dissolved in acid and diluted. After filtering the sample through a Whatman number 541 filter the sample was diluted up to a volume of 50 ml. A 50 ml. sample was required for atomic absorption spectrometer analysis of seven metals. The list below shows the metal concentrations in the most recently tested lysimeter leachate sample. The detection limits for each metal are also shown. The concentration values were obtained from the UBC co-disposal study (Atwater et al. 1981)

Table XXV - Concentration Of Metals In The Last Leachate Sample

<table>
<thead>
<tr>
<th>Metal</th>
<th>Concentration (mg/l)</th>
<th>Detection Limit (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Cr</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Fe</td>
<td>83</td>
<td>0.05</td>
</tr>
<tr>
<td>Pb</td>
<td>0.09</td>
<td>0.5</td>
</tr>
<tr>
<td>Ni</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>Zn</td>
<td>9.5</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The smallest metal concentration was Cd so it was used as the limiting metal for the calculations. To ensure that Cd could be detected a sample containing five times the cadmium detection limit should be collected.

Sample volume*[detection limit] = minimum mass
0.05 \times 0.05 = 0.0025 \text{ mg}

\text{Minimum mass/}[\text{concentration in leachate}] = \text{minimum volume}

\frac{0.0025}{0.03} = 0.083 \text{ L}

0.083 \times 5 = 0.412 \text{ L}

So a volume of 400 ml. was collected for the liquid sample volume.
APPENDIX J - DATA AND CALCULATIONS FOR ESTIMATE OF SOLID SAMPLE VOLUME

The following calculations were based on concentration data from the Atwater et al. study (1981). All the calculations were for Cd as it was available in the smallest quantities. A sample volume of 0.05 L was assumed for atomic absorption spectrometer samples.

Minimum moles of Cd for detection = 0.05x0.05 = 0.0025 mg.
Mass desired = 5 x 0.0025 = 0.0125 mg.
Net mass in Tank F = 0.57 - 0.033 = 0.537 gms.
Volume of Tank F = 70 x (30)^2 x 0.25 x π = 50 L.

Note: the actual height is 95 cm. but there are 15 cm. of topsoil and at least 5 cm. of dead space (top and bottom) which do not contain metals. So 25 cm. were subtracted from the actual height.

Concentration of Cd in Tank F = 0.537/50 = 10 mg/L.

To allow for 28 extraction samples and 35 chelation samples (63 total):

\[65 \times 0.0125 = 0.8125 \text{ mg.}\]

\[0.81 / 10 = 0.08 \text{ L of solid sample are required.}\]

This calculates the compressed volume of solids that would be required. The mulching of the sample during drilling increases the sample volume so the sample size must be based on the size of the cavity created by sampling.
APPENDIX K — DANGEROUS PROPERTIES OF CHEMICALS

Ammonium Acetate — No hazards listed.

Ethanic Acid (Acetic acid) — Irritant, caustic, flammable if exposed to flame. Dangerous with HNO₃. Can burn, penetrates skin easily causing dermatitis and ulcers. Irritates mucous membranes of the nose.

Ethanol — Rapidly oxidizes, irritates eyes and nose if exposed to 5000-10000 ppm. May cause drowsiness if exposure is prolonged over an hour.

Glycine — No hazards listed.

Hydroxylamine hydrochloride — Toxic chloride fumes on contact with acid, dangerous when heated.

Hydrogen peroxide — Tissue irritant, can burn at 35% concentration. Eyes are very sensitive to contact. If highly concentrated it is explosive.

L-Histidine — No hazards listed.

8-hydroxyquinoline — Acrid fumes when heated, slight fire hazard. Also called 8-quinolinol.

Methyl-iso-butyl ketone — Dangerous when exposed to heat or flame. Skin irritant and inhalation irritant.

Nitrilo-tri-acetic acid — No hazard listed.

Nitric Acid — Moderate fire hazard, fumes are highly irritant to eyes and nose.

Pyrrophosphate — No hazard listed.

Oxalic Acid — Corrosive, vapour irritates eyes and nose. Prolonged exposure to vapour has severe side effects.

Sodium citrate — No hazard listed.

Sodium dithionite — No hazard listed.

Scientists around the world have been using fluorescent dyes as aids for detecting microscopic bodies for the past three decades. Acridine orange (AO) has been one of the more popular dyes and was used until recently. Staining techniques have now been developed, using 4', 6 diamidino-2-phenylindole (DAPI), and mithramycin, which provided greater enhancement and specificity than AO.

When AO techniques were first being developed it was originally believed that living cells fluoresced green while dead cells fluoresced red. Bucherer later showed that the colour distinction was caused by an excess of AO glowing red. The plasma of living gram positive cells tended to have a higher sorptive capacity than gram negative cells so they glowed red even when alive. This did not stop scientists from using AO. Francisco et al. (1973) showed that the counts obtained using AO compared favourably with a Petroff-Hauser Counting chamber (PHC chamber). The PHC chamber has a detection limit of $10^7$ bacteria/ml. So it cannot be used for bacterial counts in natural waters. Francisco recommended that AO be used for total whole cell counts in fresh water. To ensure consistency, Francisco prefiltered all solutions and used AO from one manufacturer. (AO varied between manufacturers)

1. The original article was by S. Strugger in Flureszenzmikroskopie and Mikrobiologie (1949). It was referenced in the article by Trolldenier.
2. The article by Bucherer (1966) is written in German and was referenced by Trolldenier.
Trolldenier (1973) attempted to extend the use of AO to bacterial counts in soils. By adding sodium metaphosphate he was able to disperse the soil particles which gave higher bacterial counts and a random bacterial distribution. Trolldenier found that 4 to 6 fields of view/preparation and 10 preparations resulted in a 5% probability that the real value was more than 10% different from the value obtained.

Trolldenier obtained a high correlation between plate counts and fluorescent counts. He used AO to enumerate bacteria in soil. As long as the soil particles are well dispersed, accurate counts could be made, otherwise AO is non-specific for in-situ soil micro-organisms. AO is not useful for a complex mixture unless it is specific for the bacteria in the material under study or the material and bacteria can be dispersed.

The conclusions of Trolldenier and others caused researchers to look for alternate dyes and better techniques. Jones (1974) compared AO to fluorescein isothiocyanate (FITC) and euchrysine 2GNX (E-2GNX). Jones concluded that E-2GNX was consistently better than AO but that the conclusion was dependent upon the experimental method used. Daley (1975) came to a similar conclusion; the results were sensitive to sample type, contact time, concentration of fluorochrome, operator, lamp type, filter size, fixation, and procedure. AO and E-2GNX both increase the resolution but the results obtained using the experimental technique of Francisco et al. (1973) or Zimmerman and Meyer-Reil¹, were not reproducible for cell counts in
natural waters. Daley recommended using a sample which gives 15 to 30 bacteria per graticule for counting, the use of 2-5% formaldehyde solution for fixation and a black filter background.

Some researchers compared AO to other counting methods. Watson (1977) compared AO with a transmission electron microscope (TEM), a Lipopolysaccharide method, and an ATP determination. AO and TEM are direct count methods and are able to discriminate between procaryote and eucarote cells. The other techniques only estimate cell mass. So AO or other fluorescent dye techniques are the quickest and cheapest methods for obtaining bacterial counts in natural waters.

Hobbie et al. (1977) outlined general principles for direct count techniques:

a) All bacteria must be retained by the filter.

b) All bacteria must be visible at the filter surface.

c) Staining and optics must produce a high contrast between bacteria and background.

Hobbie was able to improve the background by staining 0.22 um. nucleopore filters with irgalan black and by using a Cargille type B immersion oil. His technique agreed well with the limalus lysate, scanning electron microscope, and TEM methods. In further work by Daley (1979), he found AO to be better than LPS, FITC, and E-2GNX for estimating total numbers of aquatic bacteria in natural waters. He found that aggregation of cells

3. This article was referred to by Daley (1979) and is found in the journal Kieter Meeresforschungen Vol.30, pp.24-27, 1974.
could not be avoided but that a minimum of 6 ml. of sample should be filtered giving 5-25 cells per field for best results. A control should be run because a filtered blank could still have 1-2% of typical sample counts. Costerton and Geesey (1979) found that molecular oxygen decomposes aldehydes to form acids which may decompose cells. So they recommended a 5% alkaline buffered argon and purged glutaldehyde for sample fixation.

While Hobbie, Daley, and others tried to perfect AO methods, other researchers were developing a technique for 4', 6 diamidino-2-phenylindole (DAPI). In 1975, it was shown that the DAPI-DNA complex is fluorescent. Kapuscinski and Skoczylas (1977) developed a fluorometric microassay technique which was sensitive to 5*10^{-10} gm DNA/ml. This was much better than the 5*10^{-7} gm DNA/ml. previously achieved with mithramycin. Their technique was not affected by RNA, nucleotides, histones, urea or pH (5.0 < pH < 10.0). The fluorescent intensity was found to decrease with increased ionic strength. Leeman and Ruch (1978) and Kapuscinski and Skoczylas (1980) experimented to determine how DAPI was bound to DNA. Both mithramycin and DAPI required at least two binding sites. Each dye had a specific dye-DNA site and one general site that would accept either dye. The DNA-DAPI complex was found to be very stable and formed a rigid bond. Leeman and Ruch found that DAPI-DNA banding appeared sharper after refrigeration for a few

days. Kapuscinski and Skoczylas found that fluorescent intensity increased as viscosity, solids, SDS concentration (anionic detergent) increased. Low concentrations of NaCl and superficial tensions had no effect on fluorescence.

In a 1979 paper, Salari and Ward (1979) compared DAPI with hoechst 33258 and a giesma stain. They found that DAPI and hoechst 33258 had greater sensitivity and was easier to use. They used a phosphate buffered saline solution to wash out bacteria, yeast, white cells, epithelia, and other DNA containing debris from the fixed cells they looked at. Cowell and Franks (1980) found that pretreatment with RNase (1 mg./ml. for 20 minutes at 37°C) removed the general cytoplasmic staining without affecting the nuclear staining intensity. They found that methanol in acetic acid (3:1) as a fixative gave 20% higher fluorescence than formaldehyde and methanol in acetic acid (10:85:5). Naimski et al. (1980) found that DAPI bound proportionally to DNA in the 0.032-0.320 ug. DNA range. There was some dependence upon the quantity of deoxyadenylate-thymidylate (AT nucleotides) regions. Porter and Feig (1980) used DAPI for counting and identifying shapes of natural aquatic microflora. They found DAPI far superior to AO because of increased visualization, increased storage times (up to 24 weeks), and increased stability of the DAPI-DNA complex under the microscope. Allan and Miller (1980) compared mithramycin with DAPI for staining yeast nuclei and found that mithramycin stained more consistently. Coleman (1980) compared DAPI with mithramycin for staining in natural environments. She
found that both dyes could be used in the pH 4 to 8 range and 5-30 min. of exposure to the dye ensured penetration. Although DAPI fluoresces brighter than mithramycin, it binds with some mucocyst-trichocyst material in flagellates, polyphosphate granules, and very slightly to bacterial slime trails. Both dyes gave uniform and repeatable results. She found that a pH=4.4 McIlvaine buffer solution reduced the background fluorescence and wall binding. Also, a 3:1 or 70% ethanol fixation procedure was more consistent than using formaldehyde. Coleman et al. (1981) picked DAPI in preference to ethidium bromide, AO, hoechst 33258, feugen reaction, and chromomycin A-3 because of it's greater sensitivity and slow fading.

Considering the published materials, some experimentation and personal communications with Iqubal Velgi (SFU), the procedure outlined in Appendix G was compiled.
APPENDIX M - CONCENTRATION VS ABSORBANCY PLOTS FOR CHELATION

DATA SET ONE

Chelate Test -- Conc. vs Abs. -- Cd

Chelate Test -- Conc. vs Abs. -- Cd2

Chelate Test -- Conc. vs Abs. -- Cr
Chelate Test -- Conc. vs Abs. -- Cr2

Chelate Test -- Conc. vs Abs. -- Cu

Chelate Test -- Conc. vs Abs. -- Cu2
Chelate Test -- Conc. vs Abs. -- Fe

Chelate Test -- Conc. vs Abs. -- Ni

Chelate Test -- Conc. vs Abs. -- Pb
Chelate Test — Conc. vs Abs. — Pb2

Chelate Test — Conc. vs Abs. — Zn

Chelate Test — Conc. vs Abs. — Zn2
APPENDIX N — PLOT OF $\log_{10}(K_s)$ VS LIGAND

$\log_{10}(K_s)$ vs Ligand

- Cr(III)
- Cu(II) Maximum $K_s$
- Fe(III)
- Fe(II)
- Zn(II)

Ligand: Eth, Ox, Cly, Hist, NTA, EDTA, Hyd
APPENDIX O — PERCENT SPIKE VS ABSORBANCY PLOTS FOR CHELATION DATA SET TWO

Chelates — Cr — Abs. vs Spike

Chelates — Cu — Abs. vs Spike

Chelates — Hydroxy — Abs. vs Spike
Chelates -- Fe -- Abs. vs Spike

Chelates -- Zn -- Abs. vs Spike
Assume:
1) Extraction efficiency = 1 for each complex
2) Schubert conditions occur ([ligand]>>[metal]).

Equations:
1) \( \Sigma ZMnLm = \Sigma ZBnm * Mn * (L) \)
2) \( \Sigma ZMnLm = MO - \Sigma ZMn \)
3) \( \Sigma ZMnLm = LO - L \)
4) \( \Sigma ZMnLm = ML \)

If values for \( Bnm \) can be determined experimentally then equations 1-4 can be solved for \( n=1,2 \) and \( m=1,2...6 \). In special cases the equations can be solved when \( n=3 \) (the third species is a solid). The values of \( MO \) and \( ML \) can be determined experimentally and the ligand concentration \( LO \) is controlled.

Table XVI was calculated assuming:
1) 1:1 ligand-metal complexes \((m=1)\).
2) Only one species is present in significant concentrations \((n=1)\).

The second assumption may not be valid for iron, chromium or copper. Stability constants were not available for the \( \text{Cu(I)} \) species so \( ML \) was not calculated in this case. If \( n=2 \) the value of \( ML \) cannot be determined without experimental data.

For \( n=m=1 \) equations 1-4 become:
1) \( M1L1 = B_{11} * M1 * L \)
2) \( M1L1 = MO - M1 \)
3) \( M1L1 = LO - L \)
4) \( M1L1 = ML \)

The terms \( M1L1, \ ML, \ L \) and \( M1 \) are unknowns and there are four equations so a solution can be found. The equations reduce to:
\[ ML = K * (MO-ML) * (LO-ML) \] OR \[ 0=MOLO-ML(LO+K^{-1}+MO)+ML^2 \]

\( ML \) can be solved using the solution for a quadratic equation. The initial values used for Table XVI are shown in Table XXV and the \( B_{11} \) values were taken from Table X.

Note that the equation for \( ML \) changes if there are 1:2 and 1:1 complexes.
\( ML= B_{12}*(MO-ML)*(LO-ML)^2 \) A trinomial in \( ML \).
Table XXVI - Initial Values For Calculation Of Complex Concentrations

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximum [Metal] uM/dry gm.</strong></td>
<td>0.4</td>
<td>1.8</td>
<td>1.3</td>
<td>82</td>
<td>0.18</td>
<td>3.0</td>
<td>9.8</td>
</tr>
<tr>
<td><strong>Log10(Conc.)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS1-3.6 gm.</td>
<td>-5.84</td>
<td>-5.19</td>
<td>-5.63</td>
<td>-3.53</td>
<td>-6.19</td>
<td>-4.97</td>
<td>-4.45</td>
</tr>
<tr>
<td>(moles)</td>
<td>-6.14</td>
<td>-5.49</td>
<td>-5.63</td>
<td>-3.83</td>
<td>-6.49</td>
<td>-5.27</td>
<td>-4.75</td>
</tr>
<tr>
<td>DS2-1.8 gm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(moles)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LO Values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS2</td>
<td>4.77</td>
<td>17.15</td>
<td>8.57</td>
<td>6.85</td>
<td>5.98</td>
<td>6.38</td>
<td>8.56</td>
</tr>
</tbody>
</table>
APPENDIX Q - PSEUDO MASS RATIO CALCULATIONS FOR THE CHELATION EXPERIMENT

Table XXVII - Calculation Of Rx

Data Set One -- DS1

If:

Vs = volume of MIBK added to sample before rinse -- in ml.
Vr = volume of MIBK rinse -- in ml.
Mm = measured metals -- gm.
Cm = concentration of metals in sample -- mg/l.
P = fraction of solids
W = mass of sample -- gm.
RR = mass ratio -- mass of metals/mass of sample dry weight
x = a different constant for each metal
f1 = mass correction factor
Am = absorbancy units

Then:  
RR = Mm/WP
Mm = Cm(Vs+Vr)
RR = Cm(Vs+Vr)/WP

Let:  
f1 = (Vs+Vr)/WP

Then:  
RR/x = Rx = Am(f1)

<table>
<thead>
<tr>
<th></th>
<th>EDTA</th>
<th>Eth</th>
<th>Gly</th>
<th>Hist</th>
<th>Hyd</th>
<th>NTA</th>
<th>Oxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>121</td>
<td>122.5</td>
<td>113.7</td>
<td>122.1</td>
<td>116.8</td>
<td>84.3</td>
<td>117.7</td>
</tr>
<tr>
<td>Vs</td>
<td>30.5</td>
<td>29</td>
<td>34.5</td>
<td>23*</td>
<td>29.5</td>
<td>35.5</td>
<td>32</td>
</tr>
<tr>
<td>f1</td>
<td>6.97</td>
<td>6.63</td>
<td>8.15</td>
<td>6.83</td>
<td>7.05</td>
<td>11.2</td>
<td>7.43</td>
</tr>
</tbody>
</table>

* - Spilt roughly 7 ml. of sample.
Table XXVIII - Calculations Of Xm

Data Set Two -- DS2

RR = Mm/WP
RR = CmVs/WP
RR/Vs = Cm/WP
Cm = xAm
RR/Vs = xAm/WP
x = a constant which is different for each metal.
Multiply both sides of the equation by 50 gms. to calibrate the samples to a 50 gms.

wet mass sample.
RR50/xVs = Am50/WP = Xm

Let: f2 = 50/WP
Xm = Am(f2)

<table>
<thead>
<tr>
<th></th>
<th>EDTA</th>
<th>Eth</th>
<th>Gly</th>
<th>Hist</th>
<th>Hyd</th>
<th>NTA</th>
<th>Oxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blk W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f2</td>
<td>39.5</td>
<td>41.4</td>
<td>41.1</td>
<td>41.7</td>
<td>37.4</td>
<td>39.9</td>
<td>39.2</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>33.5</td>
<td>33.8</td>
<td>33.3</td>
<td>37.1</td>
<td>34.8</td>
<td>35.4</td>
</tr>
<tr>
<td>10% W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f2</td>
<td>47.0</td>
<td>50.7</td>
<td>43.6</td>
<td>46.4</td>
<td>51.3</td>
<td>51.3</td>
<td>49.6</td>
</tr>
<tr>
<td></td>
<td>34.3</td>
<td>31.8</td>
<td>37.0</td>
<td>34.8</td>
<td>31.4</td>
<td>31.4</td>
<td>32.5</td>
</tr>
<tr>
<td>50% W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f2</td>
<td>52.2</td>
<td>48.6</td>
<td>51.4</td>
<td>47.1</td>
<td>49.3</td>
<td>51.5</td>
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<tr>
<td></td>
<td>26.6</td>
<td>28.6</td>
<td>27.0</td>
<td>29.5</td>
<td>28.2</td>
<td>27.0</td>
<td>27.2</td>
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<tr>
<td>100% W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f2</td>
<td>42.5</td>
<td>38.2</td>
<td>41.1</td>
<td>40.6</td>
<td>40.5</td>
<td>39.6</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>38.0</td>
<td>42.2</td>
<td>39.2</td>
<td>39.7</td>
<td>39.8</td>
<td>40.7</td>
<td>37.6</td>
</tr>
</tbody>
</table>

Note: P = 0.036 for Blk and 50%
P = 0.031 for 10% and 100%
APPENDIX R — METAL SPECIES FORMULAE AND DERIVATIONS

Assume:

a) Shubert conditions LO>>MO and L>>Mn.
b) Values for En, Bnm, MA and MO can be determined experimentally.
c) The value of LO is known.
d) The activity=concentration.
e) A maximum of three metal species can occur in solutions. M3=1 indicates that the third species is a solid.

Then:

1) \(\Sigma MnLm = ML\)
2) \(\Sigma MnLm = \Sigma Bnm*Mn*(L)\)
3) \(\Sigma MnLm = MO - \Sigma Mn\)
4) \(LO = L + \Sigma MnLm\)
5) \(\Sigma MnLm*Enm = MA\)
6) \(\Sigma MnLm = \Sigma Bnm*Mn*Enm*(L)\)

Abbreviations:

\[Zn = \Sigma Bnm*Enm*L\]
\[Sn = \Sigma Bnm*L\]

Solving for L the following equation is obtained:

\[(MA-Z3)*(S1-S2)-MO*(Z2*S1-Z1*S2)+S3*(Z1-Z2) = (L-LO)*(Z1-Z2-S1*Z2+S2*Z1)\]

The equation can be solved for any m but values of m greater than one require a polynomial solution. In the special case where m=1 (ie. EDTA) a quadratic solution can be found.
Values for Bnm, MO and MA should be determined experimentally. Values for LO are controlled in the ligand additions but they should be much larger than the expected metal concentrations for optimal results.
APPENDIX S -- CONCENTRATION VS ABSORBANCY PLOTS FOR EXTRACTION DATA

Extraction -- Cd -- Conc. vs Abs.

Extraction -- Cd -- Conc. vs Abs.

Extraction -- Cd -- Conc. vs Abs.
APPENDIX T - MASS RATIO CALCULATIONS FOR THE EXTRACTION EXPERIMENT

The data listed in matrix form below was obtained from the graphs in Appendix S. The values are listed in mg/l.

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>SON 0</td>
<td>1.160</td>
<td>9.940</td>
<td>65.000</td>
<td>2700.000</td>
<td>2.690</td>
<td>122.000</td>
<td>386.000</td>
</tr>
<tr>
<td>SON 1</td>
<td>0.630</td>
<td>0.390</td>
<td>10.600</td>
<td>69.000</td>
<td>0.810</td>
<td>3.920</td>
<td>47.000</td>
</tr>
<tr>
<td>SON 2</td>
<td>0.960</td>
<td>0.510</td>
<td>28.900</td>
<td>740.000</td>
<td>0.700</td>
<td>8.200</td>
<td>37.000</td>
</tr>
<tr>
<td>SON 3</td>
<td>0.970</td>
<td>0.800</td>
<td>10.900</td>
<td>320.000</td>
<td>0.370</td>
<td>11.000</td>
<td>55.000</td>
</tr>
<tr>
<td>SON 4</td>
<td>0.300</td>
<td>7.350</td>
<td>13.900</td>
<td>68.000</td>
<td>0.370</td>
<td>3.160</td>
<td>25.500</td>
</tr>
<tr>
<td>SON 5</td>
<td>0.380</td>
<td>11.100</td>
<td>1.610</td>
<td>3600.000</td>
<td>1.660</td>
<td>21.900</td>
<td>238.000</td>
</tr>
<tr>
<td>SON 6</td>
<td>0.330</td>
<td>4.810</td>
<td>28.000</td>
<td>2200.000</td>
<td>0.720</td>
<td>24.800</td>
<td>87.000</td>
</tr>
<tr>
<td>BLK 0</td>
<td>1.750</td>
<td>10.000</td>
<td>65.000</td>
<td>4000.000</td>
<td>6.900</td>
<td>146.000</td>
<td>480.000</td>
</tr>
<tr>
<td>BLK 1</td>
<td>1.360</td>
<td>0.090</td>
<td>11.100</td>
<td>74.000</td>
<td>5.700</td>
<td>1.800</td>
<td>56.600</td>
</tr>
<tr>
<td>BLK 2</td>
<td>2.170</td>
<td>0.210</td>
<td>29.500</td>
<td>48.000</td>
<td>4.940</td>
<td>5.500</td>
<td>41.800</td>
</tr>
<tr>
<td>BLK 3</td>
<td>0.960</td>
<td>0.200</td>
<td>9.700</td>
<td>76.000</td>
<td>1.850</td>
<td>8.000</td>
<td>37.000</td>
</tr>
<tr>
<td>BLK 4</td>
<td>0.490</td>
<td>3.690</td>
<td>8.200</td>
<td>940.000</td>
<td>0.340</td>
<td>26.400</td>
<td>65.800</td>
</tr>
<tr>
<td>BLK 5</td>
<td>0.040</td>
<td>3.310</td>
<td>1.460</td>
<td>2300.000</td>
<td>0.720</td>
<td>7.400</td>
<td>266.000</td>
</tr>
<tr>
<td>BLK 6</td>
<td>0.690</td>
<td>9.220</td>
<td>42.000</td>
<td>3800.000</td>
<td>2.480</td>
<td>146.000</td>
<td>236.000</td>
</tr>
<tr>
<td>FOX 0</td>
<td>1.330</td>
<td>3.510</td>
<td>53.000</td>
<td>2500.000</td>
<td>1.540</td>
<td>126.000</td>
<td>396.000</td>
</tr>
<tr>
<td>FOX 1</td>
<td>177.500</td>
<td>1.660</td>
<td>395.000</td>
<td>1630.000</td>
<td>4.900</td>
<td>134.000</td>
<td>547.000</td>
</tr>
<tr>
<td>FOX 2</td>
<td>43.400</td>
<td>0.160</td>
<td>43.000</td>
<td>45.000</td>
<td>1.660</td>
<td>6.700</td>
<td>88.600</td>
</tr>
<tr>
<td>FOX 3</td>
<td>14.000</td>
<td>0.330</td>
<td>7.900</td>
<td>230.000</td>
<td>0.620</td>
<td>5.500</td>
<td>46.400</td>
</tr>
<tr>
<td>FOX 4</td>
<td>4.290</td>
<td>21.800</td>
<td>81.000</td>
<td>390.000</td>
<td>0.715</td>
<td>5.900</td>
<td>50.000</td>
</tr>
<tr>
<td>FOX 5</td>
<td>1.460</td>
<td>4.670</td>
<td>4.790</td>
<td>4100.000</td>
<td>1.740</td>
<td>15.000</td>
<td>586.000</td>
</tr>
<tr>
<td>FOX 6</td>
<td>0.690</td>
<td>5.210</td>
<td>30.000</td>
<td>1400.000</td>
<td>0.710</td>
<td>34.500</td>
<td>105.000</td>
</tr>
</tbody>
</table>

The program on the next page reads all the aerated, blank or sonicated data and calculates the mass ratios, % of total mass ratio and cumulative % of total mass ratio. This data is outputed as x and y coordinates for a plotting program.
The purpose of this file is to calculate an output file for mass ratio vs phase plots. The mass ratio values for BLK and FOX data are calculated using the same program. All occurrences of SON are changed to BLK or FOX when the other data is calculated. A data file must be concatenated with this file.

This section inputs the data:

```
REAL SON(7,7), SONM(7,7), C(7), V, B(7), SUM(7), SONM2(7,7)
```

- J = metal number: 1 = Cd, 2 = Cr, 3 = Cu, 4 = Fe, 5 = Ni, 6 = Pb, 7 = Zn
- I = phase number
- V = volume of AA sample
- C = dry weight factor

This section adjusts the data for sample volume:

```
DO 20 I = 1, 7
   V = 0.1
   DO 10 J = 1, 7
      SUM(J) = 0
      IF(I.GE.5) V = 0.25
      SONM(I,J) = SON(I,J) * V / C(I)
   10 CONTINUE
```

This section prints the input data in matrix form:

```
PRINT 50
  FORMAT(T13,'Cd', T23,'Cr', T33,'Cu', T43,'Fe', T53,'Ni', 
        T63,'Pb', T73,'Zn', '/)
DO 25 I = 1, 7
   M = I - 1
   PRINT 100, M, (SON(I,J), J = 1, 7)
25 CONTINUE
```

This section outputs x and y co-ordinates for plotting:

```
DO 33 J = 1, 7
   B(J) = 0
   DO 33 I = 1, 7
      MMM = I - 1
      PRINT 330, MMM, SONM(I,J)
330 FORMAT(I3, F12.6)
   DD 44 I = 2, 7
   DD 44 J = 1, 7
   B(J) = B(J) + SONM(I,J)
   DO 44 J = 1, 7
```

```
CONTINUE
```

```
DD 35 J = 1, 7
DD 35 I = 1, 7
SONM(I,J) = SONM(I,J) * 100 / B(J)
M = I - 1
IF(I.LE.2) GOTO 34
SONM2(I,J) = SONM2(I-1, J) + SONM(I,J)
GOTO 37
34 CONTINUE
```

```
SONM2(I,J) = SONM(I,J)
37 CONTINUE
```

```
PRINT 200, MMM, SONM(I,J), MMM, SONM2(I,J)
200 FORMAT(I3, F12.6, I3, F12.6)
STOP
```

End
The data listed in matrix form below was obtained from the program on the previous page. All values are mass ratios of metal mass over dry mass of sample (after drying at 104°C).

<table>
<thead>
<tr>
<th></th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLK 0</td>
<td>0.035714</td>
<td>0.204082</td>
<td>1.326530</td>
<td>81.632640</td>
<td>0.140816</td>
<td>2.979592</td>
<td>9.795918</td>
</tr>
<tr>
<td>BLK 1</td>
<td>0.004345</td>
<td>0.000288</td>
<td>0.035926</td>
<td>0.281482</td>
<td>0.006852</td>
<td>0.029630</td>
<td>0.137037</td>
</tr>
<tr>
<td>BLK 2</td>
<td>0.000714</td>
<td>0.059107</td>
<td>0.026071</td>
<td>41.071420</td>
<td>0.012857</td>
<td>0.132143</td>
<td>4.750000</td>
</tr>
<tr>
<td>BLK 3</td>
<td>0.003556</td>
<td>0.000741</td>
<td>0.085417</td>
<td>0.791666</td>
<td>0.003542</td>
<td>0.275000</td>
<td>0.685417</td>
</tr>
<tr>
<td>BLK 4</td>
<td>0.005104</td>
<td>0.209545</td>
<td>0.954545</td>
<td>86.363630</td>
<td>0.056364</td>
<td>3.318181</td>
<td>3.363636</td>
</tr>
<tr>
<td>BLK 5</td>
<td>0.015682</td>
<td>0.209545</td>
<td>0.954545</td>
<td>86.363630</td>
<td>0.056364</td>
<td>3.318181</td>
<td>3.363636</td>
</tr>
<tr>
<td>BLK 6</td>
<td>0.035714</td>
<td>0.204082</td>
<td>1.326530</td>
<td>81.632640</td>
<td>0.140816</td>
<td>2.979592</td>
<td>9.795918</td>
</tr>
<tr>
<td>S0N 0</td>
<td>0.004345</td>
<td>0.000288</td>
<td>0.035926</td>
<td>0.281482</td>
<td>0.006852</td>
<td>0.029630</td>
<td>0.137037</td>
</tr>
<tr>
<td>S0N 1</td>
<td>0.000714</td>
<td>0.059107</td>
<td>0.026071</td>
<td>41.071420</td>
<td>0.012857</td>
<td>0.132143</td>
<td>4.750000</td>
</tr>
<tr>
<td>S0N 2</td>
<td>0.003556</td>
<td>0.000741</td>
<td>0.085417</td>
<td>0.791666</td>
<td>0.003542</td>
<td>0.275000</td>
<td>0.685417</td>
</tr>
<tr>
<td>S0N 3</td>
<td>0.005104</td>
<td>0.209545</td>
<td>0.954545</td>
<td>86.363630</td>
<td>0.056364</td>
<td>3.318181</td>
<td>3.363636</td>
</tr>
<tr>
<td>S0N 4</td>
<td>0.015682</td>
<td>0.209545</td>
<td>0.954545</td>
<td>86.363630</td>
<td>0.056364</td>
<td>3.318181</td>
<td>3.363636</td>
</tr>
<tr>
<td>S0N 5</td>
<td>0.003556</td>
<td>0.000741</td>
<td>0.085417</td>
<td>0.791666</td>
<td>0.003542</td>
<td>0.275000</td>
<td>0.685417</td>
</tr>
<tr>
<td>S0N 6</td>
<td>0.015682</td>
<td>0.209545</td>
<td>0.954545</td>
<td>86.363630</td>
<td>0.056364</td>
<td>3.318181</td>
<td>3.363636</td>
</tr>
<tr>
<td>AER 0</td>
<td>0.001176</td>
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<td>0.445378</td>
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<td>0.243816</td>
<td>0.002862</td>
<td>0.058352</td>
<td>0.166078</td>
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<tr>
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<td>0.055945</td>
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<td>0.218232</td>
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<td>Cu</td>
<td>Fe</td>
<td>Ni</td>
<td>Pb</td>
<td>Zn</td>
<td></td>
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<td>166.666600</td>
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<td>1.878788</td>
<td>6.590908</td>
</tr>
</tbody>
</table>
APPENDIX U — CONCENTRATION VS ABSORBANCY PLOTS FOR LIQUID DATA

The cadmium data was at detection limits so the data was not used. For all the other metals tested the concentrations are reported below. A1 was a sample of the supernatant after centrifuging the leachate sample. The total volume of the sample used was 356 ml. S2 was a mixture of supernatant and residue while S3 was mainly residue.

\[(S1+S2+S3)*0.05/0.356 = \text{mg./l concentration of each metal in the Leachate.}\]

Table XXIX — Metal Concentrations In The Leachate Sample

<table>
<thead>
<tr>
<th>Metal</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>ug/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>0.21</td>
<td>0.16</td>
<td>0.13</td>
<td>70</td>
</tr>
<tr>
<td>Cu</td>
<td>0.11</td>
<td>0.10</td>
<td>0.06</td>
<td>38</td>
</tr>
<tr>
<td>Fe</td>
<td>7.6</td>
<td>3.9</td>
<td>4.0</td>
<td>2200</td>
</tr>
<tr>
<td>Ni</td>
<td>1.9</td>
<td>3.5</td>
<td>3.5</td>
<td>1200</td>
</tr>
<tr>
<td>Pb</td>
<td>0.08</td>
<td>0.09</td>
<td>0.09</td>
<td>37</td>
</tr>
<tr>
<td>Zn</td>
<td>10.5</td>
<td>5.0</td>
<td>4.9</td>
<td>2900</td>
</tr>
</tbody>
</table>

Concentration vs Absorbancy — Leachate Sample — Cd
## APPENDIX V — REDUCTION POTENTIALS

### Table XXX — Reduction Potentials

<table>
<thead>
<tr>
<th>Equation</th>
<th>Reduction Potential In Volts</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Cd}^{+2} + 2e^- = \text{Cd}$</td>
<td>$-0.4026$</td>
</tr>
<tr>
<td>$\text{Cr}^{+3} + e^- = \text{Cr}^{+2}$</td>
<td>$-0.41$</td>
</tr>
<tr>
<td>$\text{Cr}^{+3} + 3e^- = \text{Cr}$</td>
<td>$-0.74$</td>
</tr>
<tr>
<td>$\text{Cr}^{+2} + 2e^- = \text{Cr}$</td>
<td>$-0.557$</td>
</tr>
<tr>
<td>$\text{Cr}_2\text{O}_7^{+2} + 14\text{H}^+ + 6e^- = 2\text{Cr}^{+3} + 7\text{H}_2\text{O}$</td>
<td>$+1.33$</td>
</tr>
<tr>
<td>$\text{CrO}_2^- + 2\text{H}_2\text{O} + 3e^- = \text{Cr} + 4\text{OH}^-$</td>
<td>$-1.2$</td>
</tr>
<tr>
<td>$\text{CrO}_4^{+2} + 4\text{H}_2\text{O} + 3e^- = \text{Cr(OH)}_3 + 5\text{OH}^-$</td>
<td>$-0.12$</td>
</tr>
<tr>
<td>$\text{Cr(OH)}_3 + 3e^- = \text{Cr} + 3\text{OH}^-$</td>
<td>$-1.3$</td>
</tr>
<tr>
<td>$\text{HCrO}_4^- + 7\text{H}^+ + 3e^- = \text{Cr}^{+3} + 4\text{H}_2\text{O}$</td>
<td>$+1.195$</td>
</tr>
<tr>
<td>$\text{Cu}^+ + e^- = \text{Cu}$</td>
<td>$+0.522$</td>
</tr>
<tr>
<td>$\text{Cu}^{+2} + e^- = \text{Cu}^+$</td>
<td>$+0.158$</td>
</tr>
<tr>
<td>$\text{Cu}^{+2} + 2e^- = \text{Cu}$</td>
<td>$+0.3402$</td>
</tr>
<tr>
<td>$\text{Fe}^{+2} + 2e^- = \text{Fe}$</td>
<td>$-0.4092$</td>
</tr>
<tr>
<td>$\text{Fe}^{+3} + 3e^- = \text{Fe}$</td>
<td>$-0.036$</td>
</tr>
<tr>
<td>$\text{Fe}^{+3} + e^- = \text{Fe}^{+2}$</td>
<td>$+0.770$</td>
</tr>
<tr>
<td>$\text{Ni}^{+2} + 2e^- = \text{Ni}$</td>
<td>$-0.23$</td>
</tr>
<tr>
<td>$\text{O}_2 + 4\text{H}^+ + 4e^- = 2\text{H}_2\text{O}$</td>
<td>$+1.229$</td>
</tr>
<tr>
<td>$\text{O}_2 + 2\text{H}_2\text{O} + 4e^- = 4\text{OH}^-$</td>
<td>$+0.401$</td>
</tr>
<tr>
<td>$\text{Pb}^{+2} + 2e^- = \text{Pb}$</td>
<td>$-0.1263$</td>
</tr>
<tr>
<td>$\text{S} + 2\text{H}^+ + 2e^- = \text{H}_2\text{S(aqu)}$</td>
<td>$+0.141$</td>
</tr>
<tr>
<td>$\text{Zn}^{+2} + 2e^- = \text{Zn}$</td>
<td>$-0.7628$</td>
</tr>
</tbody>
</table>

After Weast (1971)

**Note:** A positive potential indicates that the reaction will proceed to the right.
APPENDIX W - LIST OF SYMBOLS USED

AO = Acridine Orange
ASV = anodidc strip voltametry
AW = atomic weight
Bnm = bulk stability constant
Ci = metal concentration in sample
EDTA = ethylene-diamine-tetra-acetic acid
Enm = extraction efficiency of ligand m when complexed with metal species n.
DAPI = 4,6-diamidino-2-phenylindole
Ks = stability constant
LO = moles of ligand added
m = number of ligands complexed
MA = measured moles of complexed metal
ML = measured moles of complexed metal if the extraction efficiency is 100%
Mm = metal mass in sample
Mn = moles of metal species n
MnLm = moles of complex with species n and m ligands
MO = initial moles of a metal
n = metal species number
NTA = nitrilo-tri-acetic acid
P = solids fraction determined by oven drying at 104°C
PGA = polygalacturonic acid
RR = mass ratio
Rx = pseudo mass ratio for chelation data set one
Vi = MIBK volume recovered
$W = \text{weight of sample}$

$X_m = \text{pseudo mass ratio for chelation data set two}$