DYNAMICS OF THE MICROBIAL POPULATIONS IN THE SUBSTRATE OF THE CONSTRUCTED WETLAND AT THE BIG FIVE TUNNEL, IDAHO SPRINGS, COLORADO

by
Wafa H. Batal
November, 1989

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

T-3813

A thesis submitted to the Faculty and Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirements for the degree of Master of Science (Engineering Ecology).

Golden, Colorado

Date 11/9/89

Signed: Wafa Batal
Wafa H. Batal

Signed:
J. C. Emerick
Thesis Co-Advisor

Signed:
R. R. Cohen
Thesis Co-Advisor

Golden, Colorado

Date 11/9/89

Signed:
Dr. John C. Emerick
Associate Professor and
Acting Head,
Environmental Sciences
and Engineering Ecology
Department

ABSTRACT

The ecology of various bacterial groups that have geochemical significance in mineral transformations, was studied in a constructed wetland receiving acid mine drainage from the Big Five Tunnel, Idaho Springs, Colorado. Used as "soils" were a compost mixture in one cell (A), and a mixture of equal amounts of aged manure, decomposed wood products and peat from Colorado in the other two cells (B and C). Samples collected over a one-year period were analyzed for sulfate-reducing, autotrophic and heterotrophic iron-oxidizing, and hetertrophic manganese-oxidizing bacteria.

Results of the study suggest that the system is mostly anaerobic with a very thin aerobic layer. Sulfate reducers were dominant throughout the wetland substrates, and the metal-oxidizing bacteria were limited to the surface.

Summer temperatures adversely affected sulfate reducers population, by increasing the redox potential of the soil through plant root activity. However, populations of iron and manganese-oxidizing bacteria increased with warmer temperatures, suggesting iron and manganese oxidation. The aerobic formation of these oxidized ions is required for the reduction of Fe^{3+} and Mn^{4+} at the aerobic-anaerobic interface.

The compost mixture in cell A was the most effective at removing metals from the mine drainage, and mineralizing sulfur as sulfides. Nutrient concentrations and organic matter of the substrate, as well as inputs of sulfate and ferrous iron from the mine drainage supported the intrinsic microflora and encouraged dissimilatory sulfate reduction. Reducing conditions, maintained by water saturation of the substrate, stabilize metal sulfides preventing them from reaching Clear Creek. Effective manganese removal, however, might require a second phase in the system.

TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

LIST OF FIGURES

LIST OF FIGURES
(Continued)

LIST OF FIGURES
(Continued)

LIST OF TABLES

LIST OF TABLES
(Continued)

LIST OF MAPS

ACKNOWLEDGEMENTS

This is to acknowledge my appreciation to my thesis advisors, Dr. John C. Emerick and Ronald R. Cohen for all the assistance they provided throughout this project. In addition, I would like to acknowledge the Environmental Protection Agency and Camp, Dresser and McKee, Inc., particularly Holly Fliniau and Edward R. Bates, for the financial support of the project. I also wish to thank my thesis committee members, Dr. Edward A. Howard III, Dr. David Updegraff of The Colorado School of Mines and Dr. Steve Schmidt of The University of Colorado, Boulder, for their scientific assistance and support.

I would like to thank my family for enduring the evolution of this thesis. This thesis is dedicated to them.

CHAPTER I

INTRODUCTION

Coal mining in the Eastern United States and both coal and hard-rock mining in the Western United States are major causes of water pollution. Acid mine drainage (AMD) resulting from the oxidation of pyrite in such mines is a major pollution source. The acidity and dissolved minerals in AMD are produced through a complex chemical-biological process. Acid water draining from these sites and especially from underground mines that have been abandoned for decades, adversely affects streams, rivers and underground waters, therefore impacting aquatic and terrestrial life. Estimates are that 11,000 miles of streams in Appalachia alone have been polluted by AMD[1]. In Colorado, 18,500 miles of waterways fail to meet federal water quality standards; of this mileage, 1,400 miles are the result of 10,000 mines that are no longer active[1,2]. Due to the lack of sufficient funding for the identification of the parties responsible for the cleanup of these sites, the Colorado Water Quality Control Division (CWQCD) has considered discharges from abandoned mines as non-point sources[3,4].

Conventional treatment of AMD to reach discharge standards is expensive, costing over $1,000,000 per day[5], per state. Methods used for treating AMD include chemical

treatments, where toxic constituents are neutralized and made insoluble, and physical storage to create an anoxic environment that inhibits the growth of iron-oxidizing bacteria that catalyze the oxidation of pyrite[6]. Due to the many limitations of these methods, especially in remote mountain areas, the high cost involved, the large area impacted and the high number of sites, the use of wetland habitats as an alternative, low maintenance, low cost, effective, broadly applicable treatment technology has been suggested in the last few years[5,7,8,9]. Wetland ecosystems have long been known to affect the chemistry of water passing through them. Man-made wetlands have been used for the treatment of stormwater and municipal wastewaters[10,11,12,13,14,15]. They have been extensively used to treat drainages from coal mines [7,16,17,18,19]. However, very few wetlands are being used to treat waters derived from metal ores and mineral mining.

Only a few wetlands have been constructed to treat AMD from non-coal mines in the Rocky Mountains of Colorado[20]. The efficacy of the above-mentioned wetlands is variable. All are at least partially successful. Many are functioning and treating drainages so well that several government agencies have supported the construction of such systems, thereby reducing water treatment costs[1,8]. The Colorado

School of Mines was selected by the Environmental Protection Agency (EPA) to construct a wetland and study the effectiveness of such a passive treatment system, as part of the Remedial Investigation and Feasibility Study process on Clear Creek, Colorado.

The purpose of this project was to determine the fate of metals in the system, the adaptation of vegetation to the metal concentration and climate, to identify the organic substrates appropriate for such a system, and to issue a design handbook for treatment of AMD in the Rocky Mountain Region.

The specific objective of this thesis is to report on the occurrence and spatial distribution of species of bacteria in the substrate of the constructed wetland that have geochemical significance in mineral transformations. Further objectives were to make interpretations concerning the effects of environmental factors, such as pH, Eh and temperature on the bacterial populations in the system, and the role of these bacteria in metal removal and retention by the wetland.

CHAPTER II

BACKGROUND

WETLAND TREATMENT SYSTEMS

Wetlands were long considered unproductive areas without significant value, however, this view has changed. They are now recognized as having an important role in: floodpeak reduction, providing food and habitat for wildlife, shoreline stabilization, ground water recharge, and water quality improvement[21]. Various federal agencies now have policies for the protection and enhancement of natural wetlands[4]. The best alternative to the use of natural wetlands for AMD treatment is to construct artificial wetlands, thereby avoiding the ethical and legal ramifications of discharging AMD in natural systems and resolving the problem of finding a wetland in the vicinity of mine effluents.

Man-made wetlands upgrade water quality by retaining certain metals and modifying the pH of the water. Mechanisms controlling metal removal are summarized in Figure 1. Adsorption and cation exchange of metals by peat and organic matter were believed to be the primary mechanisms in a wetland[22]. Chelation of metal ions by organic compounds, physical filtration, and uptake by plants and algae are also important

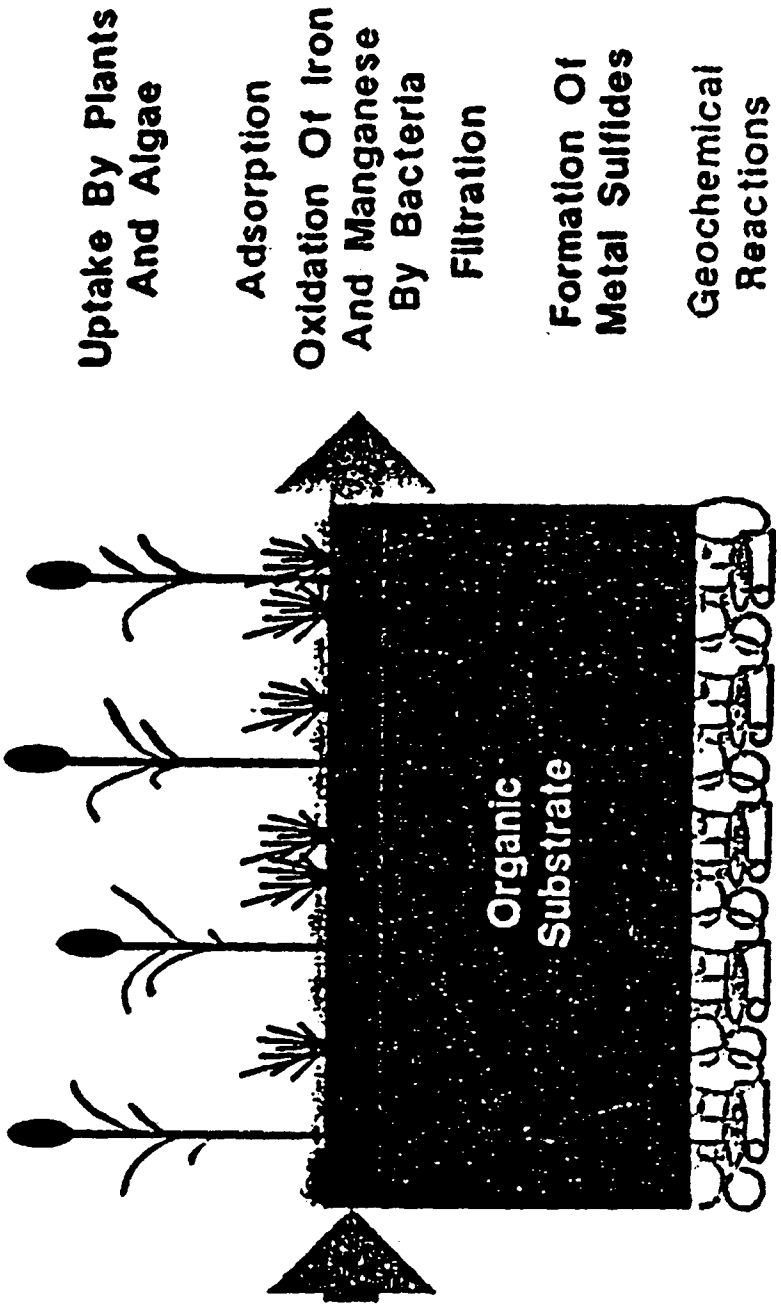


FIGURE 1. Metal Removal Processes in Wetlands[8].

processes. Other geochemical processes as well as microbial activities take place in the organic-rich substrate.

Precipitation of metals and pH adjustment through the activity of the intrinsic bacterial microflora in a wetland system have not been extensively studied and are often ignored. In the aerobic zone[23] (Figure 2), two groups of heterotrophic metal-utilizing bacteria are present, the iron and manganese oxidizers, as well as one group of obligate autotrophic iron oxidizers (Thiobacillus ferrooxidans)[24]. In the anaerobic zone, sulfate reducers are known to be present.

ACID MINE DRAINAGE FORMATION

1. Chemical Oxidation

The formation of AMD has been documented by several authors[22,23,25,26,27]. Presence of the mineral pyrite (FeS_2) initiates the process. It is a very widespread mineral associated with high sulfur coal reserves in the eastern United States and base metal sulfide ore deposits in the western United States. Both the iron and sulfur in pyrite are in reduced states. Mining activities expose pyrite to the atmosphere, and thereby to oxygen and water. The following reaction takes place:

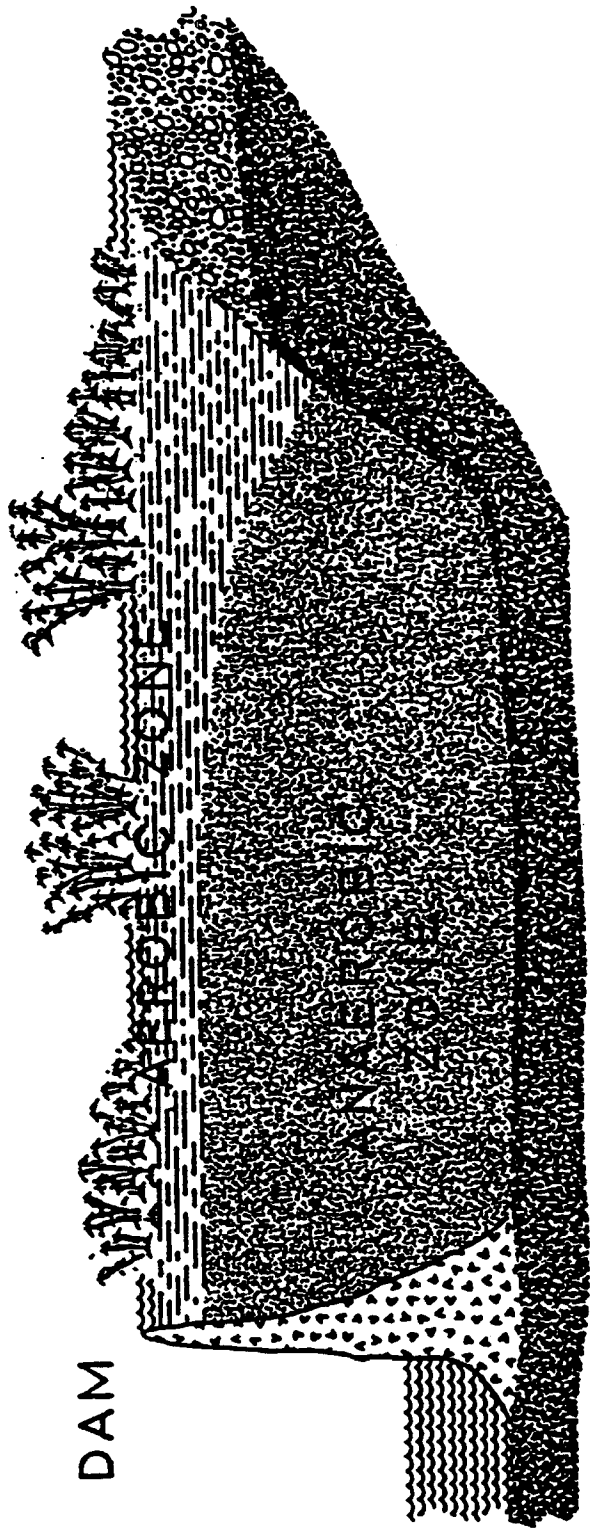


FIGURE 2. Wetland Aerobic and Anaerobic Zones[23].

$$2FeS_2 + 7O_2 + 2H_2O \longrightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \quad (I)$$

As a result of the release of hydrogen ions into solution, acidity is produced and more pyrite is dissolved. Ferrous ions are released into solution and oxidized to the ferric state. The reactions occurring are as follows:

$$2Fe^{2+} + 1/2O_2 + 2H^+ \longrightarrow 2Fe^{3+} + H_2O \quad (II)$$

$$2Fe^{3+} + 6H_2O \longrightarrow 2Fe(OH)_3 + 6H^+ \quad (III)$$

Ferric hydroxide precipitates as "yellow-boy," and the H^+ ions increase the acidity, but precipitation stops at pH <3 and ferric iron remains in solution, oxidizes pyrite and goes back to the ferrous state. The process becomes self-propagating with no need for oxygen, and more acid is continually produced, which mobilizes other metals[28].

2. Microorganisms in AMD

The above-mentioned reactions (II, III) are very slow. Ferrous iron is stable at pH 3.0. A catalyst is involved that speeds up the process. This catalyst is a group of bacteria that are capable of pyrite oxidation and of increasing its rate by one million times the inorganic oxidation rate[29]. The isolation of the bacteria responsible took place in the 1940's and 1950's[30]. Two autotrophic bacteria were isolated

from acid mine water, Thiobacillus thiooxidans and Thiobacillus ferrooxidans. T. ferrooxidans was found to significantly accelerate pyrite oxidation with subsequent acid production. Thiobacillus thiooxidans was found to be capable of oxidizing sulfur to sulfuric acid but unable to oxidize Fe^{2+}. T. thiooxidans derives energy from elemental sulfur and thiosulfate to produce acid. It always coexists with T. ferrooxidans. Both of these bacteria are involved in AMD formation.

T. ferrooxidans oxidizes iron and can utilize elemental sulfur, tetrathionate ($S_4O_6^{2-}$), thiosulfate ($S_2O_3^{2-}$), and non-iron sulfide minerals as sources of energy.

T. ferrooxidans accelerates pyrite oxidation either directly (enzymatic), or indirectly (nonenzymatic) by oxidizing Fe^{2+} to Fe^{3+} which in turn oxidizes the sulfide minerals[25,26,27]. During the first stage of this process, fine-grained pyrite is oxidized by T. ferrooxidans and by air. The ability of the T. ferrooxidans to lower the pH rapidly is very important. As long as alkalinity exceeds acidity, the only major downstream effect is an increase in sulfate concentration.

As the pH decreases (<3), abiotic oxidation of Fe^{2+} slows down or stops, and T. ferrooxidans starts oxidizing Fe^{2+},

thereby producing acidity and ferric hydroxide. This is defined as the "bacterial catalysis-controlled stage," shown in reaction IV.

$$Fe^{2+} + 1/4O_2 + H^+ \longrightarrow Fe^{3+} + 1/2H_2O \qquad (IV)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \longrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad (V)$$

These reactions are also referred to as the "Propagation Cycle"[22]. Reaction V shows that pyrite can be oxidized without the presence of oxygen.

As the pH drops to less than 3, iron solubility increases, $Fe(OH)_3$ precipitation rate decreases and Fe^{3+} activity increases (Figure 3[31]). At drainage pH 3.5-4.0, bacterial oxidation of Fe^{2+} (Reaction IV) and reduction of Fe^{3+} by the pyrite (Reaction V) cause a decrease in pH and iron concentration[25].

Another bacterium of the genus Metallogenium was isolated from acid mine drainage[32]. Walsh and Mitchell (1972) described it as an acid tolerant, stalked bacterium that catalyzes iron oxidation only in pH range 3.5 to 5.0. This could be the major cause of the initial production of acidity since T. ferrooxidans grows at pH less than 3.5.

Other thiobacilli, such as T. neopolitans, oxidize sulfur compounds at pH values close to neutral and produce sulfuric acid.

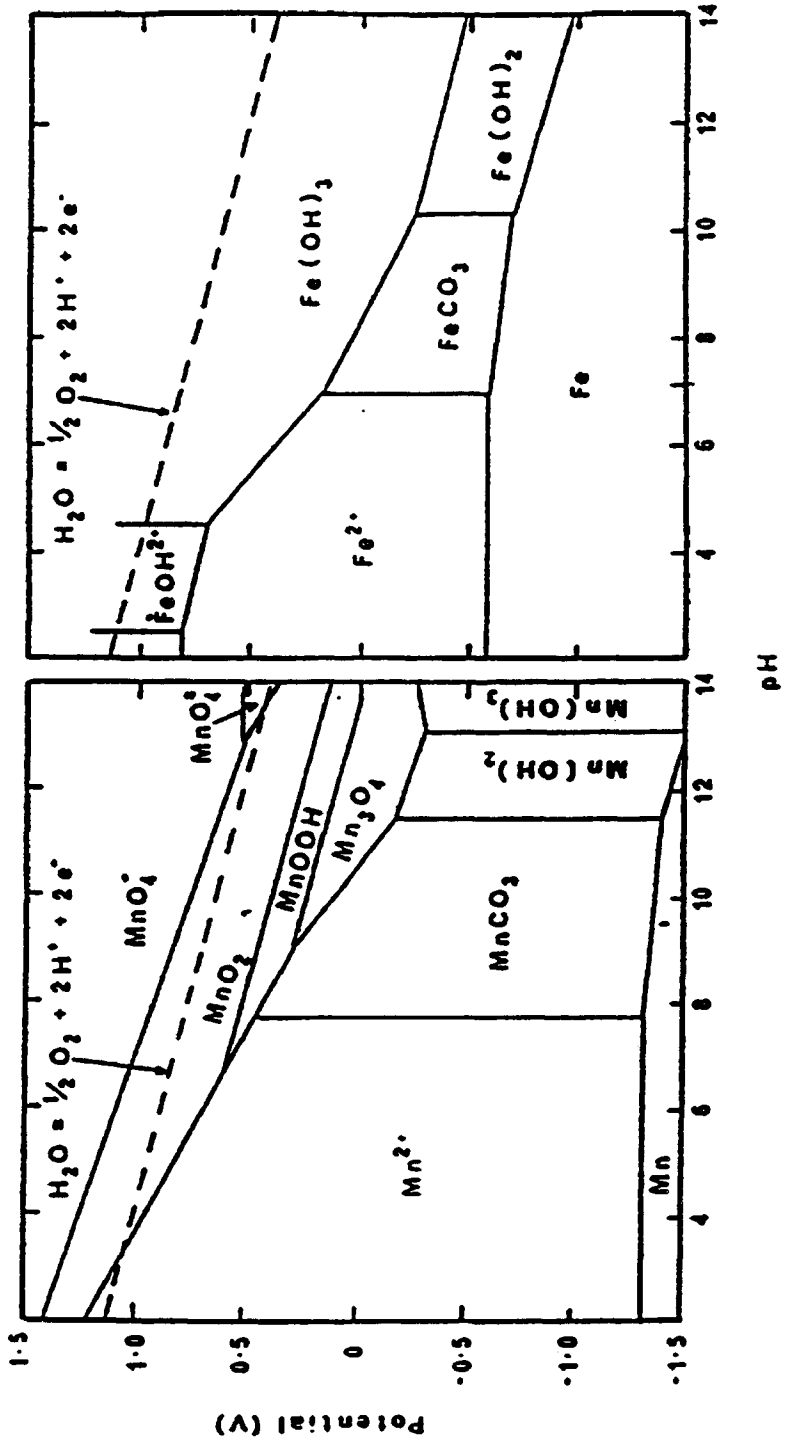


FIGURE 3. Eh-pH Stability Diagrams for Manganese and Iron at 25°C and Activity of 10^{-5} M[31].

BACTERIA IN WETLANDS IMPORTANT TO METAL REMOVAL

1. AEROBIC BACTERIA

1.1. Iron-Oxidizing Bacteria (IOB)

1.1.1. Iron Chemistry

Iron has two oxidation states, Fe(II) and Fe(III). Stabilities of various inorganic iron phases in solution are summarized in Figure 3. Iron oxidation is usually fast and affected by pH and oxygen concentration. Oxidized iron, Fe(III), hydrolyzes in solution producing hydrated and oxidized phases. Iron can also be readily chelated by a variety of organic molecules, resulting in increases in the amount of iron in solution. At pH <3, oxidation of Fe^{2+} to Fe^{3+} is very slow and precipitation of $Fe(OH)_3$ decreases or stops. Iron-oxidizing bacteria have been found to catalyze the oxidation and precipitation of Fe^{2+}.

1.1.2. Autotrophic Iron Oxidizers

Iron-oxidizing and/or iron oxides-accumulating bacteria are divided into two groups: the acidophilic (acid-tolerant) forms, and bacteria growing at neutral pH[33]. The acidophilic group is the only group shown to oxidize Fe^{2+} to Fe^{3+} enzymatically. Leptospirillum ferrooxidans is one organism in this

group that oxidizes iron only. The most extensively and thoroughly studied bacterium is Thiobacillus ferrooxidans (Ferrobacillus ferrooxidans and F. sulfooxidans were considered strains of, or even synonyms of T. ferrooxidans[33,34,35]). T. ferrooxidans grows well between pH 2.5 and 3.5. It is a motile, acidophilic, mesophilic, chemoautotroph; it is a gram-negative, non-spore-forming, rod-shaped organism. Its energy requirements for CO_2 fixation and other metabolic functions are derived from oxidation of ferrous iron and reduced sulfur compounds.

In the acidic environments, Fe^{2+} is stable and its oxidation releases enough energy for the T. ferrooxidans. Sulfate ion is required for the growth of the bacteria[33,34,36,37]. Studies showed the involvement of T. ferrooxidans in the formation of acid sulfate soils[36]. In an experiment, Lazaroff et al.[36] showed that SO_4^{2-} is required for iron oxidation resulting in the formation of jarosite-type minerals ($MFe_3(SO_4)_2(OH)_6$; where M= K^+, NH_4^+, Na^+, or H_3O^+). The enzymatic iron oxidation is shown in reaction IV which can also be written as:

$$4FeSO_4 + O_2 + 2H_2SO_4 \longrightarrow 2Fe_2(SO_4)_3 + 2H_2O^{(38)}$$

followed by the chemical reaction III or

$$Fe_2(SO_4)_3 + 6H_2O \longrightarrow 2Fe(OH)_3 + 3H_2SO_4$$

Ferric ions from reaction IV might oxidize pyrite (reaction V), producing Fe^{2+} which can, again, be oxidized by the bacteria.

Since pyrite in AMD has already been oxidized to Fe^{2+}, reaction V will not be taking place unless pyrite is present in the soil. In a wetland substrate, Fe^{3+} is expected to precipitate as hydroxide and would therefore be removed from the AMD and accumulated in the soil.

Neutral pH autotrophic iron bacteria include Gallionella and Leptothrix groups. Gallionella ferruginea is able to use Fe^{2+} autotrophically and to precipitate it; ferric ion is bound to the organic matter of the G. ferruginea stem. Formation of ferric hydroxide particles takes place on aging stems and precipitation is mainly chemical[39] (at Temp. 6°C, pH 6.4). Leptothrix bacteria are thought to be autotrophs; however, some species are facultative autotrophs. Crenothrix grows both autotrophically and heterotrophically. Clonothrix and Lieskeella are other genera of iron oxidizers[39,40].

1.1.3 Heterotrophic Iron-Oxidizing Bacteria (HIOB)

Heterotrophs are also known to be associated with ferric iron precipitation[39,40]. Iron can be bound as iron-organic complexes in the soil. Many heterotrophic bacterial species

can degrade these complexes and release the bound iron[39,40]. Precipitation then results from a direct action on the organic portion of the complex. Leptothrix ochracea can utilize glucose as a source of energy. Crenothrix is another heterotroph[40]. Optimum temperatures for the growth of these two bacteria are 25°C and 18-24°C and pH ranges are 5.8-7.8 and 5.5-6.2, respectively[40]. Pseudomonas, Baccillus, Serratia, Acinetobacter, Klebsiella, Mycobacterium, and Corynebacterium can precipitate Fe^{3+} by attacking the organic portion of iron salts. Metallogenium species catalyze iron oxidation between pH 3.5 and 5.0[32,38]. Metallogenium symbioticum have been successfully cultured by some researchers; however, the presence of this genus has been disputed, especially since other researchers have not been able to obtain pure cultures in laboratories or have failed to reproduce results of others[33,41]. Metallogenium, Pedomicrobium and Seliberia genera attack iron-organic compounds, which results in coating of the bacteria by $Fe(OH)_3$. Some strains of Thiobacillus ferrooxidans have adapted to growing heterotrophically on glucose but this occurrence is variable even with different cultures of a single strain[42]. Heterotrophic iron oxidation is summarized by Zajic[40] in the following reactions (the equations are not balanced, as they appear in the reference):

$$2FeCO_3 + O_2 + 3H_2O \longrightarrow 2Fe(OH)_3 + 2CO_2$$

$$2C_3H_6O_3 + 2Fe(OH)_2 \longrightarrow 2CH_3COOH + 2Fe(OH)_3 + 2CO_2 + 3H_2$$

$$CH_3COOH + 2Fe(OH)_2 \longrightarrow 2CO_2 + 2Fe(OH)_3 + 3H_2$$

1.2 Heterotrophic Manganese-Oxidizing Bacteria (HMOB)

1.2.1 Chemistry of Manganese

Manganese occurs in the soil in the tetravalent Mn^{4+} form and as the divalent ion Mn^{2+}. Mn^{4+} is insoluble (MnO_x) and Mn^{2+} is water soluble. It can also occur in the trivalent form, mostly bound in organic complexes. Eh and pH of the soil determine the stability of each oxidation state of manganese (Figure 3). Mn^{2+} is present at pH 5.5 and, at pH >8, under oxidizing conditions, manganic oxides are formed (MnO_x). Solubility of manganese is six or seven times that of iron (Figure 3). Oxidation of Mn^{2+} takes place at pH >8.5. Removal of Mn^{2+} from solution can be achieved in the presence of fine particulate matter[43]. MnO_x adsorbs Mn^{2+} and increases the rate of reaction[44]. Bicarbonates and sulphates can form complexes with manganese that are more resistant to oxidation than free Mn^{2+}[45]. Manganese also forms ligands with organic compounds[45]. Between pH 5.5 and 8.0, oxidation reactions are minimal and are bacterially catalyzed.

1.2.2 Bacterial Oxidation of Manganese

Below pH 8 and above Eh +200 mv, manganese oxidation is by microorganisms[46]. No estimate of rate of bacterially catalyzed oxidation has been obtained; however, several factors suggested that biological activity was taking place: rates of oxidation were too high to be accounted for by chemical oxidation, presence of Mn^{4+} at neutral pH, and finally amount of Mn^{2+} removal was too great to be only through adsorption onto particulates and uptake by bacteria[44]. In the past few years, research has been conducted, leading to a better understanding of bacterial oxidation of Mn^{2+}.

Bacteria involved in manganese oxidation include strains of the following genera: Arthrobacter, Bacillus, Corynebacterium, Klebsiella, Metallogenium, Pedomicrobium, and Pseudomonas[38]. Growth of these bacteria requires organic carbon as a source of energy; thus, manganese oxidizers are heterotrophs. Bacterial Mn^{2+} oxidation is fastest at pH 6.0 to 7.5[38].

In an experiment, 45 strains of Mn oxidizers were isolated from a marine environment and were found to grow at extreme temperatures and salinity[47]. A pH of 9.0 or above inhibited oxidation. In general, optimal growth conditions

are at pH 5.5-8.0, Eh greater than +200 mv, and temperature range 10-44°C[46,48].

The mechanism for oxidation and deposition of manganese is divided into two major classes: enzymatic and non-enzymatic[40,45].

1.2.2.1. Non-Enzymatic or Indirect Oxidation

Microbial manganese oxidation can occur indirectly as a result of microbial growth and metabolism, which alter Eh and pH of the soil. Eh modification is a result of O_2 production or consumption, or by excretion of acidic or alkaline metabolic byproducts. Streptomyces species produce a secondary metabolite which oxidizes Mn^{2+} at pH 5. Pseudomonas strains oxidize Mn^{2+} at pH 7 by an interacelluar protein, and in the absence of O_2[45,46].

Microbes can also utilize organic complexes with manganese, releasing Mn^{2+} into solution which precipitates if Eh and pH conditions are favorable. This type of oxidation is promoted by a large number of bacteria[42].

1.2.2.2. Enzymatic Oxidation

The direct oxidation can proceed by several paths. Arthrobacter, Pseudomonas, Citrobacter, and Leptothrix can oxidize manganese as follows[42]:

$$Mn^{2+} + 1/2O_2 + H_2O \longrightarrow MnO_2 + 2H^+.$$

Arthrobacter siderocapsulatus, Metallogenium and Lepthothrix pseudoochracea can catalyze Mn^{2+} oxidation using prebound Mn^{4+}[34,42,45].

$$Mn^{2+} + H_2MnO_3 \longrightarrow MnMnO_3 + 2H^+ \text{ (Chemical reaction)}$$

$$MnMnO_3 + 1/2O_2 + 2H_2O \longrightarrow 2H_2MnO_3 \text{ (Bacterial)}.$$

Some bacteria can oxidize Mn^{2+} by H_2O_2 produced in their metabolism:

$$Mn^{2+} + H_2O_2 \longrightarrow MnO_2 + 2H^+.$$

Some species of the iron bacteria can precipitate manganese, such as: Crenothrix polyspora, Lepthothrix ochracea, Cladothrix dichtoma, and Clonothrix fusca.

Gallionella and the budding bacteria Metallogenium and Pedomicrobium can deposit both Mn and Fe at neutral pH as depicted by Ghiorse et al.[49].

Ferrous iron can be oxidized by iron oxidizers or by MnO_2

$$MnO_2 + Fe^{2+} + 4H^+ \longrightarrow Fe^{3+} + Mn^{2+} + 2H_2O,$$

and Mn^{2+} can be oxidized by microbes[40].

2. ANAEROBIC BACTERIA

Table 1 shows the vertical sequence of microbially mediated reactions taking place in organic-rich soils and sediments. The reducing zone is stratified and the reduced products are shown at each level[50].

2.1. Sulfur Chemistry

Sulfur is an essential element for plants and animals. It occurs in many chemical forms with oxidation states ranging from +6 to -2 as in sulfate (SO_4^{2-}) and hydrogen sulfide (H_2S), respectively. Sulfite (SO_3^{2-}), thiosulfate ($S_2O_3^{2-}$), polythionate ($S_nO_6^{2-}$), and elemental sulfur (S^o) are some of the inorganic intermediates in nature[48]. The most oxidized (sulfate) and the most reduced (sulfide) states have the greatest stability (Figure 4[51]). Sulfate is predominant in oxidized environments while H_2S dominates anoxic soils and sediments. Sulfur is involved in many biochemical and inorganic reactions, many of which are slow and need microorganisms to catalyze them. It occurs in the organic form in the protein bound amino acids cysteine and methionine[52]. It is also a constituent of enzymes and thus is important in metabolism. Organic sulfur can occur as ester sulfates (polysaccharide sulfates) formed by animals, plants and

TABLE 1.
VERTICAL SEQUENCE OF MICROBIALLY MEDIATED REACTIONS IN ORGANIC-RICH SOILS AND SEDIMENTS[50]

Reaction Zone	Sediment Depth Sequence	Hydrogen Acceptor	Reduced Product
1. Aerobic respiration	Surface	O_2	H_2O
2. Nitrate reduction		NO_3	NO_2, N_2O, N_2
3. MnO_2 reduction		MnO_2	Mn^{2+}
4. Fe_2O_3		Fe_2O_3	Fe^{2+}
5. Sulfate reduction		SO_4	H_2S
6. Methanogenesis	Depth	CH_3COOH	CH4

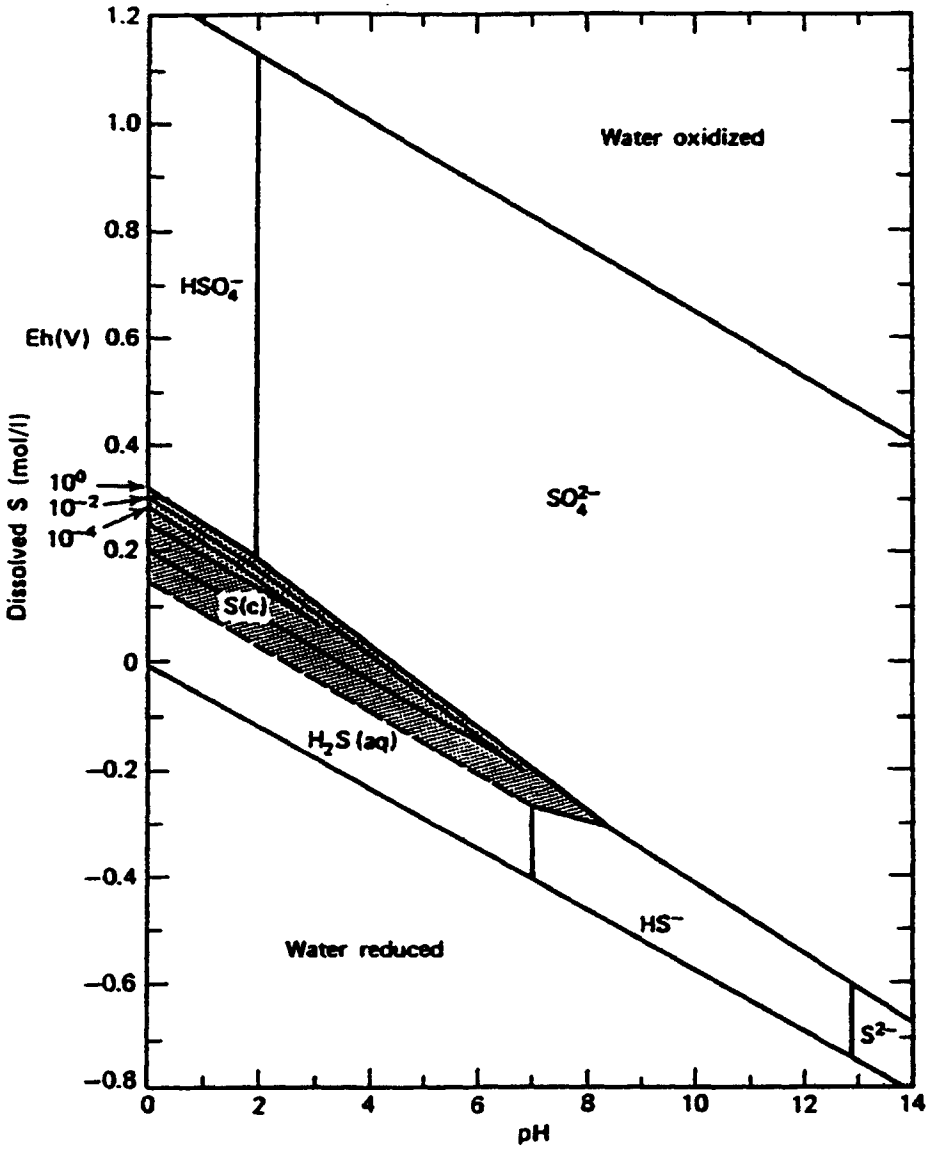


FIGURE 4. Stability Fields of Aqueous Sulfur Species and Elemental Sulfur in the System $S+H_2O+O_2$ at 25°C and 1 ATM Pressure[51].

microorganisms. In aquatic ecosystems, sulfur is in the inorganic form in general, and a small percentage is in the organic form[51]. The sulfur cycle is summarized in Figure 5 showing the interaction between the organic and inorganic sulfur compounds[51].

The various forms of sulfur are connected by microbial processes. Microbial transformations of sulfur can be divided into four groups[38,53]: a) assimilation of sulfur, resulting in immobilization of sulfur, b) decomposition of organic sulfur forms, or mineralization of sulfur, c) oxidation of organic and inorganic sulfur compounds, and d) dissimilatory sulfate reduction as well as reduction of other inorganic sulfur forms.

Sulfate is assimilated by bacteria, algae and plants, reduced to sulfide and transferred to amino acids of cells where it is immobilized. Upon decomposition of these cells, sulfur is released from cysteine and methionine as sulfate and gases such as H_2S (from cysteine) and volatile organic sulfur forms, methane thiol (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$) (from methionine). Ester sulfates are also biologically hydrolyzed into inorganic sulfate. Volatile organic sulfur compounds are believed to be microbially metabolized to CH_4, CO_2 and H_2S. Hydrogen sulfide is, in turn, oxidized

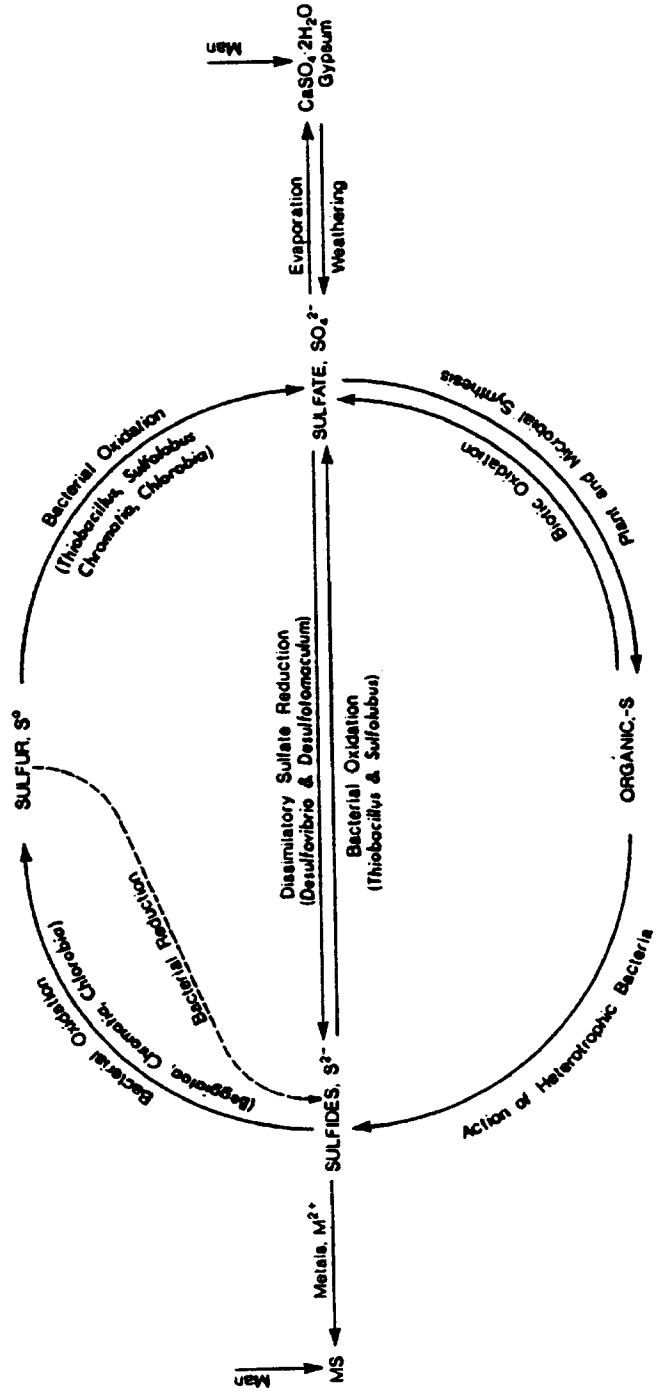


FIGURE 5. Sulfur Cycle and Transformations in Nature[51].

back to SO_4^{2-} and S^o through bacterial oxidation[38,51]. The oxidation of sulfide depends on the initial sulfide concentration, dissolved oxygen concentrations, pH, Eh, temperature and presence of catalysts.

Biological decomposition of organic sulfur compounds results in the mineralization of sulfur but the sulfide produced accounts for a very small percentage of the total mineral sulfur in soils. Microorganisms perform dissimilation of sulfur compounds, incorporating small amounts of sulfur into their cells, while the remaining sulfur is used as an electron acceptor or donor in oxidation of organic matter and metabolism. Dissimilatory sulfate reduction under natural conditions at less than 200°C occurs only through microorganisms[52,54]. It takes place in anoxic environments where organic matter and sulfate are present. Sulfate reduction rate depends on the number of bacteria, sulfate concentrations, temperature (<70°C) and the amount of nitrate, which is energetically favored as an electron acceptor. It results in the formation of H_2S which can either escape as a gas or react with metal ions in the soil and precipitate them as metal sulfides. Hydrogen sulfide produced can be oxidized to sulfate, polythionate, elemental sulfur, or to the unstable

sulfite and thiosulfate. Oxidation of sulfide by oxygen at neutral pH is rapid, but sulfide minerals are stable.

The presence of organic or inorganic sulfur compounds in the soil depends on environmental factors affecting the activity and types of microflora. Immobilization of sulfur compounds in amino acids is higher in soils with low sulfur contents while mineralization is dominant in high sulfur soils[38].

Microbial sulfate reduction and subsequent formation of metal sulfides is the most important process in this study. Sulfates in AMD, as well as metal ions in solution, can be removed as sulfides and stabilized in the anoxic zone of wetlands. Tuttle et al.[55] reported on the potential use of sulfate-reducing bacteria to reduce sulfate to sulfide in AMD. Ferric and other metal ions are precipitated and pH of the soil is increased by metabolism of the bacteria. Wieder et al.[7] in their study conducted on Tub Run Bog in the Appalachian Mountains in West Virginia, suggested that AMD treatment was mainly achieved through sulfate reduction and precipitation of metal sulfides. Hedin et al.[56] concluded that such precipitation is preferable to the hydroxide forms, due to the stability of the sulfide minerals and their location in the anoxic zone of the substrate.

2.2 Sulfate-Reducing Bacteria (SRB)

The sulfate-reducing bacteria are strict anaerobes which respire by reducing sulfate to sulfide[57]. The most extensively studied genera are the spore-forming Desulfotomaculum and the non-sporulating Desulfovibrio. They are heterotrophs utilizing pyruvate, lactate, and malate as their sources of energy.

A third genus Desulfomonas, very similar to Desulfovibrio has also been isolated[57]. Pfennig and Widdel[58] isolated additional genera from anaerobic environments: Desulfobacter, Desulfobulbus, Desulfonema, Desulfococcus, Desulfuromonas, Desulfosarcina[52,59,60,61] which are metabolically versatile, morphologically different, and can utilize a wide range of carbon sources (CO_2 and fatty acids).

Desulfovibrio species cannot oxidize organic carbon to CO_2 but rather to acetate. They can utilize H_2 as an energy source in the presence of SO_4^{2-}[40,52,57,62,63]. Desulfotomaculum oxidizes lactate and pyruvate to acetate and CO_2. Sulfate reduction, accompanied by organic matter oxidation, in general results in the production of CO_2 and H_2S[64].

The biology of the sulfate-reducing bacteria has been described by several researchers, and reviewed in detail by Postgate[57,65].

Several factors affect sulfate-reducing bacteria: reducing conditions, availability of sulfate, organic matter as an available energy source, temperature and nutrients. The Eh of the reducing environment must be negative, starting around -100 mV in pure cultures[57]. The depth of the aerobic zone in soils is determined by the rate of oxygen diffusion downward and the rate of respiration of microorganisms[66]. Sulfate reduction occurs between the oxidized upper layer of soil and the deep methanogenic zone (Table 1) and was found to occur in the upper 10-15 cm of sediments[59,67,68,69], controlled by the concentration of sulfate ions and Eh. Sulfate reducers tolerate pH values ranging from below 5.0 to 9.5[57]. Wakao et al.[70] found that sulfate reduction did not occur below pH 4.0 but started between pH 4.0 and 5.2. Acid mine drainage flowing through organic material decreases the pH to <4. The establishement of sulfate-reducing bacteria in such soils was found to be achieved through the increase of the pH and decomposition of organic matter by two groups of bacteria, cellulolytic and cellulose-utilizing bacteria[71]. Cellulose digesters, which can survive in acidic conditions, convert cellulose to available nutrients for sulfate-reducing bacteria. Cellulose utilizers that do not completely degrade cellulose but possibly produce oxycellulose, increase the pH

of the soil[71]. The process of cellulose degradation is exothermic, which could be beneficial in treatment of AMD in cold climates.

Sulfate concentrations in sediments and soils determine the activity of sulfate reducers[58]. However, Sorensen et al.[72] determined that sulfate reduction was not limited by low sulfate concentrations, but by Eh and temperature. Sulfate in AMD is at high levels, and therefore is not limiting to sulfate-reducing bacteria in substrates of wetlands receiving AMD.

Sulfate reducers are heterotrophs. Therefore, the availability of organic material is an essential factor determining the rate of sulfate reduction in soils. More important is the rate at which utilizable energy becomes available. This is a function of both organic matter concentration and its degradability[52]. In the past, it was believed that Desulfovibrio were the bacteria responsible for sulfate reduction, and their only source of energy was lactate. Desulfovibrio convert lactate to acetate, and in turn, acetate is converted by methanogenic bacteria to CH_4 and CO_2. With the many new genera of sulfate-reducing bacteria recently isolated, organic matter can be completely mineralized. Acetate-oxidizing sulfate reducers were

discovered (Desolfotomaculum acetoxidans and Desulfobacter postgatei)[58,73,74,75]. Competition between methanogens and sulfate reducers for H_2 and acetate have been demonstrated by several researchers[76,77,78]. Sulfate reduction was predominant in high sulfate concentration while methanogenesis was inhibited. Thus, in AMD treatment, sulfate reducers should be expected to be the dominant genera, because they are energetically favored.

Hydrogen sulfide is toxic to anaerobic microorganisms[52,64]. Methanogenic bacteria are therefore inhibited by high H_2S concentrations[79]. Hydrogen sulfide may also inhibit the growth of sulfate-reducing bacteria by reacting with Fe needed by the cells. At the same time, it can protect them from heavy metal poisoning. Iron in AMD is continuously supplied, therefore, H_2S toxicity is not likely to occur because of reactions between Fe and sulfide. Other requirements for sulfate reducers growth are nitrogen as ammonium, nitrate or amino acids, and phosphorus for the formation of ATP. Seasonal temperature has been reported to influence bacterial sulfate reduction in the upper 15 cm of sediments but no physiological response of sulfate-reducing bacteria to temperature was detected[80,81]. Counts of Desulfovibrio in a saltmarsh sediment at 15°C and 30°C were not significantly

different, while the in situ rates of reduction in the summer were 1.5 orders of magnitude higher than in the winter[82]. This seasonal variation was explained by Cutter et al.[83] to be a result of the presence or absence of photosynthetic activity of plants injecting oxygen into the sediments, thereby changing the redox potential of these sediments. In a marine sediment, the bacteria were found to be active at a temperature ranging from 0 to 40°C with an optimum around 30-35°C[52].

The sulfate reduction reaction with lactate as the energy source can be written as follows[52]:

$$2CH_3CHOHCOO^- + SO_4^{2-} \longrightarrow 2CH_3COO^- + 2HCO_3^- + H_2S.$$

When H_2 is utilized as an electron donor, in the presence of the enzyme hydrogenase, the reaction would be:

$$4H_2 + SO_4^{2-} + 2H^+ \longrightarrow 4H_2O + H_2S.$$

The respiratory metabolism of organic matter by the newly isolated genera can be summarized as:

$$2[(CH_2O)_c(NH_3)_n(H_3PO_4)_p] + c(SO_4^{2-}) \longrightarrow$$
$$2c(HCO_3^-) + 2n(NH_3) + 2p(H_3PO_4) + cH_2S,$$

where c, n, and p represent the C:N:P ratio of the organic matter[84]. Rates of sulfate reduction have been measured by several researchers with the use of radiotracers ($^{35}SO_4^{2-}$) and are summarized in Table 2.

TABLE 2.
RATES OF SULFATE REDUCTION

MEDIUM	SULFATE REDUCTION WINTER	SUMMER
Acidic Peat (from a New Jersey Pinelands Cedar Swamp[69]	1.0 nmol SO_4^{2-} cm^{-3} day^{-1}	173.4 nmol cm^{-3} day^{-1}
Surface Sediment of Lake Mendota (Wisconsin) (euthrophic)[85]	50 nmol cm^{-3} day^{-1}	600 nmol cm^{-3} day^{-1}
Sediment of Contrary Creek Arm of Lake Anna[86]	0.4-5.5 mmol m^{-2} day^{-1}	2-225 mmol m^{-2} day^{-1}
Coastal Marine Sediments (Denmark)[72]	2.6-7.6 mmol m^{-2} day^{-1}	9.8-15.1 mmol m^{-2} day^{-1}
Coastal Marine Sediments (Denmark)[81]	10-50 mmol cm^{-3} day^{-1}	50-400 nmol cm^{-3} day^{-1}
Saltmarsh Sediment[82] (United Kingdom)	$4x10^{-6}$ mg atoms S g^{-1} sediment day^{-1}	$8x10^{-4}$ mg atoms S g^{-1} sediment day^{-1}
Laboratory Marine Sediment Model: Sand and Chopped Zostera marina[87]	Start: 80 nmol S cm^3 day^{-1} End: 25 nmol S Cm^3 day^{-1}	

Hydrogen sulfide is produced by sulfate reduction. It is also formed by the degradation of organic sulfur compounds in the anaerobic zone. The degradation of organic sulfur contributes only 3 percent of the total sulfide production. Hydrogen sulfide in the anoxic zone precipitates metals as metal sulfides. Only 10 percent of the H_2S produced in coastal marine sediments precipitated iron and other metals in the anaerobic zone. Most of the remaining H_2S was re-oxidized in the sediment surface and some of it was found bound to ferric iron. Very small amounts reached the atmosphere[52,67].

The formation of metal sulfide is usually discussed in terms of iron sulfide since it is more abundant than the other metals. Pyrite and marcasite (both FeS_2) are the most common iron sulfides. Furthermore, the stability fields for most heavy-metal sulfides fall in the stability region of pyrite[51].

Pyrite precipitates (indirectly) according to the following reactions:

$$2FeOOH + H_2S \longrightarrow S_o + 2Fe^{2+} + 4OH^-;$$

$$Fe^{2+} + H_2S \longrightarrow FeS + 2H^+;$$

$$FeS + S^o \longrightarrow FeS_2.$$

RXN 9

The final reaction is very slow, therefore FeS and S° coexist in sediments[52].

At low H_2S concentrations and pH <6.5, pyrite is formed directly:

$$Fe^{2+} + S + H_2S \longrightarrow FeS_2 + 2H^{+}.$$

Pyrite is found in reducing sediments that are subject to oxidizing influence (around root zone[60], and in the oxic-anoxic interface)[88]. Pyrite and elemental sulfur are the end products of sulfate reduction[89] with pyrite being the major product[90].

CHAPTER III

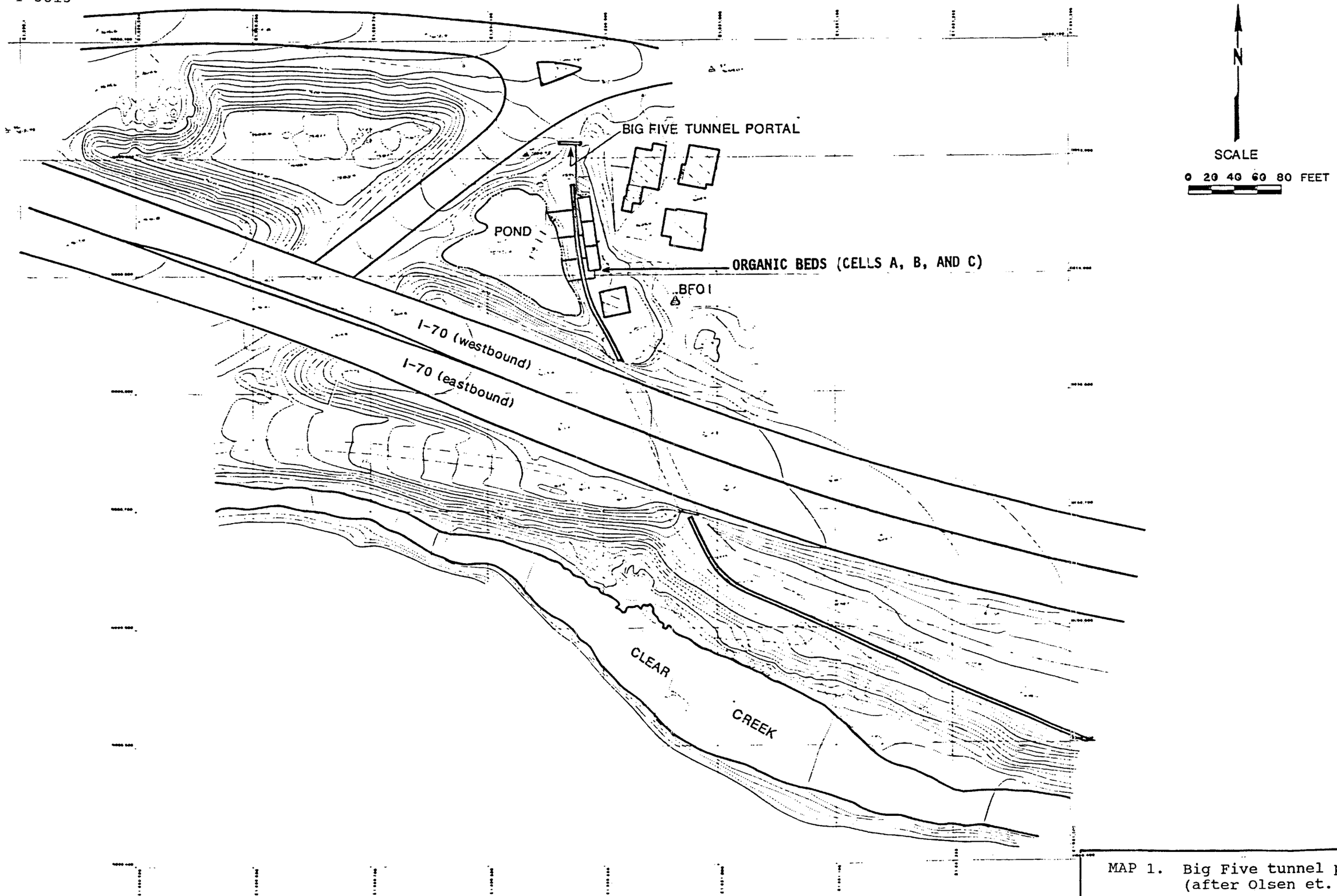
MATERIALS AND METHODS

SITE DESCRIPTION

1. Location and Problem Identification

The pilot plant wetland ecosystem has been built at the mouth of the Big Five tunnel in Idaho Springs, Colorado. Idaho Springs is located in the foothills of the Colorado Front Range. Since the 1800s, this region has been actively mined for precious metal ores. As a result, considerable amounts of mine tailings and a large number of abandoned mines became sources of water pollution. The EPA included several sites, in the Idaho Springs mining district on the National Priorities List under Superfund (the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA)).

The EPA determined in a study on Clear Creek that the Argo and Big Five tunnels are sources of metal loadings to the Creek, introducing 336 kg/day from the Argo tunnel and 11 kg/day from the Big Five tunnel[91]. The mouth of the Big Five tunnel provided a good site for the construction of a wetland and testing its effectiveness in the abatement of AMD (Map 1[92]). The tunnel drains several abandoned and working mines in this mining district. The drainage flow is 1.27×10^{-3} cms,



MAP 1. Big Five tunnel portal (after Olsen et. al[92]).

and the water characteristics are summarized in Table 3. Discharge from the tunnel collects in a shallow pond and eventually seeps under Interstate Highway 70 into Clear Creek. The wetland was built at this site through a cooperative effort of Colorado School of Mines (CSM), Camp Dresser and McKee Inc. (CDM), and EPA. Sulfate at 2100 mg/l in the drainage is not limiting for the sulfate-reducing bacteria in the wetland. The drainage is also a good source of iron, therefore, conditions are favorable for sulfate reduction and metal precipitation (as sulfides).

2. The Constructed Wetland

The construction of the wetland is described in detail in Howard et al.[93] The wetland has three cells with dimensions of 1m in depth, 3.05 m in width, and 18.3 m in length. Soils in the cells are a compost (consisting of a well composted mixture of 50 percent animal manure and 50 percent barley mash wastes from a local brewery) in cell A, a mixture of equal amounts of aged manure, decomposed wood shavings and sawdust, and Colorado peat in cell B, and the same soil as in cell B underlain with 10-15 cm of 5-8 cm diameter limestone rock in cell C. Each cell has inlet valves

TABLE 3.
WATER CHARACTERISTICS OF THE MINE
DRAINAGE OF BIG FIVE TUNNEL,
IDAHO SPRINGS, COLORADO

pH = 2.6
Temperature = 13°C

Conductivity at 25°C
= 2700 umho/cm
Flow = 0.045 CFS

Substance	Concentration (mg/1)	Substance	Concentration (mg/1)
Al	18.00	Cd	0.03
Fe	50.00	Pb	0.01
Mg	150.00	Na	46.00
Ca	370.00	K	9.20
Mn	32.00	As	0.02
Cu	1.60	SiO_2	40.00
Zn	10.00	SO^{4}_{2-}	2100.00

and a rock basket at its upstream end for better dispersal of the mine drainage throughout the substrate (Figure 6[94]). Mine drainage from the tunnel was diverted into the system through PVC lines. Drains were also installed. Inputs and outputs of the system were thereby controlled to aid in studying the mechanisms of metal removal. Six wells were placed in each of the cells to allow sampling of the interstitial waters. Each well had a screen at depth of 30.5, 61, or 91 cm. Figure 6(a) shows their location in each cell. Cattails (Typha angustifolia, T. latifolia), sedges (Carex utriculata, C. aquatilis), and rushes (Juncus arcticus) were transplanted from nearby wetlands in September, 1987.

Plants as well as soils were sampled by Colorado School of Mines' staff and were then analyzed by contract laboratories to determine initial metal and nutrient contents. Bacterial enumeration was conducted on the initial soils, and, from January, 1988, through January, 1989, on the wetland substrates. Bacteria under study are the four types mentioned earlier: the obligate autotroph Thiobacillus ferrooxidans, a group of aerobic heterotrophic iron-oxidizing bacteria, a similar group of heterotrophs oxidizing Mn^{2+} ions and, in the anaerobic zone, the sulfate-reducing bacteria.

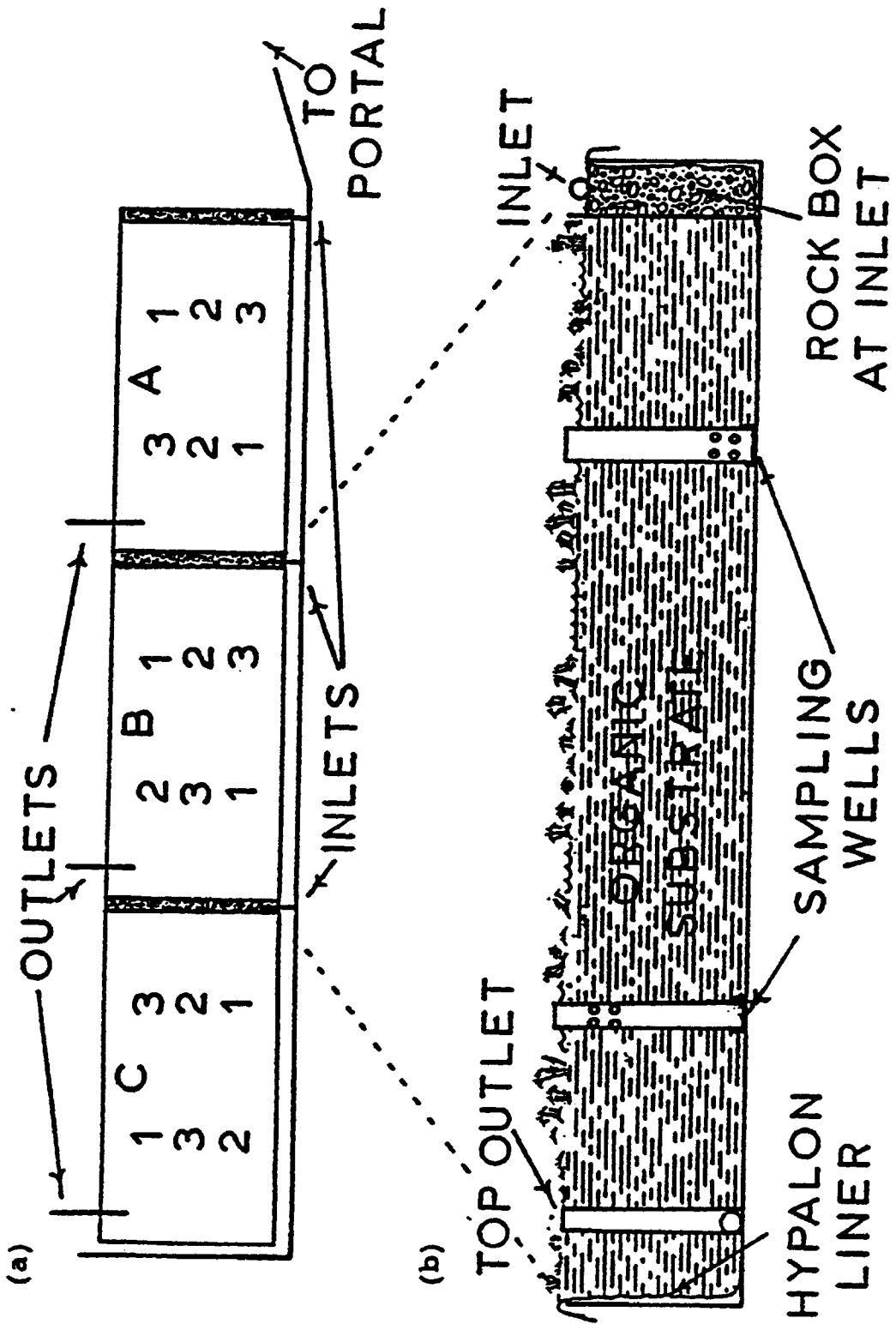


FIGURE 6. Plan view of the Wetland Pilot System at the Big Five Tunnel(a), and Cross Section of Cell B (b). Numbers 1, 2, and 3 Indicate the Well Screen Depth (in Feet)[34].

SAMPLING PROCEDURES

Soils in the wetland were highly heterogeneous. Soil composites were collected throughout the year (January, June, August, November, 1988 and January, 1989) from two different locations in each of cells B and C. One Sampling location was near the inlet and the other near the outlet, close to the wells, avoiding plants (Figure 6). Sampling of cell A was last done in November due to the reconstruction of the cell. The depths at which the soil composites were collected were 15 and 90 cm. Later in the year, additional samples were obtained at 2.5 cm from the surface.

Wide-mouthed 1/2 gal. Nalgene$^{(R)}$ jars were soaked in 1:4 nitric acid for 24 hours, rinsed with deionized water, and autoclaved at 250^{o}F for 20 minutes, prior to sampling. Soil composites from the top 2.5 cm and 15 cm were collected with a hand trowel. To "rinse" the jar and trowel, a small amount of sample was transferred to the jar using the trowel. The jar was capped and shaken and the soil sample was discarded. Samples collected at the 90 cm depth were taken with a hand auger. The auger and jar were "rinsed" in the same manner as with the shallow samples. Enough sample was collected to fill the jar approximately 3/4 full.

After bacterialogical tests were performed, the remaining sample was spread out on polyethylene sheets and allowed to air dry at room temperature.

The sample was ground in a blender. The blender was decontaminated between samples by grinding filter paper, then a small amount of the sample, then more filter paper. Finally, the sample was ground.

One split (250 gm) was placed in a precleaned jar to be sent to the laboratory for analysis. 250 gm splits were placed in plastic bags and stored in a refrigerated area at the CSM Chemistry Department.

FIELD MEASUREMENTS

Field determination of the pH, Eh, specific conductivity and temperature was performed on water samples collected from the wells placed in each cell: wells 1, 2, and 3 near the inlet, and wells 4, 5, 6 near the outlet. For January, June and August, readings of the pH, Eh, conductivity and temperature were taken from wells corresponding to the soil sampling locations (near the inlet or outlet), such that the screen depth corresponded closely to the depth of the soil samples, even if the well was not immediately adjacent to the sample location. For the November, 1988 and January, 1989 samples,

readings were taken from the soil samples immediately after collection.

Temperature measurements were made using the temperature probe on the YSI model 33 S-C-T meter (calibrated in the CSM laboratory). For pH measurements, a Leeds and Northrup model 7417 meter and Orion 91-05 electrodes were used. The pH meter was calibrated with pH 4 and 7 buffers prior to the measurements.

For Eh measurements, a second Leeds and Northrup model 7417 meter with a platinum electrode and a saturated calomel reference were used, calibrated with Light's solution.

LABORATORY PROCEDURES

Autotrophic iron-oxidizing bacteria were cultured using 9K and ferrous sulfate solution of Silverman an Lundgren[95]. Inoculations of the sulfate-reducing bacteria were made on a modification of medium B developed by Postgate[57,96]. Enumeration of both bacteria were made using the Most Probable Number Method (MPN)[6,97,98].

Heterotrophic iron- and manganese-oxidizing bacteria were assessed using the spread plate method[99,100,101]. Agar plates contained $FeSO_4$ and $MnSO_4$ solutions for iron and manganese oxidizers, respectively. Ingredients of the culture media and detailed analytical procedures are presented in Appendix A.

The moisture content of each soil sample (thus the dry weight) was determined by shaking the sample in its container, placing a portion of it (4g) (with a sterilized spatula) in each of two tared glass petri dishes and allowing them to air dry to a constant weight. The final weights were then averaged and the moisture content was determined. Appendix B presents a worksheet used for these calculations. The weight of dry soil in the first dilution (Appendix A) was then calculated.

The most probable number of bacteria was obtained from the MPN table in Page et al.[97] based on the number of positive tubes at the different dilution levels (Appendix B). Finally, populations of bacteria were reported as number of bacteria per gram of dry soil.

The soil samples were also analyzed by the contracted laboratory for minor and trace elements, total sulfur and sulfur forms (organic sulfur, sulfate sulfur, pyritic sulfur), total carbon, nitrogen and phosphorus using EPA approved standard methods.

Total sulfur was determined at the Colorado School of Mines Chemistry Department using the ASTM method D-3177[102] (for samples taken in August and November, 1988, and January, 1989).

The same analyses were performed on the original substrate materials[93] in October, 1987, prior to flow of AMD in the wetland. Assessment of the heterotrophic oxidizers populations was only started in June, 1988 and was not done on the initial substrates.

PRECISION OF THE MOST PROBABLE NUMBER METHOD

The most probable numbers of iron-oxidizing and sulfate-reducing bacteria in the samples were determined from the fraction of cultures that failed to show growth, i.e. from the negative responses in a series of tubes, when a suitable culture medium was used. This method has a low order of precision[98]. Many tubes must be innoculated to obtain precise numbers, precision increases rapidly when the number of tubes used at each dilution level is increased from 1 to 5 tubes. However, the increase is much smaller when 10 tubes are innoculated at each level, instead of 5 tubes[98].

Determination of the IOB and SRB populations in the constructed wetland was obtained using 5 tubes at each 10-fold dilution level.

The 95% confidence limits can be obtained by multiplying the MPN by 3.3 (for the upper limit) and dividing the MPN by 3.3 (for the lower limit); 3.3 is the confidence factor

obtained from Table 100-2 in Page et al.[97]. Two MPNs are considered to be different if their confidence intervals do not overlap. This method is most useful when the sample contains many different organisms and no selective method is available for the culture of the organism of interest. Additionally, if this organism produces a detectable product (such as the orange color produced by IOB and the black color produced by SRB), growth of the other organisms present in the sample will not interfere with the estimation of the organism under study.

STATISTICAL ANALYSIS

The objective of this thesis is reached by reporting on the occurrence and spatial distribution of geochemically important bacteria. However, a comparative analysis of environmental factors such as Eh, pH and temperature on one hand, with bacterial count data on the other, may reveal the existence of informative relationships between them.

Correlation analyses were performed on the data using a statistical analysis computer software known as "Statgraphics" by Statistical Graphics Corporation (release 2.6).

In performing such analyses, the main concern is whether two variables are interdependent. The calculated correlation

coefficient (R) is an expression of the linear relationship between these two variables. Correlation is a descriptive technique estimating the degree of association among the variables involved, and can lead to reasoning about causal relationships[103].

Due to the unavailablity of duplicate samples in this study, and the errors involved in obtaining the data, the correlation is unlikely to be perfect. However, the sign of R can tell us about the type of association among the variables involved. Correlation coefficients usually range from +1 to -1, +1 indicating a perfect direct relationship, and -1 indicating that one variable changes inversely with relation to the other. No correlation exists between the two variables when R = 0[104].

CHAPTER IV

RESULTS AND DISCUSSION

The constructed wetland at the Big Five tunnel has organic matter for a substrate; SO_4^{2-} and reactive iron are continuously supplied by the AMD, and water flow does not fluctuate throughout the year. Metal sulfide production and retention is favored by these conditions and was visibly evident by the black substrates (typical in sulfide deposition). Plant roots in the wetland provide the conditions favoring pyrite formation (where sulfide generated by sulfate reduction becomes oxidized to elemental sulfur or polysulfides, which are required for pyrite formation[60]).

An oxidizing zone was also noticeable, delineated by a brown layer at the surface of the substrate, and "yellow boy" precipitation in some areas.

Initial bacterial counts in the wetland substrates are presented in Table 4[94].

Results of the bacterial, metals, sulfur forms, and nutrient analyses, as well as parameters determined in the field are provided in Appendix C.

Correlation coefficients among populations of the bacterial groups under study and the pH, Eh and temperature in

TABLE 4.
INITIAL BACTERIAL COUNTS IN
THE SUBSTRATE(94)

Component	Iron-Oxidizing Bacteria MPN/g	Factor of Confidence	Sulfate-Reducing Bacteria MPN*10^4/g	Factor of Confidence
Aged Manure	0		9.0	3.3
Wood Products	0		0.3	3.3
Mushroom Compost*	0		50.0	3.3
Peat	200	3.3	0.3	3.3
Peat/Manure/Wood**	0		2.0	3.3

*Average of three samples.

**Average of two samples.

cell A, B, and C are presented in Table 5. Counts of iron-oxidizing bacteria population near the outlet of cell A during January 1988 were omitted due to problems experienced during the sampling and innoculation procedures.

OCCURRENCE AND SPATIAL DISTRIBUTION OF THE BACTERIAL GROUPS IN THE WETLAND SUBSTRATES

1. Sulfate-Reducing Bacteria.

Viable counts for sulfate-reducing bacteria ranged from 19 x 10^2 to >28 x 10^6 (Appendix C). Their populations were large in January, 1988 (larger than initial substrate population), but dropped in June mostly at the 15 cm depth. Counts increased at 90 cm in August and November of the same year. This pattern was observed in all three cells (Figures 7, 8, 9).

During the summer of 1988, sulfate-reducing bacteria were more abundant at 90 cm depth than at 15 cm in cell A. Based on confidence intervals of the SRB counts, no variation between inlet and outlet counts was observed. In cells B and C, counts showed variation between samples taken near the inlet and those collected near the outlet: SRB populations were growing better near the inlet, at 90 cm from the surface in C, and at 15 cm near the outlet in B. This variation in populaton size could be a result of different flow patterns

TABLE 5.
CORRELATION ANALYSIS OF BACTERIAL POPULATIONS
AND Eh, pH, AND TEMPERATURE
(CELLS A, B, AND C)

		pH		Eh		Temperature	
BACTERIA	CELL	$R^{(a)}$	R^2(%)	R	R^2	R	R^2
SRB[(b)]	(A)	0.3291	10.83	-0.4103	16.84	-0.2118	4.49
SRB	(B)	0.1575	2.48	-0.1016	1.03	-0.2109	4.45
SRB	(C)	0.3352	11.24	-0.2048	4.19	0.0280	0.08
IOB[(c)]	(A)	-0.6145	37.76	0.6570	43.17	-0.3122	9.75
IOB	(B)	-0.4839	23.41	0.2777	7.71	-0.1368	1.87
IOB	(C)	-0.2862	8.19	0.2366	5.60	0.1029	1.06
HIOB[(d)]	(A)	-0.0100	0.01	-0.0270	0.07	0.2719	7.40
HIOB	(B)	-0.4728	22.35	0.2878	8.29	0.5510	30.36
HIOB	(C)	-0.3174	10.07	0.4158	17.29	0.4697	22.06
HMOB[(e)]	(A)	0.2035	4.14	-0.1952	3.81	0.5126	26.28
HMOB	(B)	-0.2234	4.99	0.2213	4.90	0.6240	38.94
HMOB	(C)	-0.3186	10.15	0.4128	17.04	0.4637	21.50

(a) Linear Correlation Coefficient.
(b) Sulfate-reducing bacteria.
(c) Iron-oxidizing bacteria.
(d) Heterotrophic iron-oxidizing bacteria.
(e) Heterotrophic manganese-oxidizing bacteria.

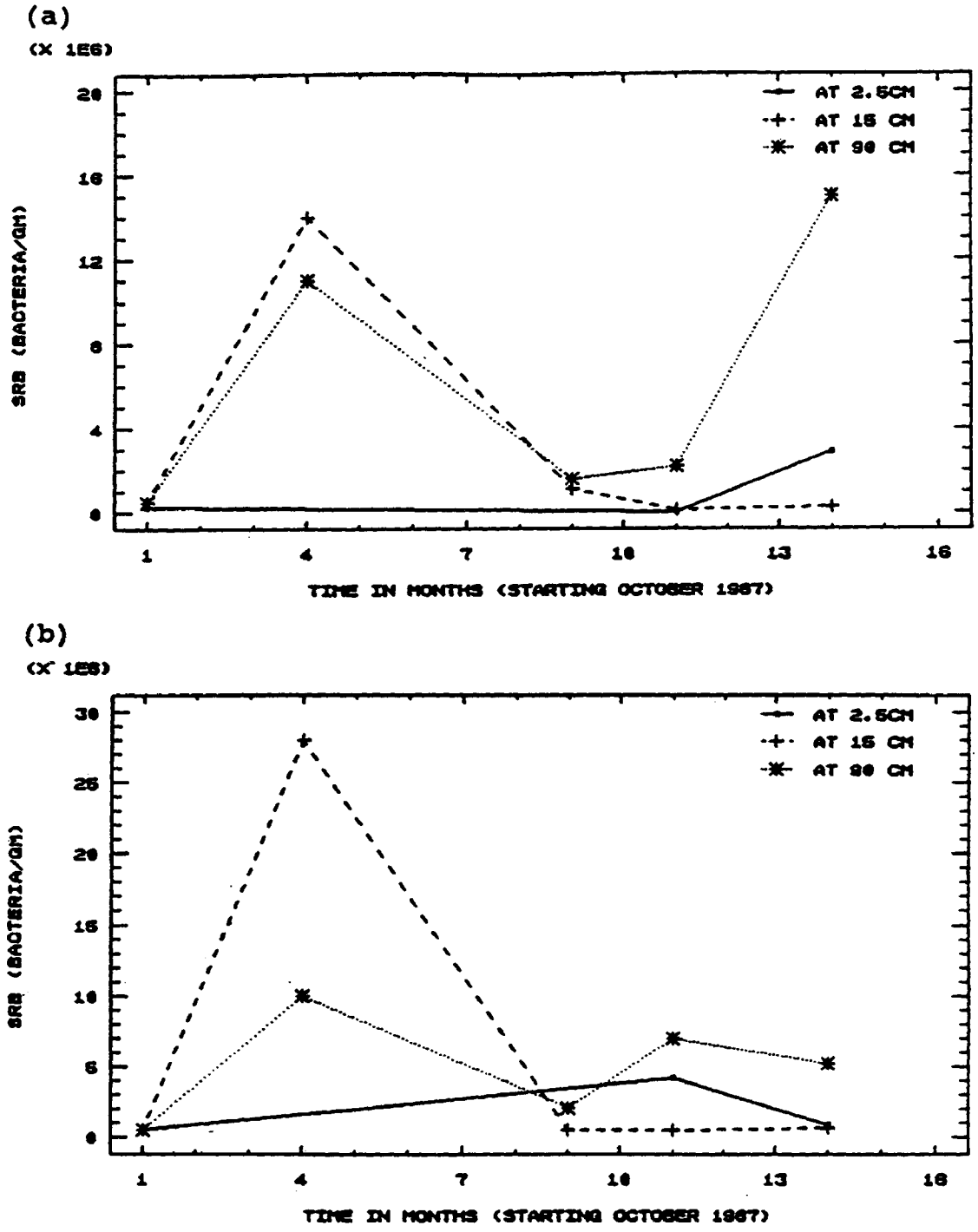


FIGURE 7. Sulfate-Reducing Bacteria Population in Cell A Substrate (a) Near the Inlet, and (b) Near the Outlet.

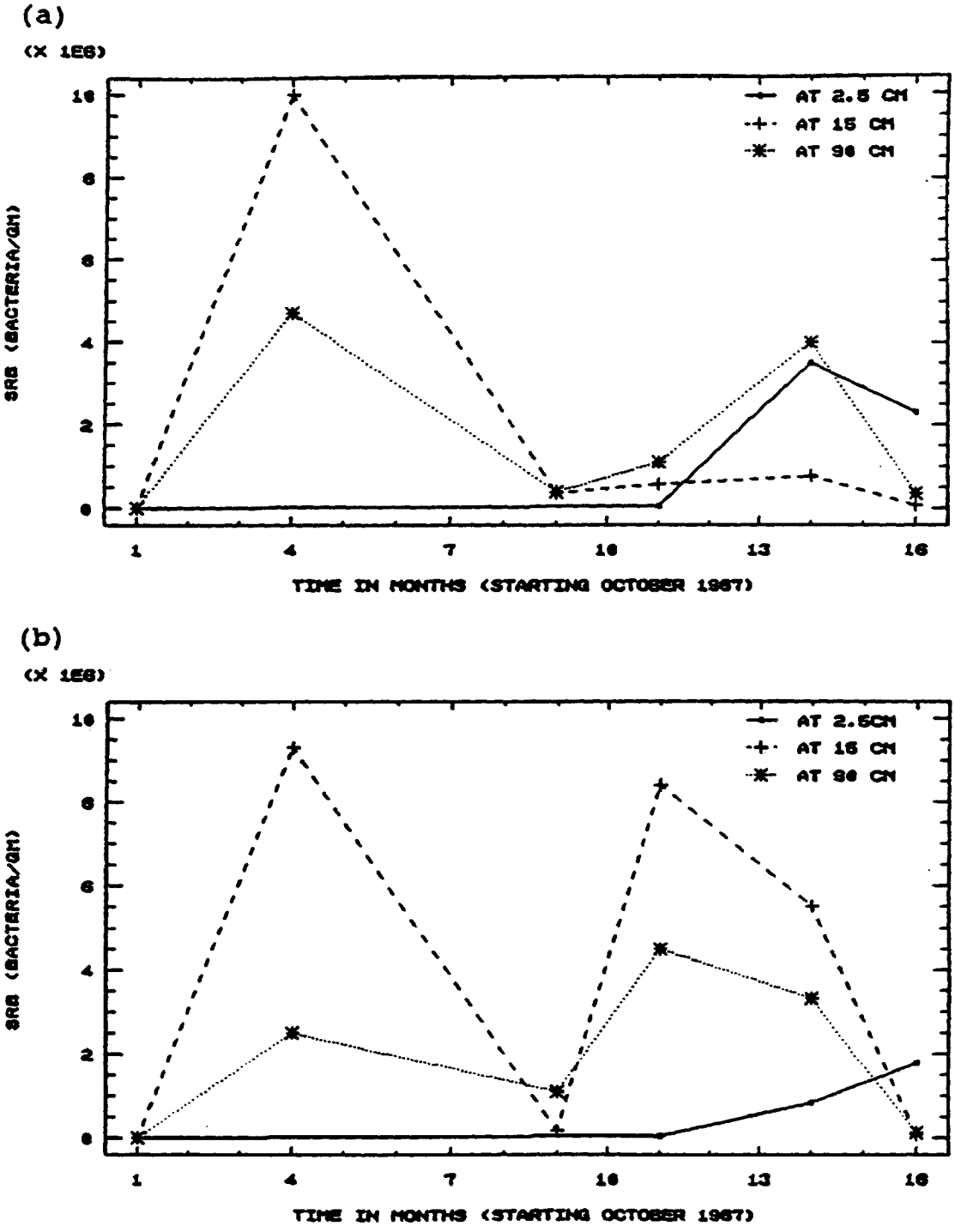


FIGURE 8. Sulfate-Reducing Bacteria Population in Cell B Substrate, (a) Near the Inlet, and (b) Near the Outlet.

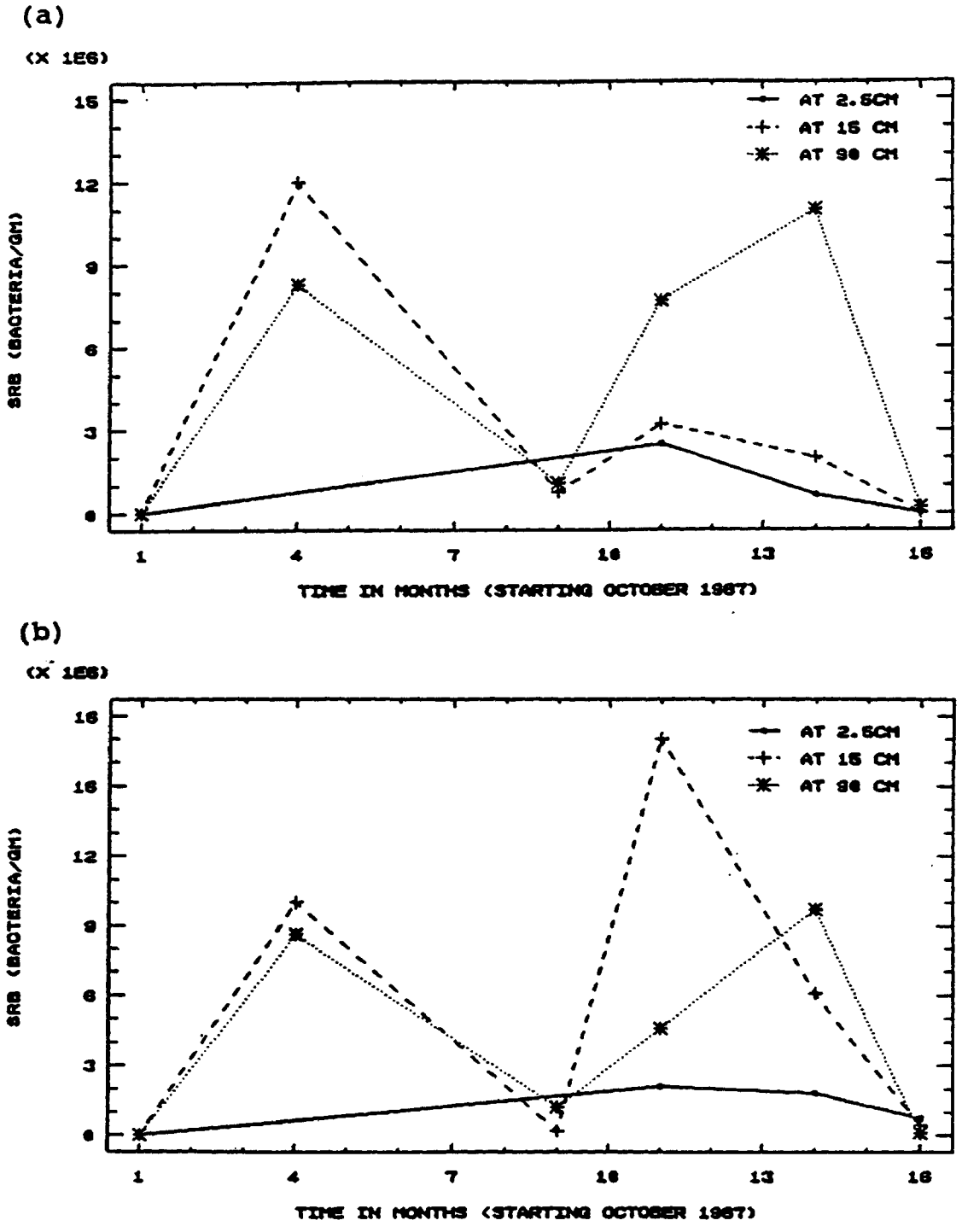


FIGURE 9. Sulfate-Reducing Bacteria Population in Cell C Substrate, (a) Near the Inlet, and (b) Near the Outlet.

within the substrates of the cells; mine drainage from the Big Five tunnel was not evenly dispersed throughout the substrate, instead it followed specific flow patterns across each cell. Sulfate-reducing bacteria were also present in the oxidized surface layer of the wetland soil in cells A, B, and C; counts were lower than at greater depth, but still much higher than expected. Such occurrences of SRB are regularly encountered in aerobic environments such as the sea, and coastal and estuarine sediments[105]. Jorgensen[106] discovered that bacterial sulfate reduction took place in the oxidized layer of a coastal marine sediment inside reduced pellets 50-200 um in diameter; such pellets, made of organic material, were found at 1 cm depth, with highest frequency at Eh near zero.

2. Iron-Oxidizing Bacteria

Viable counts of iron-oxidizing bacteria ranged from 0.003 x 10^4 to 95 x 10^4, and were higher in cell A than in cells B and C. They occurred in very large numbers in the upper 2.5 cm of the substrate surface and showed no variation between the inlet and outlet of cells A and C. Populations of IOB were larger near the inlet of cell B than its outlet (Figures 10, 11, 12). In addition, based on comparison of confidence intervals, counts showed no difference between 15 and 90 cm.

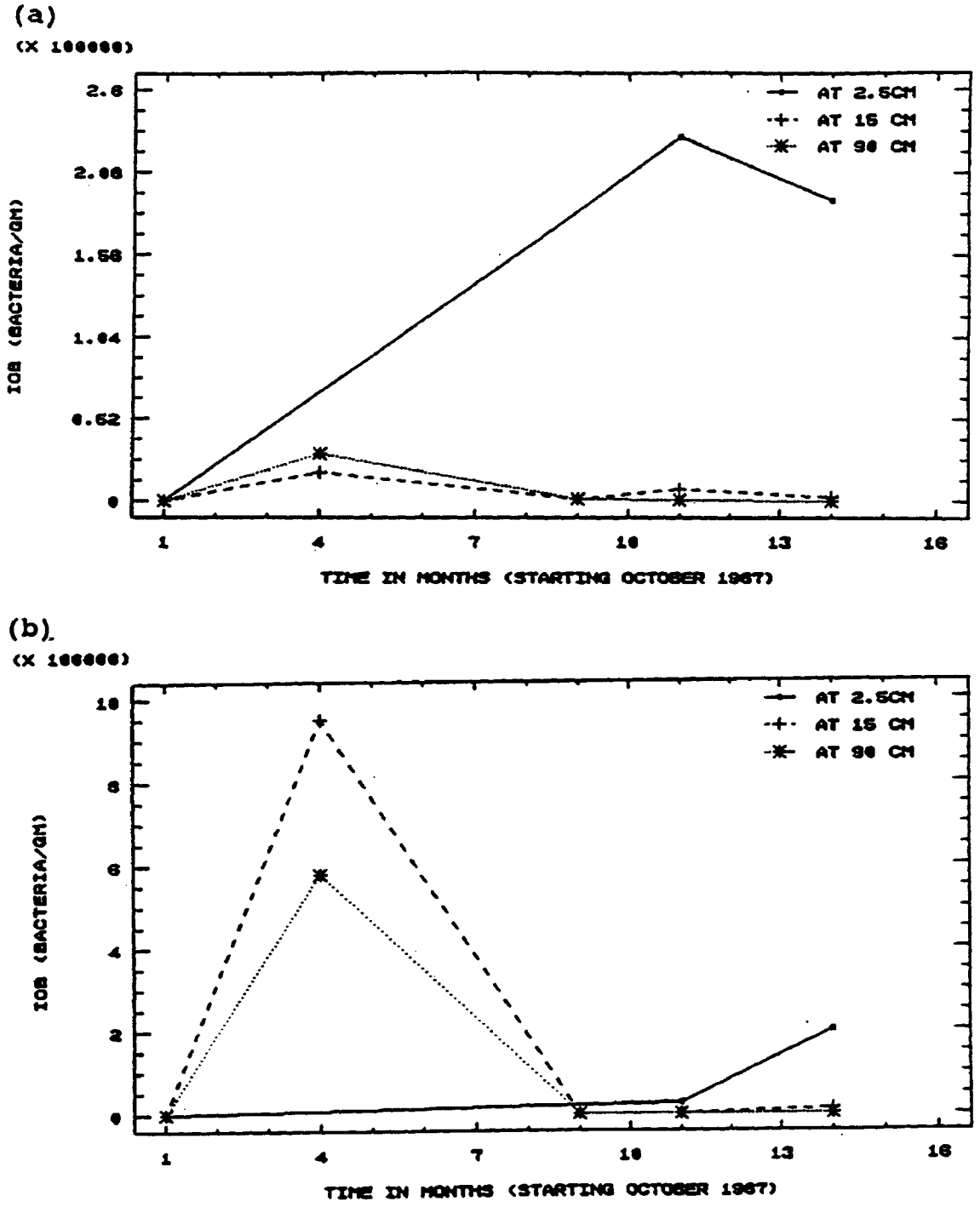


FIGURE 10. Iron-Oxidizing Bacteria Population in Cell A Substrate, (a) Near the Inlet, and (b) Near the Outlet.

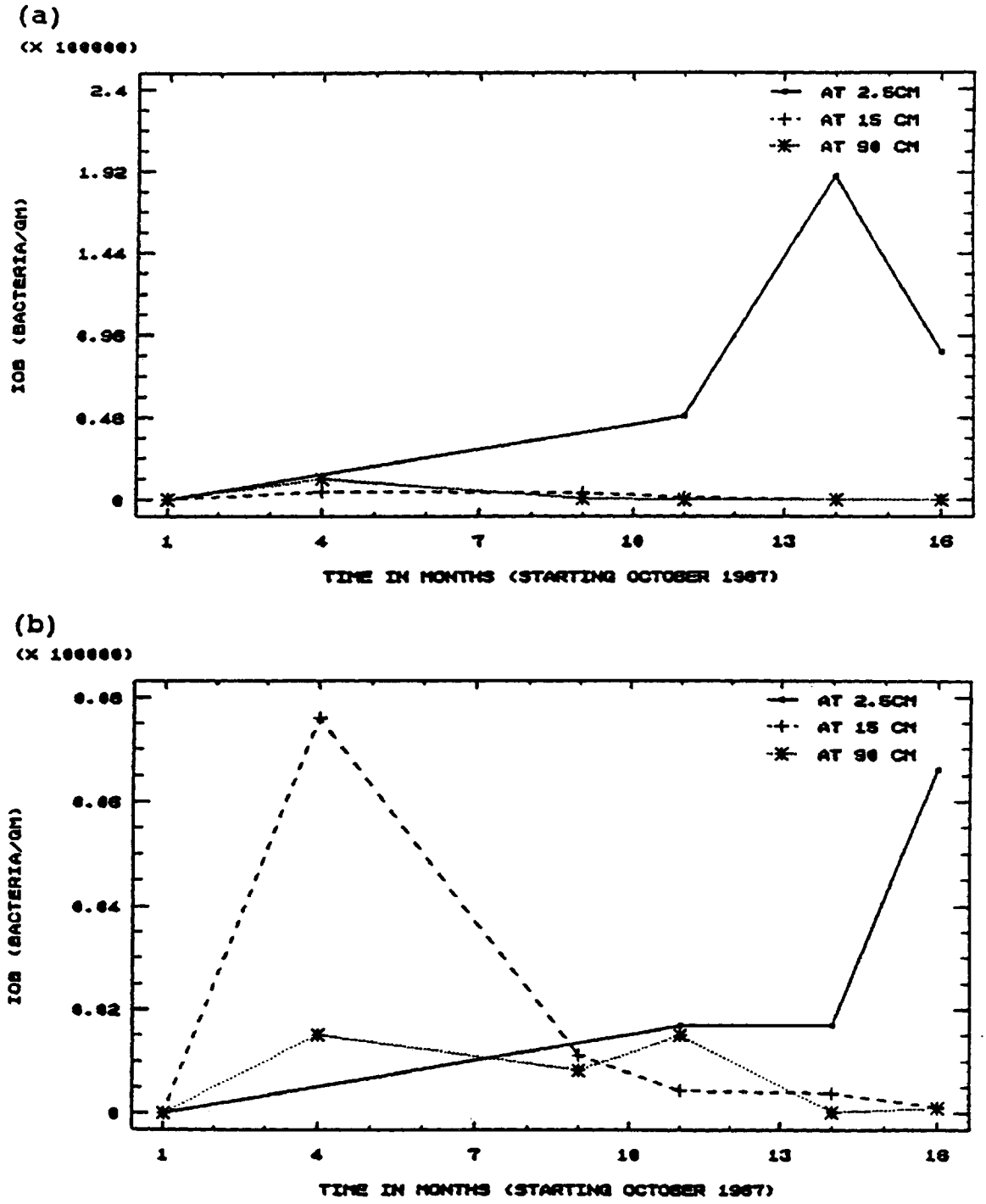


FIGURE 11. Iron-Oxidizing Bacteria Population in Cell B Substrate, (a) Near the Inlet, and (b) Near the Outlet.

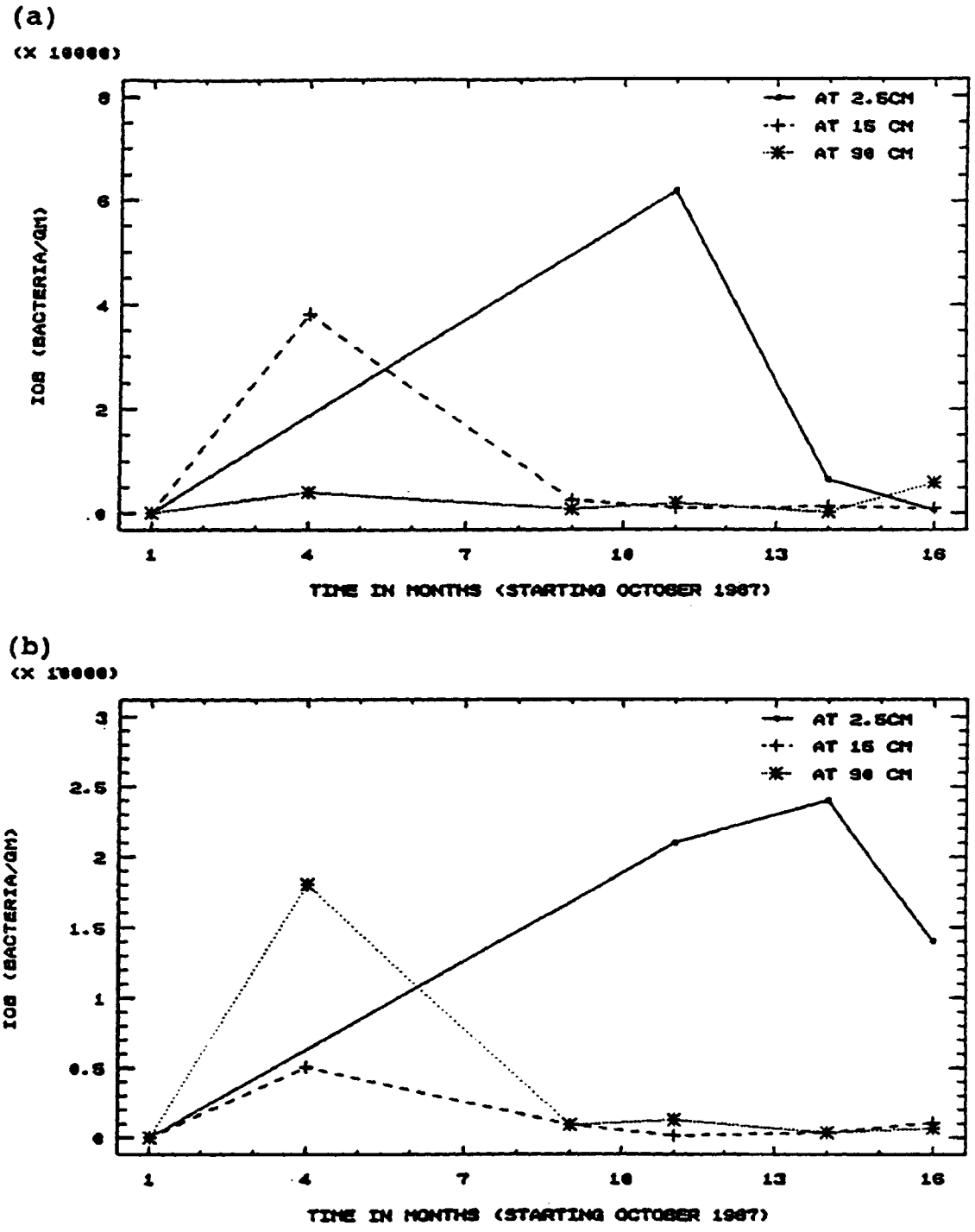


FIGURE 12. Iron-Oxidizing Bacteria Population in Cell C Substrate, (a) Near the Inlet, and (b) Near the Outlet.

These results suggest that an aerobic zone was present at the surface, especially near the inlet where oxygen is introduced by the incoming water.

Similar to SRB, IOB populations were large in January, 1988 (in all three cells, at 15 and 90 cm depths), and decreased in June. The decrease in SRB and IOB counts could be caused by many environmental factors such as Eh, pH and temperature of the substrates. A slight increase of the IOB observed at 15 cm might be a result of plant growth, and plant roots penetrating in the soil and injecting oxygen[107], creating aerobic conditions in microzones around the roots for those strict aerobes. In an attempt to confirm such a possibility, soil samples were collected in August, 1988, near the plant roots in cell A. Counts for iron-oxidizing bacteria were >160 x 10^3 bacteria/g at depth of 15 cm, and 14 x 10^3 bacteria/g at 90 cm from the surface. These counts were 1-2 orders of magnitude higher than in soil samples collected in cell A (Appendix C) away from plant roots. However, further investigation appears necessary.

In cell C, smaller population sizes were noticed during November, 1988 and January, 1989. Lower temperatures and formation of ice at the surface of the wetland can reduce the mesophilic IOB population.

3. Heterotrophic Iron-Oxidizing Bacteria

Heterotrophic iron-oxidizing bacteria plate counts indicated an increase in their population at 15 cm depth during the month of August, 1988. This increase was noticed in all three cells near the inlets and the outlets (Figures 13, 14, 15). The highest counts were at the surface suggesting, again, oxidizing conditions. Soil composites collected at 90 cm from the surface near the inlet were also analyzed for presence of HIOB, and results showed that HIOB existed at that depth but in very low numbers.

Cell C showed slightly larger populations than the other cells. All interpretation of the results for HIOB are limited by the limited sampling of these organisms.

4. Heterotrophic Manganese-Oxidizing Bacteria

Heterotrophic manganese-oxidizing bacteria counts appeared to be similar to those of HIOB (Figures 16, 17, 18). Perhaps the same bacterial genera are oxidizing both manganese and iron as explained in an earlier section. Leptothrix, Crenothrix and Metallogenium can heterotrophically oxidize both metal ions.

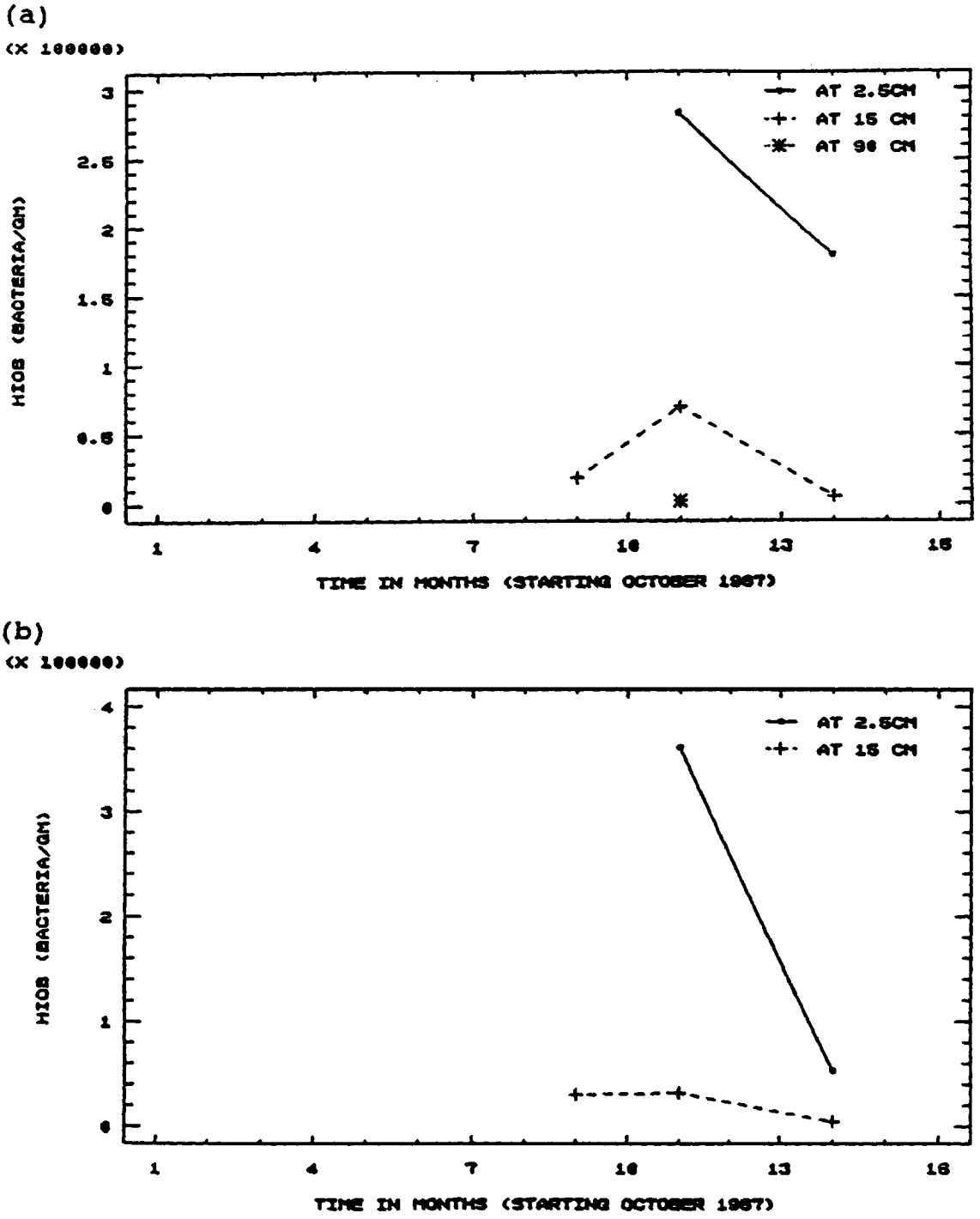


FIGURE 13. Heterotrophic Iron-Oxidizing Bacteria Population in Cell A Substrate, (a) Near the Inlet and (b) Near the Outlet.

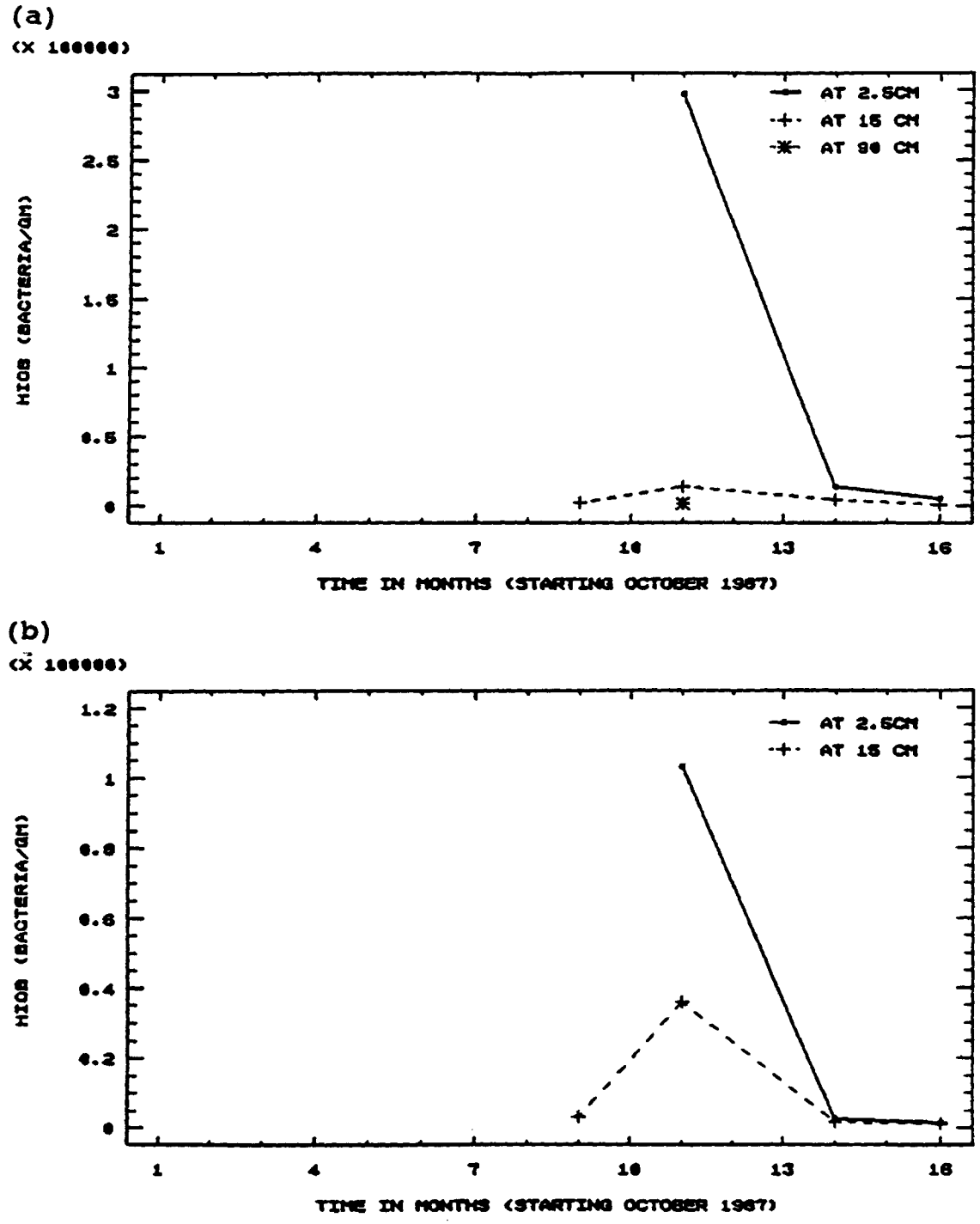


FIGURE 14. Heterotrophic Iron-Oxidizing Bacteria Population in Cell B Substrate, (a) Near the Inlet and (b) Near the Outlet.

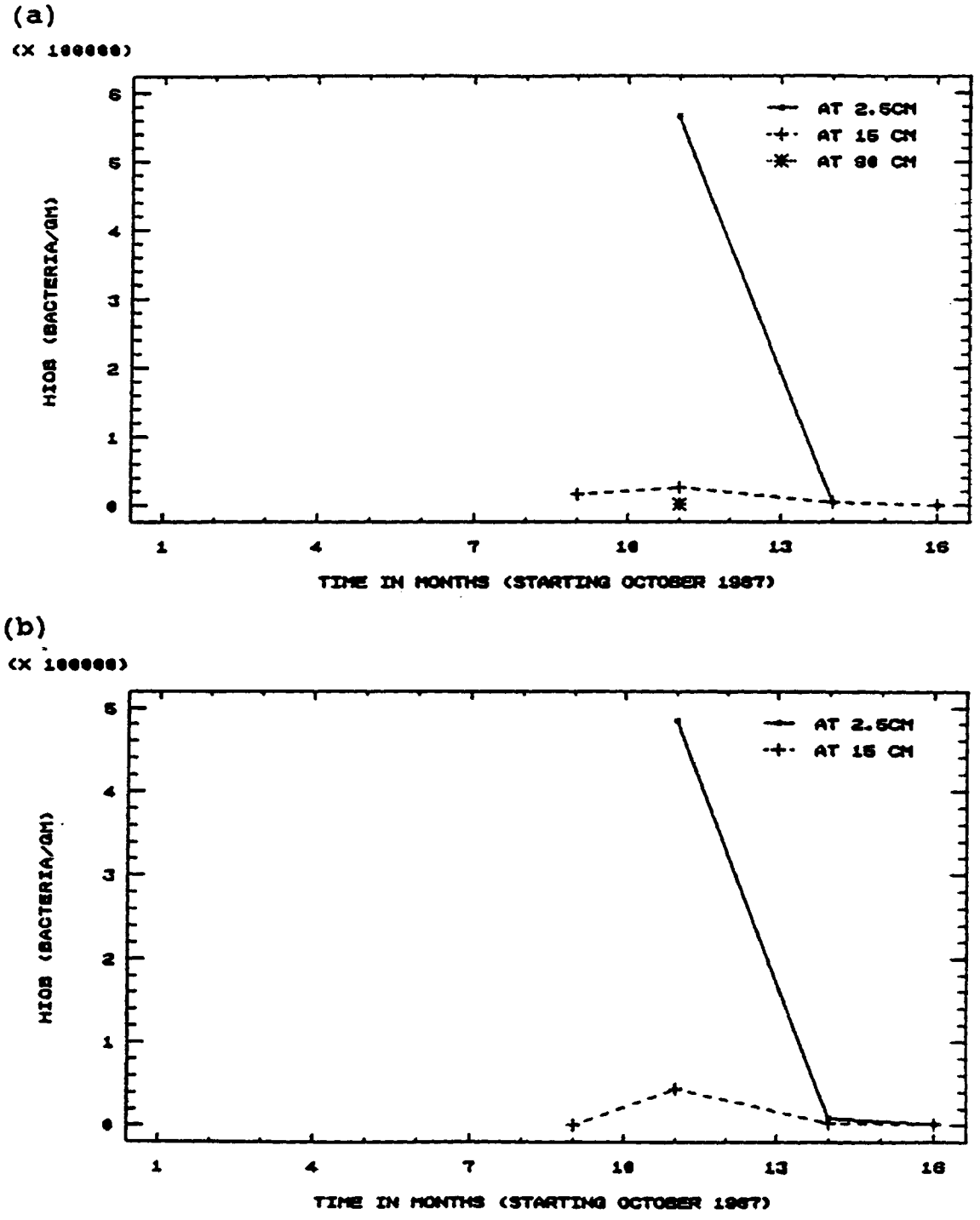


FIGURE 15. Heterotrophic Iron-Oxidizing Bacteria Population in Cell C Substrate, (a) Near the Inlet and (b) Near the Outlet.

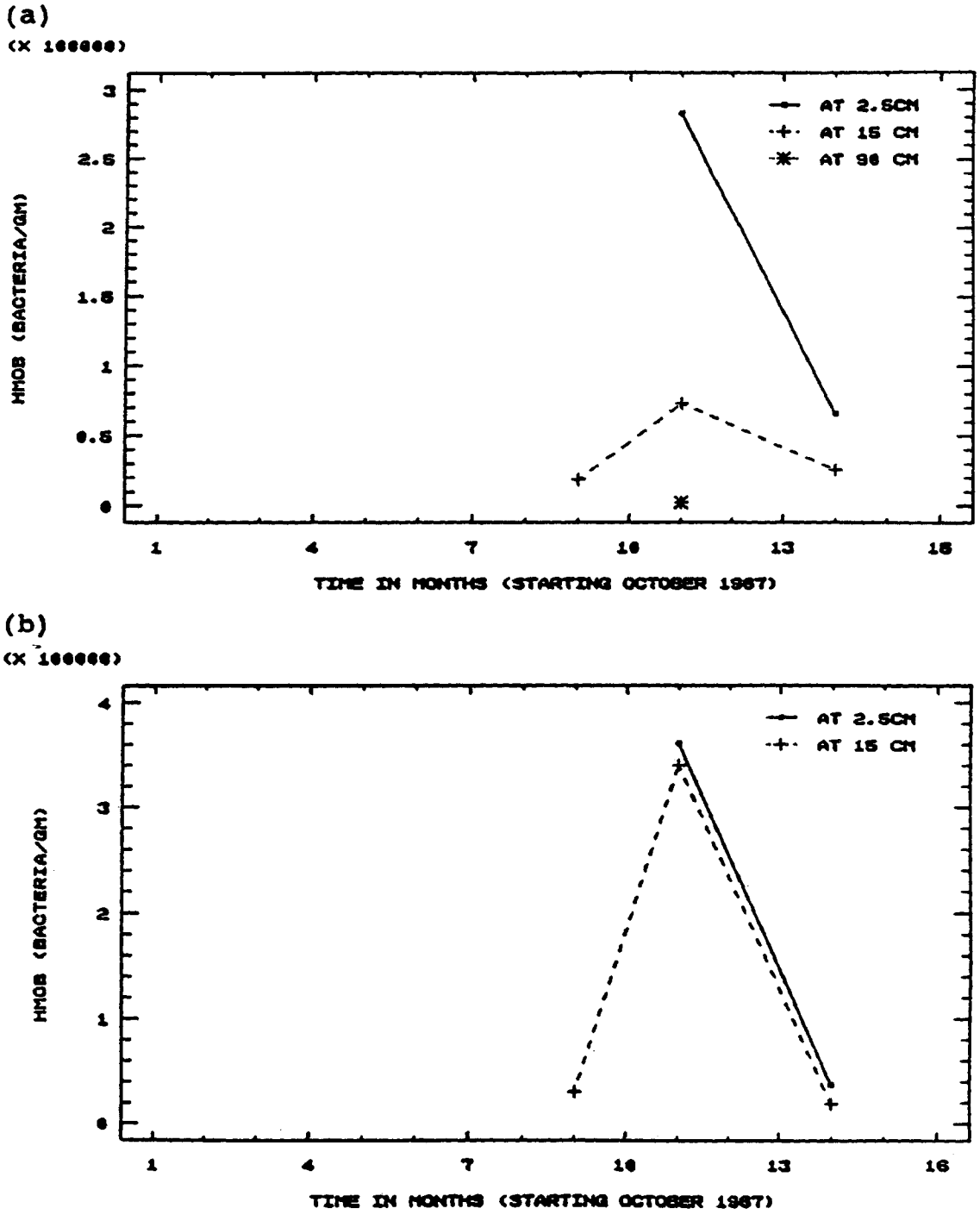


FIGURE 16. Heterotrophic Manganese-Oxidizing Bacteria Population in Cell A Substrate, (a) Near the Inlet and (b) Near the Outlet.

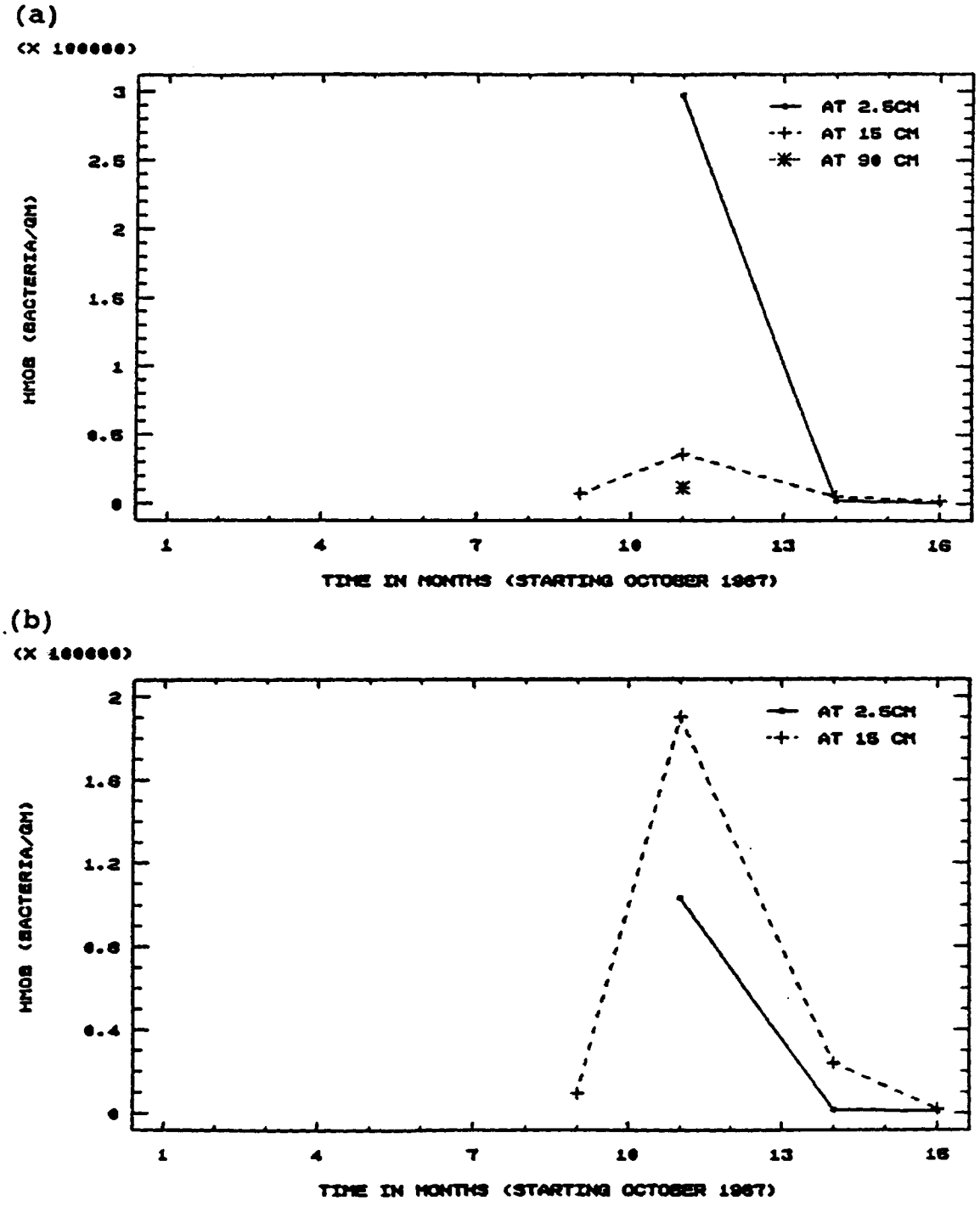
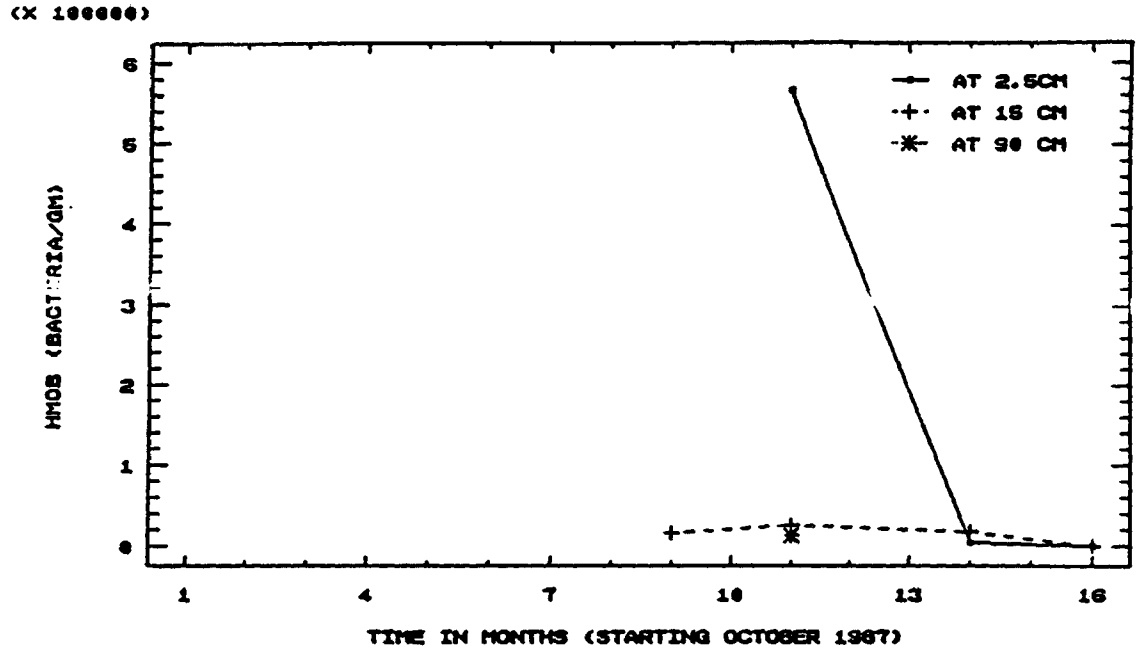


FIGURE 17. Heterotrophic Manganese-Oxidizing Bacteria Population in Cell B Substrate, Near the Inlet and (b) Near the Outlet.

(a)



(b)

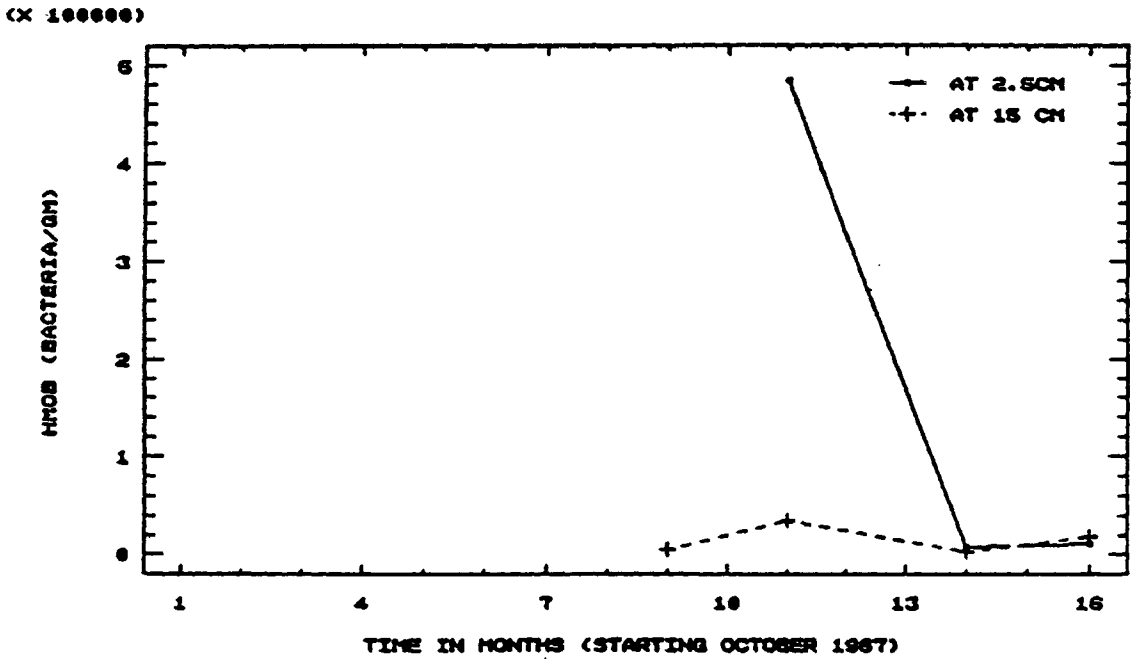


FIGURE 18. Heterotrophic Manganese-Oxidizing Bacteria Population in Cell C Substrate, (a) Near the Inlet and (b) Near the Outlet.

However, populations of HMOB at 15 cm from the surface in cells A and B, near their outlets were equal to or larger than populations at the surface. Additional genera of HMOB could be present which tolerate the prevailing environmental conditions in this layer.

ECOLOGY OF THE FOUR BACTERIAL POPULATIONS

The study of the ecology of the four groups of bacteria studied in the constructed wetland consists of two parts: a) the environmental conditions essential for their growth and multiplication, (nutrients, Eh, pH and temperature), and b) the effect of growth and multiplication of the bacteria on the wetland environment.

1. Environmental Conditions

1.1. Nutrients in the Soil

Nutrients are needed by bacteria for three separate functions: for protoplasmic synthesis, for energy necessary for biosynthetic reactions and cell growth, and as electron acceptors in energy yielding reactions[38].

Energy sources could be sunlight, hydrogen, ammonium, nitrite, inorganic sulfur compounds, iron, and organic substances.

Carbon sources are organic material and carbon dioxide. Oxygen, nitrate, carbon dioxide, sulfate, organic molecules are all electron acceptors for a variety of microorganisms. Inorganic nutrients are also needed. A source of potassium, magnesium, sulfur, iron and probably calcium, manganese, copper, zinc, cobalt and molybdenum are essential in protoplasm of cells. Organic material provides several nutrients to microorganisms. Nitrogen is an indispensable component of the protoplasm of cells. Phosphorus is second to nitrogen as an inorganic nutrient requirement essential for cellular metabolism. Sulfur is also an important element for synthesis of proteins.

Initial nutrient contents of the compost in cell A and the mixture of peat, manure and wood products in cells B and C were favorable for bacterial growth (Appendix C). Total nitrogen, phosphorus, carbon, iron, potassium, magnesium and sulfate as well as other nutrients were in high concentrations in the initial soil components. However, total nitrogen and phosphorus in the substrates decreased during the months of June and August, 1988. Concentrations in cell A are graphically represented in Figure 19 for nitrogen and in Figure 20 for phosphorus. Concentrations in cells B and C substrates showed the same pattern as in cell A, indicating a similar

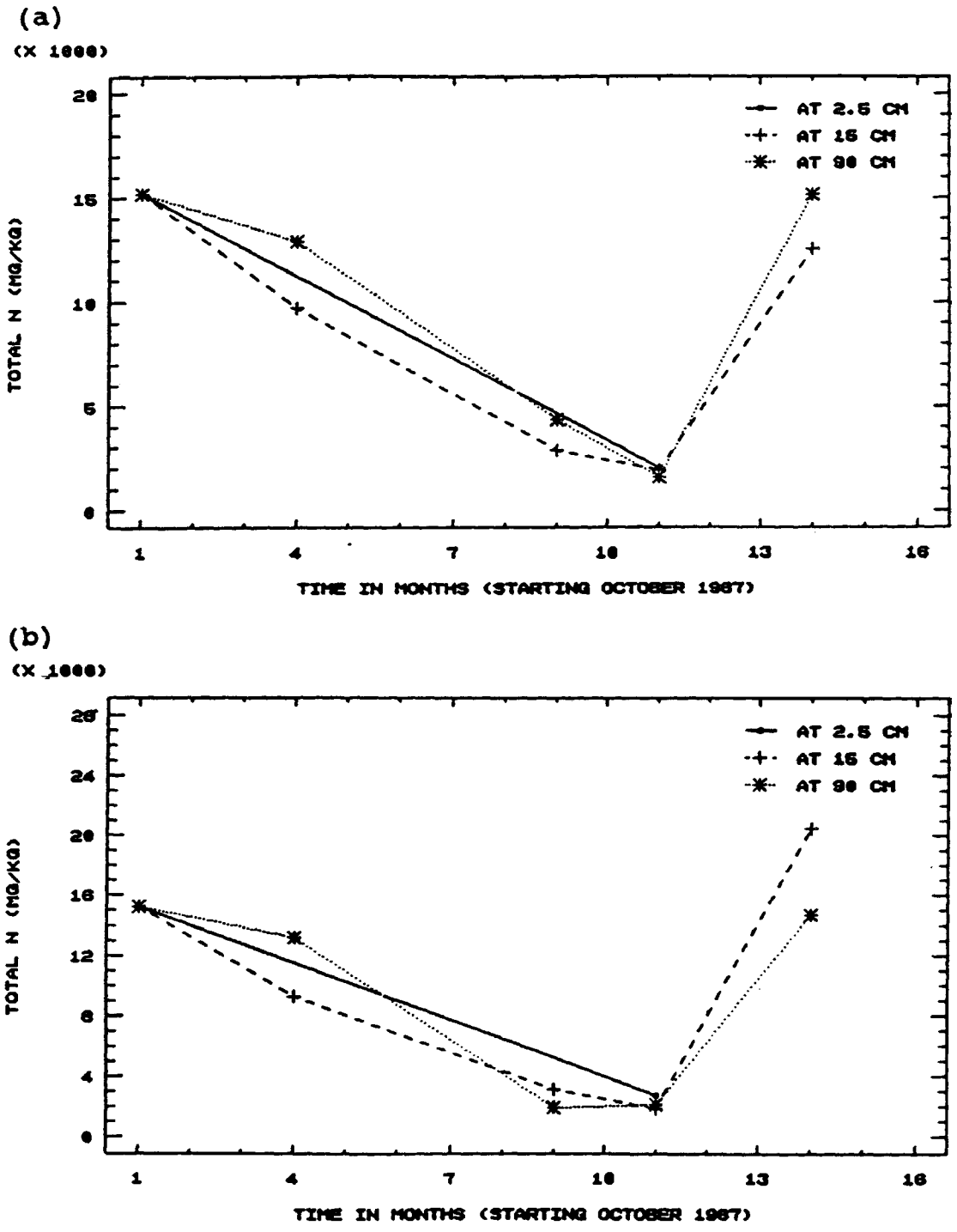
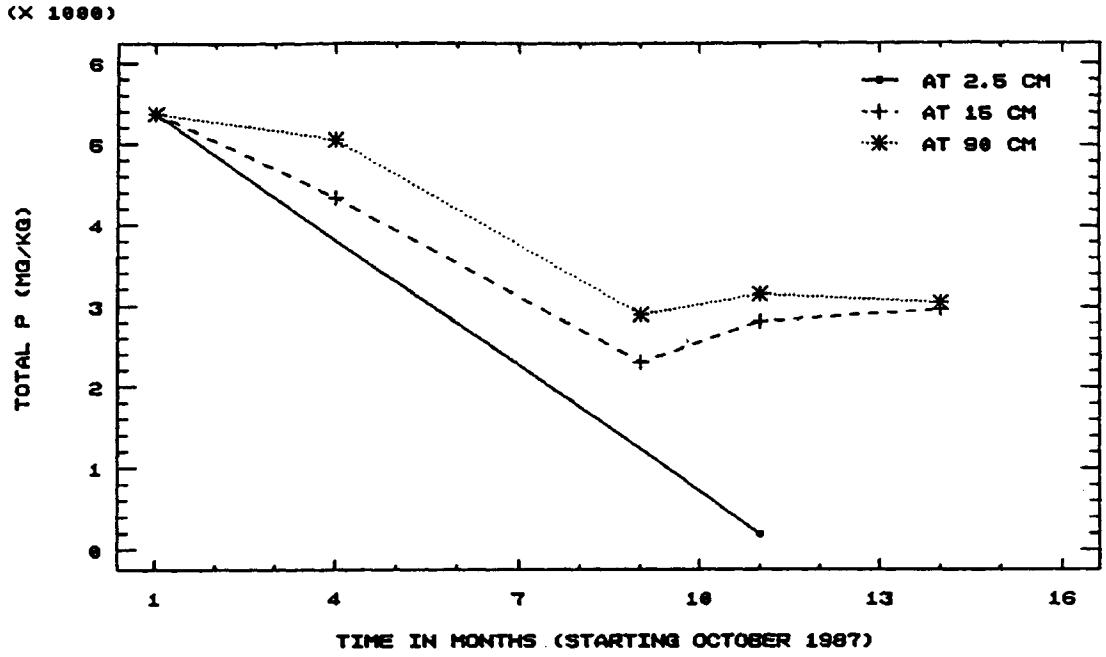


FIGURE 19. Total Nitrogen in Cell A Substrate, (a) Near the Inlet, and (b) Near the Outlet.

(a)



(b)

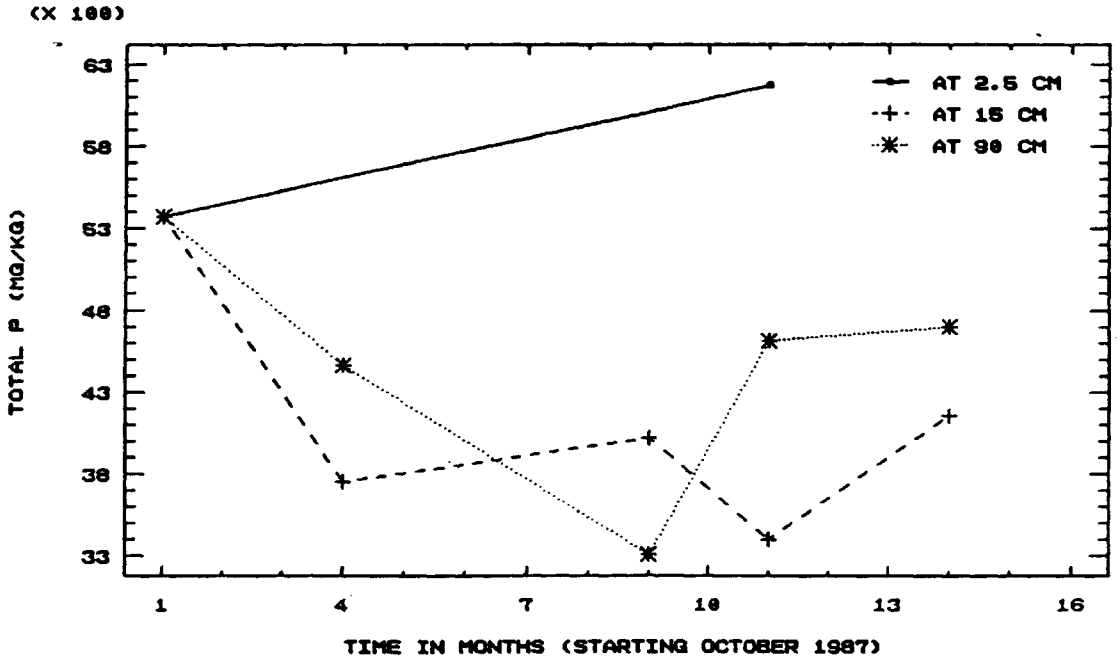


FIGURE 20. Total Phosphorus in Cell A Substrate, (a) Near the Inlet and (b) Near the Outlet.

decrease in the summer. Levels of N and P were again higher in November of the same year.

A possible explanation for the decrease in nitrogen and phosphorus concentrations at 15 cm from the surface, could be plant uptake of these nutrients at the root zone during the growing season. Low nitrogen and phosphorus levels at the surface could be a result of the algal growth noticed during the summer. Total nitrogen and phosphorus at the bottom of the substrate demonstrated similar low levels which suggests that plants were able to use these nutrients even though their roots did not reach that far. Flow patterns in the substrate could have made N and P available to plant roots.

Nitrogen can also be depleted by denitrifying bacteria which reduce nitrate to N_2. Denitrification, sulfate reduction and methane production have been demonstrated to occur simultaneously in terrestrial ecosystems[108]. It has been reported that the major product of nitrate reduction in flooded soil is gaseous nitrogen rather than nitrite or ammonium[38]. Denitrifying bacteria are facultative anaerobes which can utilize nitrate in the absence of O_2. Thiobacillus denitrificans, a sulfur-oxidizing chemoautotroph utilizes sulfide, elemental sulfur, or thiosulfate as energy sources, and has the ability to grow aerobically or anaerobically in

the presence of nitrate, converting it to N_2, which is volatile and is lost to the atmosphere.

Other bacteria reduce nitrate to ammonium which is another necessary form of nitrogen for protein synthesis, adding to the nitrogen availability to plants.

Insoluble phosphates in soils are usually mobilized by the production of acid (such as nitric and sulfuric acids)[38]. However, in flooded soils, phosphate in insoluble ferric phosphate may be mobilized by the reduction of the iron. H_2S produced by sulfate-reducing bacteria reacts with ferric phosphate yielding ferrous sulfide and liberates phosphate[42]. Many phosphate-dissolving microorganisms in the vicinity of plant roots can bring phosphate into solution, hence making it available to plants[38].

Increases in total nitrogen and phosphorus during November may be a result of lower uptake by plants and decomposition of plant material. Bacterial nitrogen fixation could be responsible for higher nitrogen concentrations.

1.2 Eh of the Substrates

Redox potential values ranged from -160 to +600 mV. Sulfate-reducing bacteria are known to grow only at negative redox potentials (Table 6). Comparing SRB population over

TABLE 6.
OPTIMAL CONDITIONS FOR GROWTH
OF THE BACTERIA

	pH	Temperature(°C)	Eh
Thiobacillus ferrooxidans	2.5-3.5	25-40	+
Heterotrophic Iron Oxidizers	3.5-7.8	18-25	
Heterotrophic Manganese Oxidizers	3.5-8.0	10-44	+(>200)
Sulfate Reducers	4.0-9.5	0-40	-

the sampling period with the Eh values obtained concurrently (Figures 21, 22, 23), a correlation can be seen where SRB population decreased with higher Eh and increased with lower Eh values with the exception of cell A near the inlet which showed some variation. Furthermore, calculated correlation coefficients R (Table 5) between SRB population and Eh values were negative for all three cells.

The redox potentials at 15 and 90 cm from the surface followed the same pattern over time but were slightly higher at 15 cm. Redox potential of the surface substrate (at 2.5 cm) was much higher than at depth and positive, which confirms that an aerobic layer exists at this depth and explains the large population of IOB, HIOB and HMOB in this layer. Iron-oxidizing bacteria population correlated positively with Eh in cells A, B, and C (Table 5). A similar positive correlation was obtained for HIOB and HMOB in cells B, and C but not for A (Table 5).

Redox potential values were low in January, 1988 and increased in June, which might be the reason why the SRB population was smaller during that period.

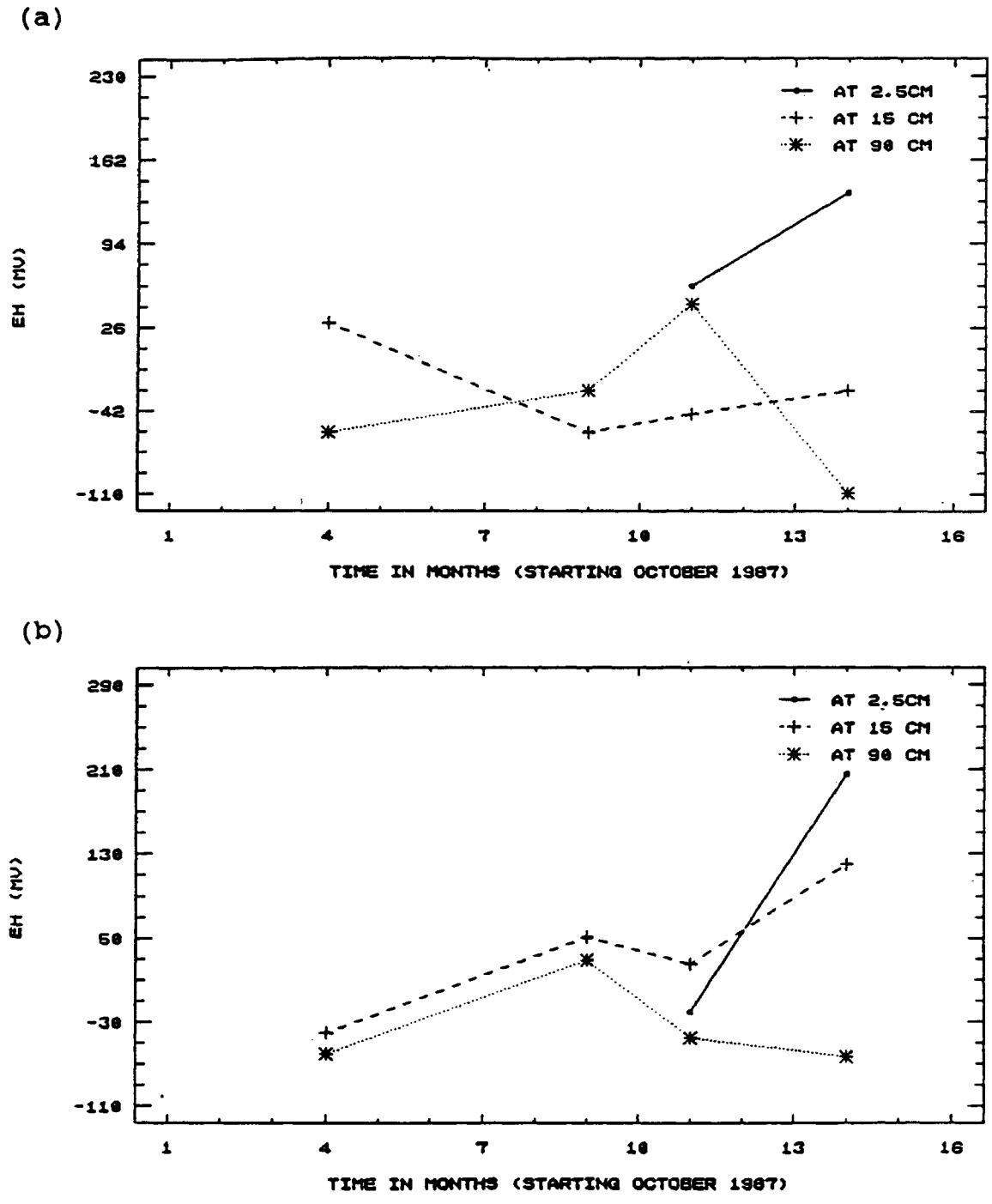
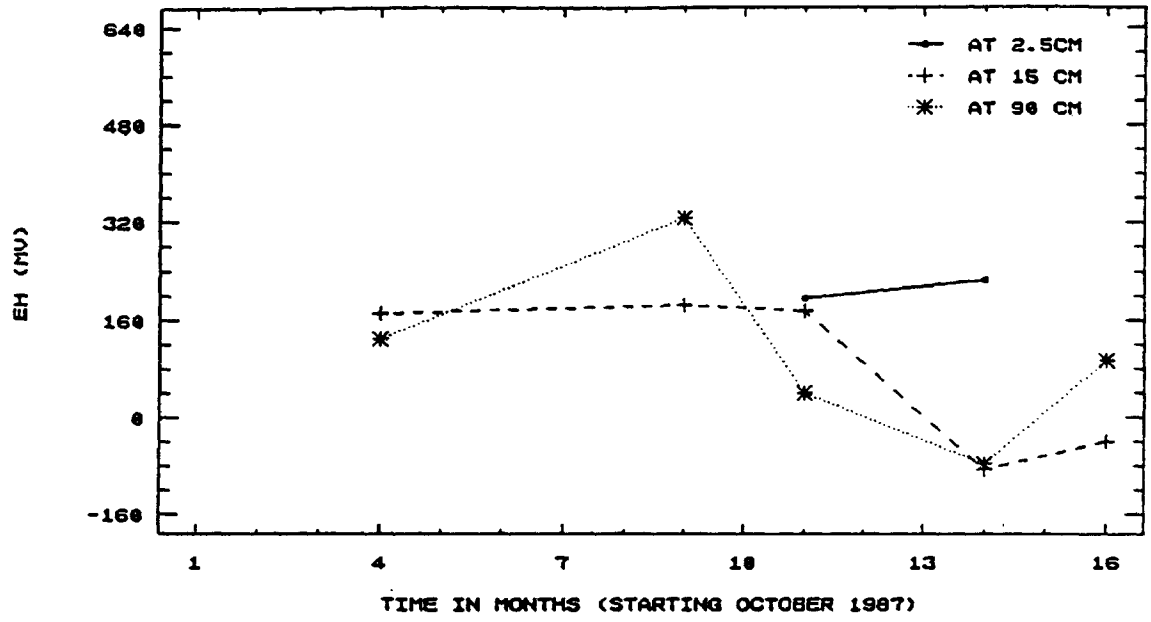


FIGURE 21. Redox Potential of Cell A Substrate, (a) Near the Inlet and (b) Near the Outlet.

(a)



(b)

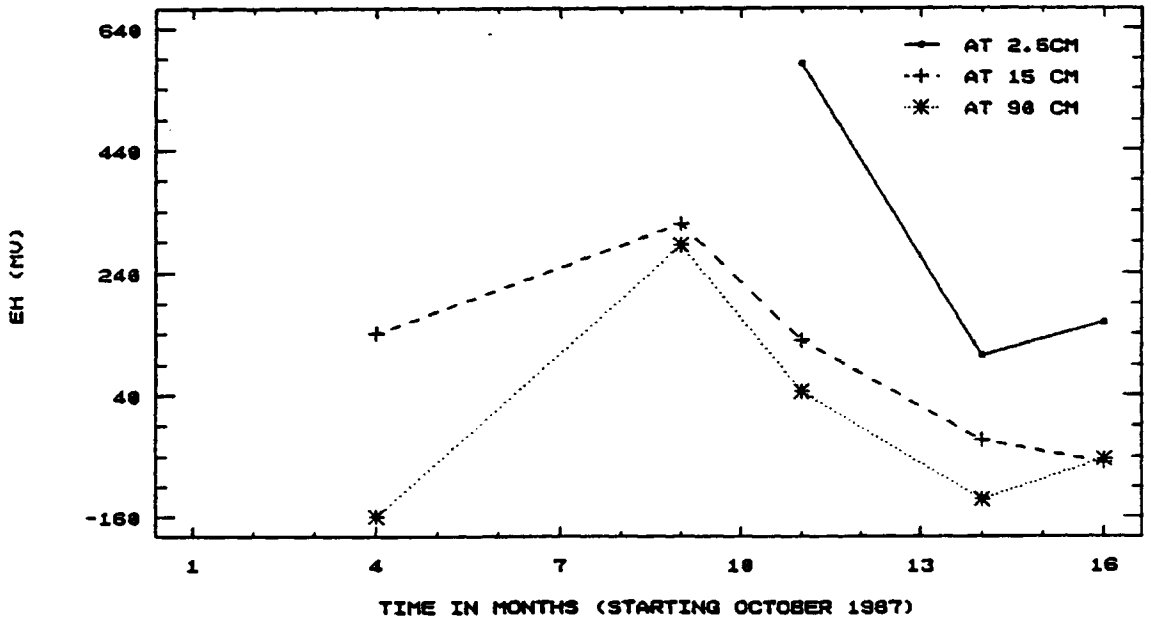
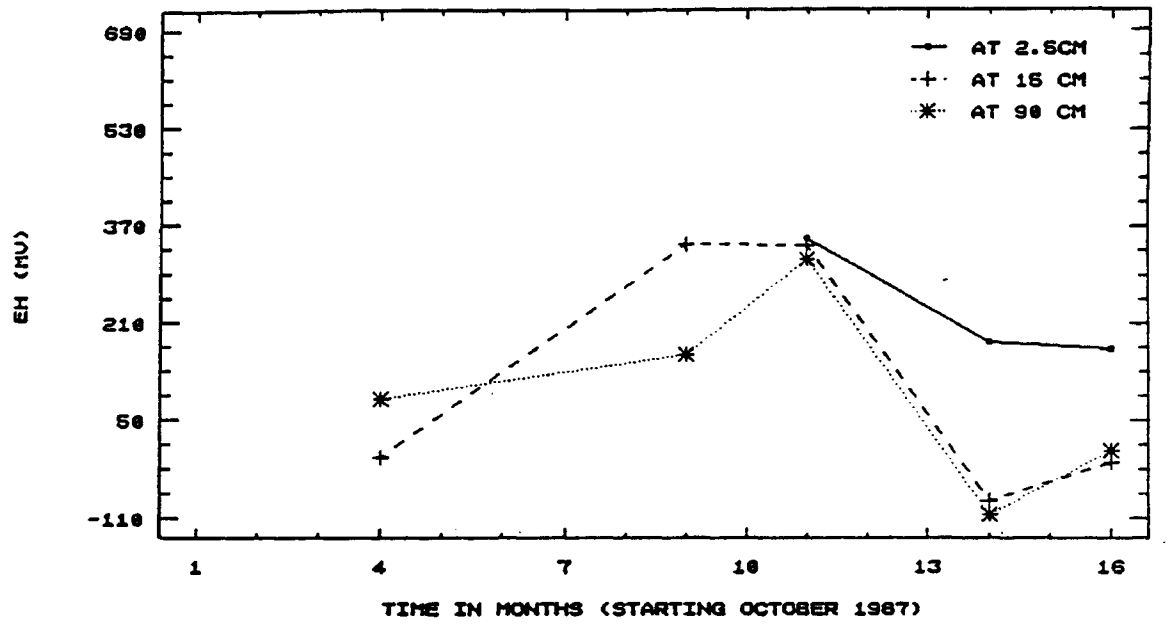


FIGURE 22. Redox Potential of Cell B Substrate, (a) Near the Inlet and (b) Near the Outlet.

(a)



(b)

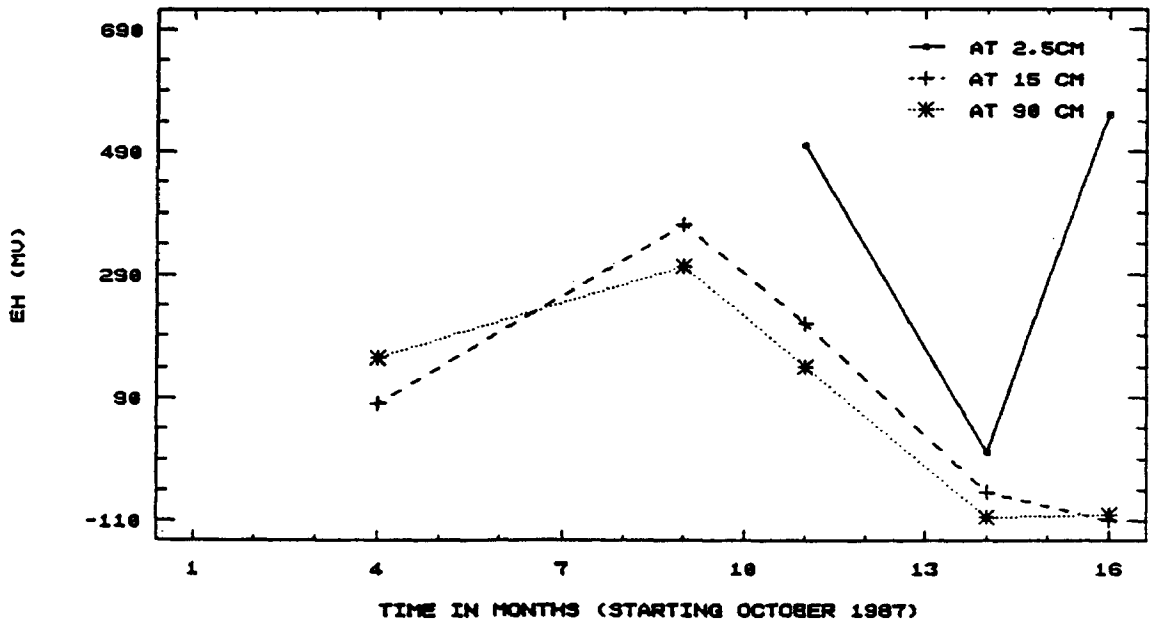


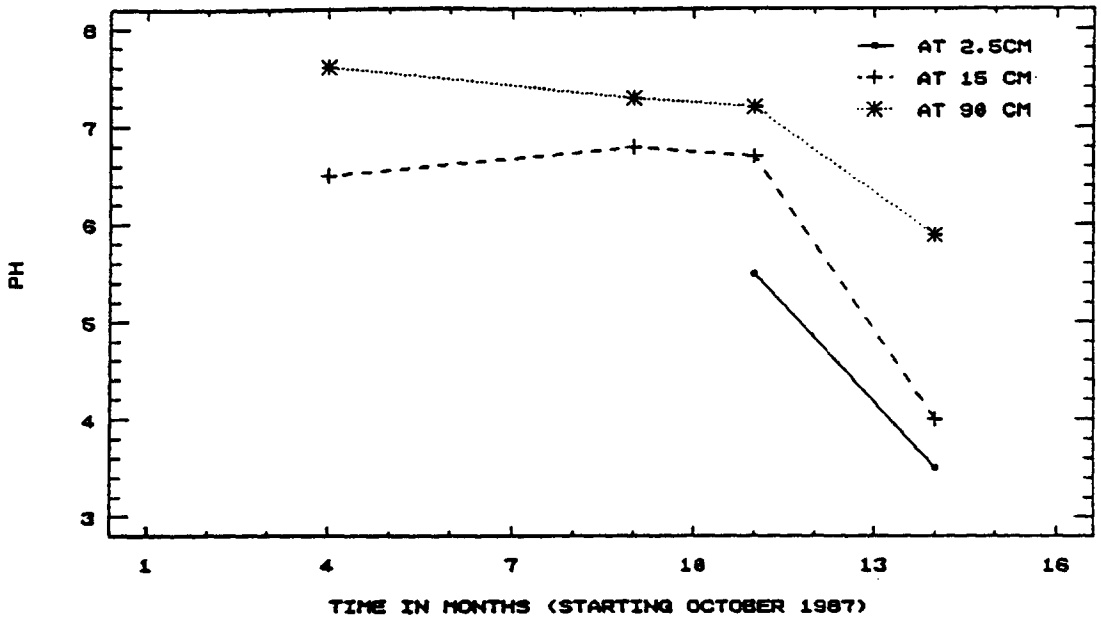
FIGURE 23. Redox Potential of Cell C Substrate, (a) Near the Inlet and (b) Near the Outlet.

1.3. pH of the Substrates

The pH of the substrate was near neutral at 15 and 90 cm and acidic at the surface. It was highest at 90 cm in all three cells (Figures 24, 25, 26). In cell A, pH decreased in November especially at 15 cm, accompanied by an increase in Eh (Figure 21) resulting in low SRB populations in the substrate (Figure 7). The pH in cells B and C did not show much variation except for a slight increase in cell C near the outlet (Figure 26). Correlation coefficients for SRB and pH were positive, therefore, SRB counts would be higher with high pH values. Iron-oxidizing bacteria require a pH of 2.5-3.5 (Table 6) and would not be active at higher values, and their correlation coefficients with pH were negative, as expected (Table 5). Populations of IOB at 15 and 90 cm were much smaller than at the surface, which is explainable by higher pH and lower Eh at these depths.

Heterotrophic iron- and manganese-oxidizing bacteria populations correlated negatively with pH which is a result of a different environmental factor, such as temperature, especially since HIOB and HMOB can grow in substrates at a pH between 3.5-7.8 (Table 6). Another explanation is that only

(a)



(b)

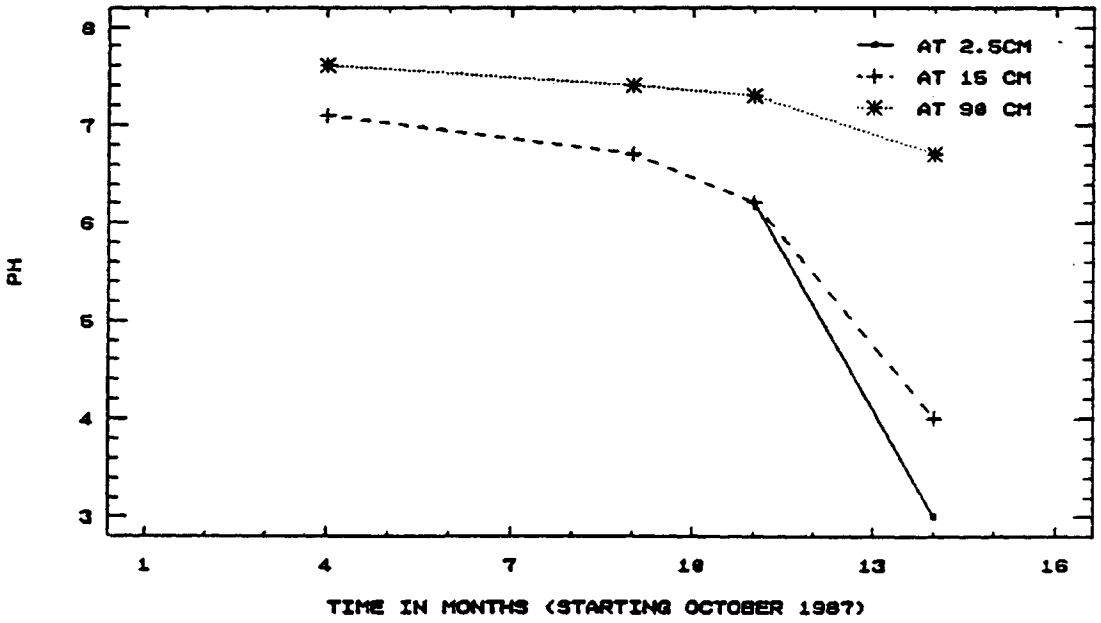
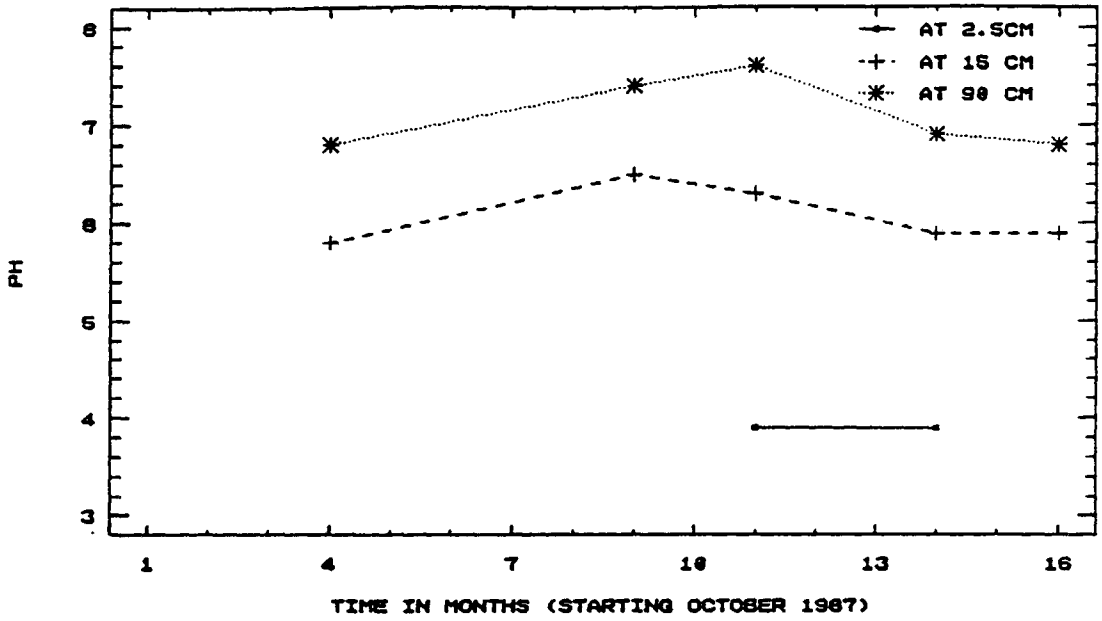


FIGURE 24. pH of Cell A Substrate, (a) Near the Inlet and (b) Near the Outlet.

(a)



(b)

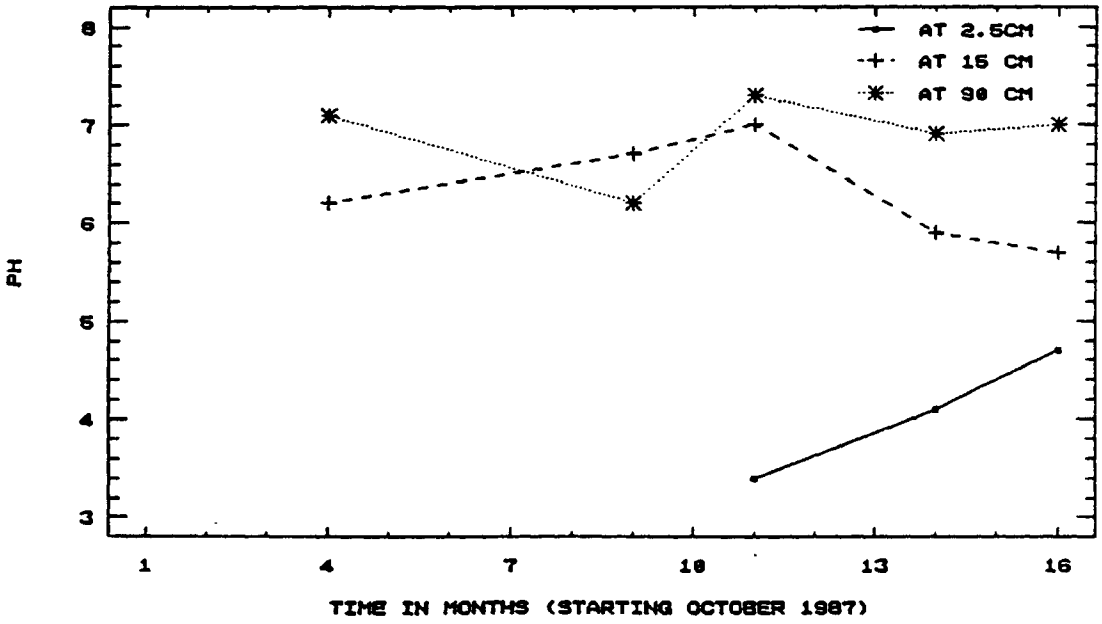
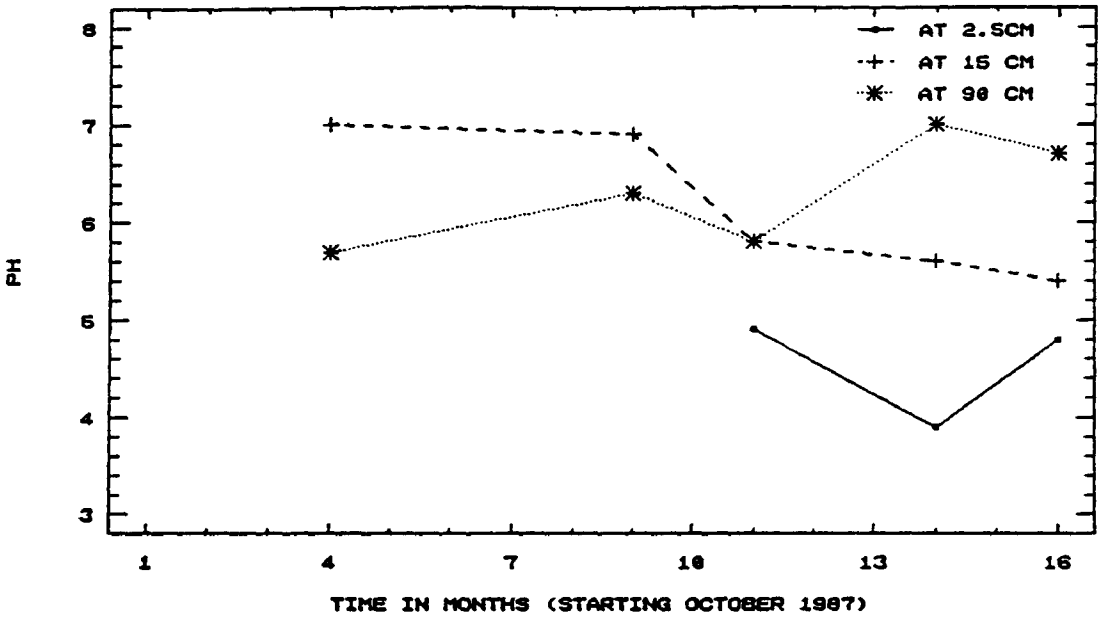


FIGURE 25. pH of Cell B Substrate, (a) Near the Inlet and (b) Near the Outlet.

(a)



(b)

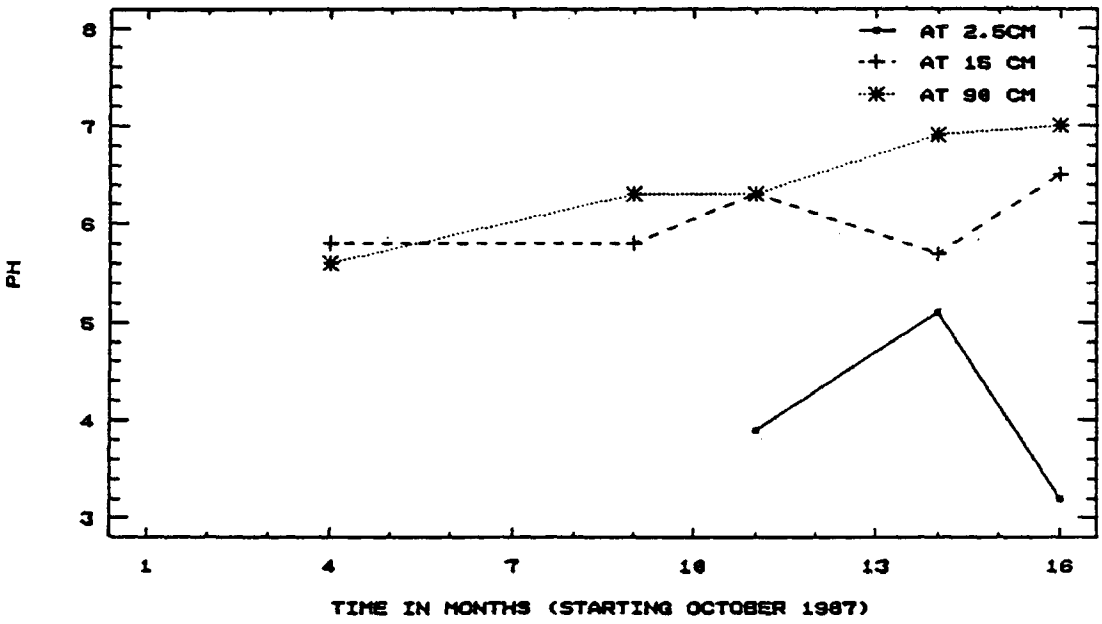


FIGURE 26. pH of Cell C Substrate, (a) Near the Inlet and (b) Near the Outlet.

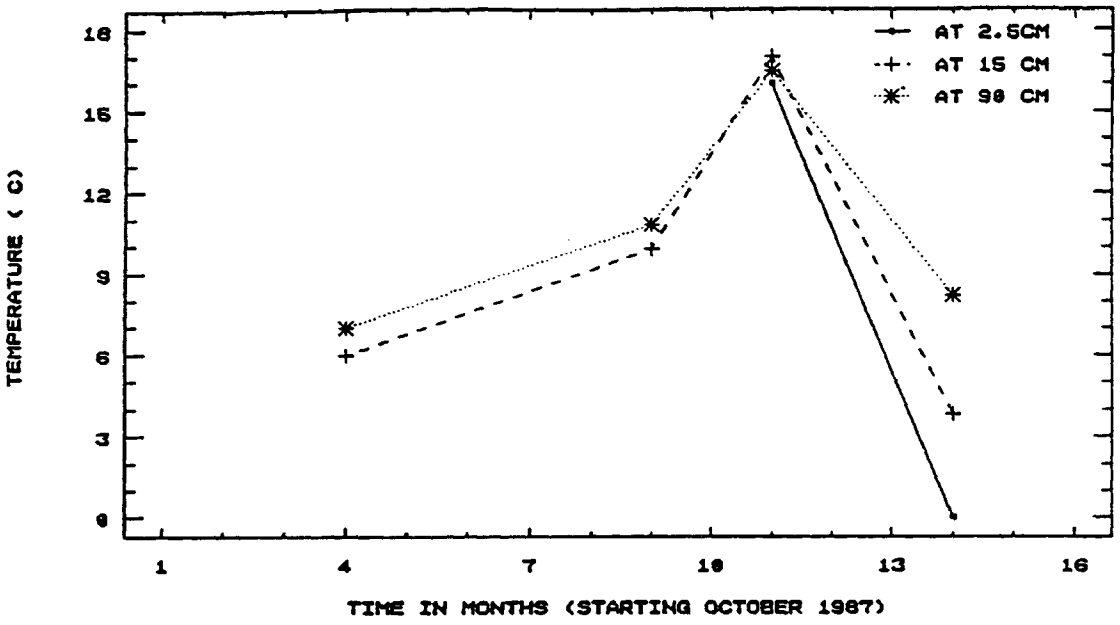
one of the HIOB and HMOB genera is present, such as Metallogenium which requires a pH of 3.5-5.0. Therefore, an increase in pH above 5.0 would cause lower bacterial counts.

1.4. Soil Temperatures

Temperatures of the soils were, as expected, low in the winter and high in the summer. The temperatures ranged between 0-17°C. Surface soils were a few degrees warmer in the summer or colder in the winter than at greater depths (Figures 27, 28, 29). Snow or ice was seen across the surface during some winter periods.

From the correlation analyses, populations of SRB and IOB seem to correlate negatively with temperature with the exception of cell C. This negative correlation is most probably not a direct one but a result of the effect of Eh, pH and nutrient concentrations on the bacteria. As temperature increased during the summer, the dormant plants started growing and removing nutrients from the substrate, injecting oxygen into the soil, and increasing Eh conditions. The temperature range under study is not very large and is still within the tolerance limits of SRB. Heterotrophic iron- and manganese-oxidizing bacteria showed a relatively high correlation with temperature as expressed by R^2 in Table 5. These

(a)



(b)

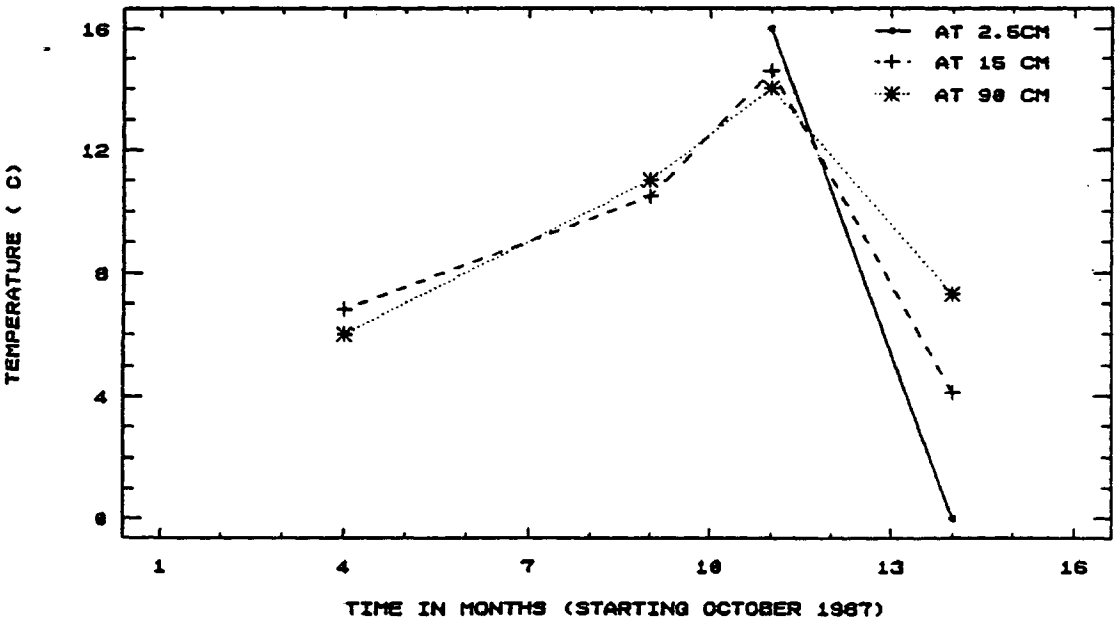
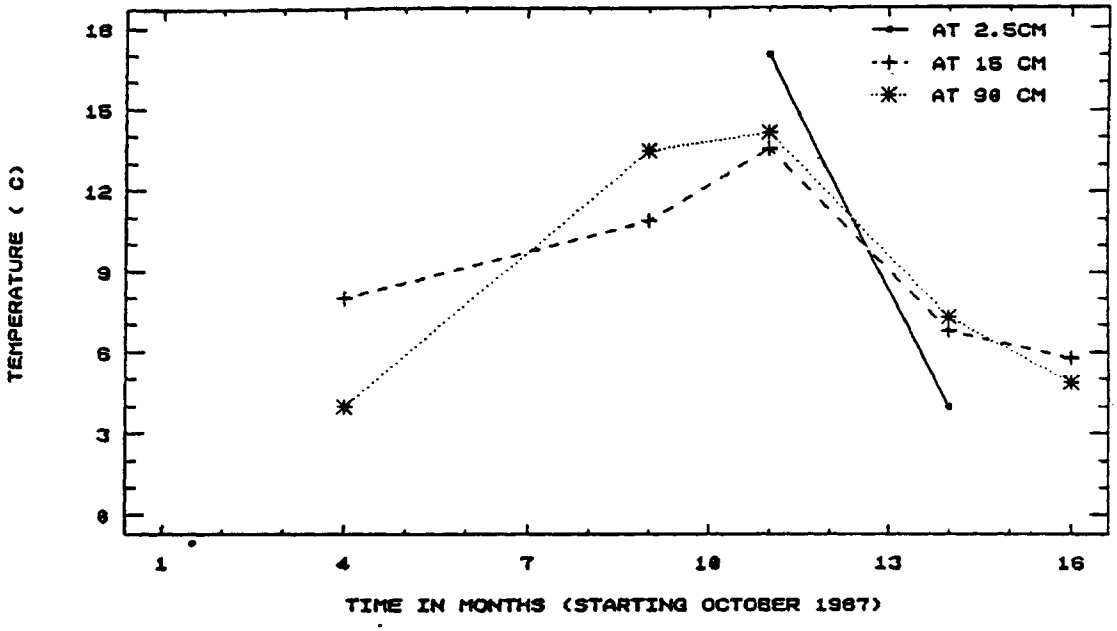


FIGURE 27. Temperature of Cell A Substrate, (a) Near the Inlet and (b) Near the Outlet.

(a)



(b)

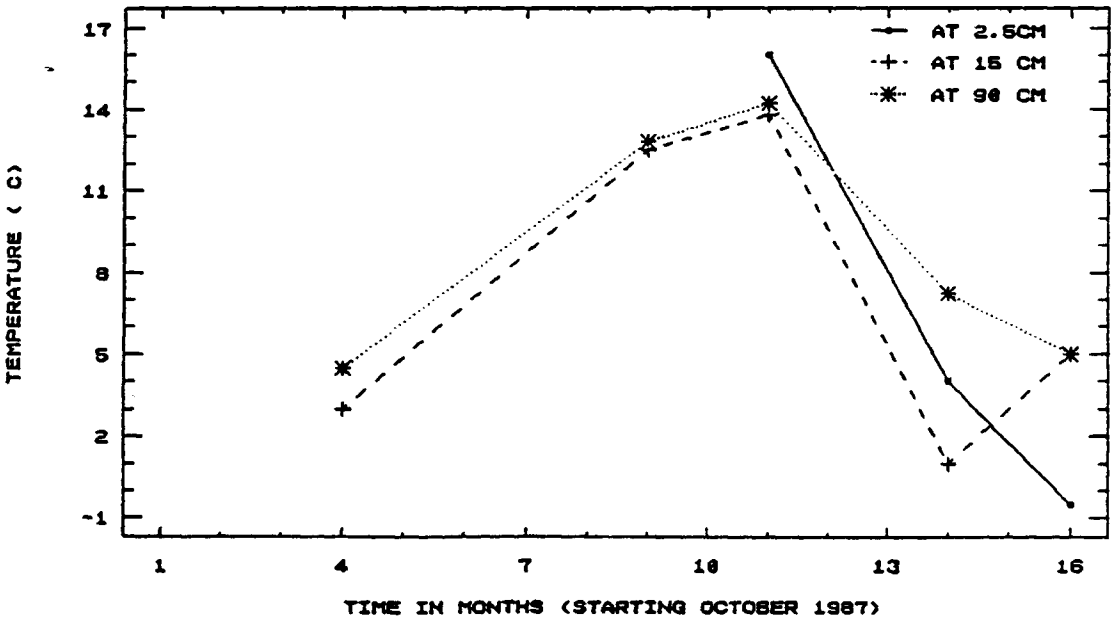
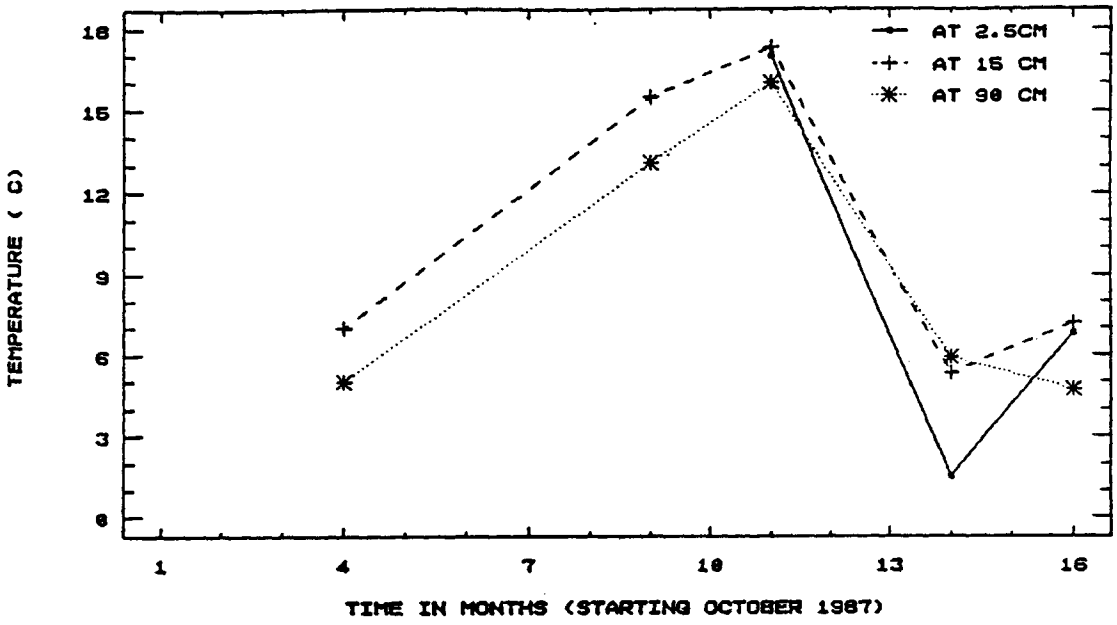


FIGURE 28. Temperature of Cell B Substrate, (a) Near the Inlet and (b) Near the Outlet.

(a)



(b)

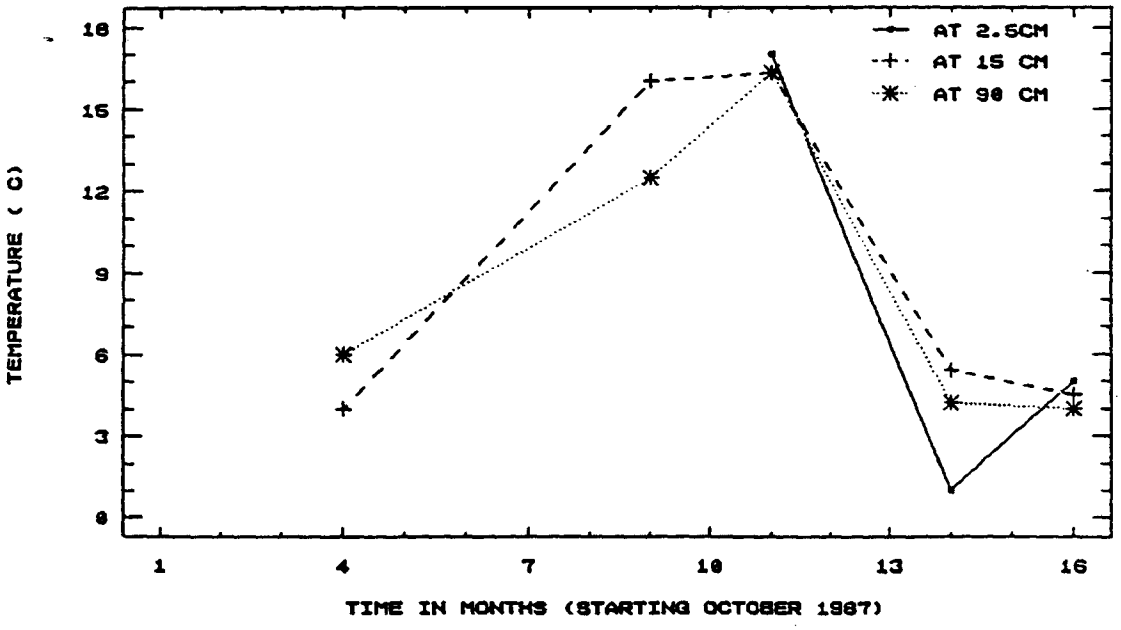


FIGURE 29. Temperature of Cell C Substrate, (a) Near the Inlet and (b) Near the Outlet.

bacteria require temperatures between 10-44°C and were therefore affected by cold conditions in the soil.

1.5. Summary

The decrease in SRB counts noticed in June in all three cells was most probably caused by an increase in the redox potential of the substrate even though the pH did not vary. Temperature seemed to have a negative effect on their populations which is contrary to findings in previous research. This interpretation is complicated by the multivariate changes in Eh, pH and nutrients along with the temperature changes.

In a study on saltmarsh sediments[83], photosynthetic activity of plants was found to be injecting oxygen into the sediment during spring and early summer, decreasing SO_4^{2-} reduction. In the autumn, sulfate reduction predominated. Iron sulfides continued to precipitate during the winter due to upward diffusion of Fe^{2+} and slow production of H_2S, even though oxidation and reduction rates were slow.

Thus, the effect of temperature variation on SRB populations is indirect. Changes in temperature affect redox potentials of soils, which increase or decrease SRB populations. High SO_4^{2-} concentrations were available to SRB at all depths

in the substrate and therefore could not have limited SRB growth.

Populations of IOB were smaller in the summer even though the Eh of the soils was optimal for their growth. However, pH was still in the neutral range which can kill Thiobacillus ferrooxidans. Higher temperatures of the summer did not seem to enhance growth and multiplication of these mesophilic bacteria at 15 and 90 cm depths, but increased their populations at 2.5 cm whenever pH and Eh conditions were favorable. Numbers of HIOB and HMOB correlated positively with Eh, but were not favored by higher pH even though different genera can exist under a wide pH range. Populations of HIOB and HMOB in cells A, B, and C, did not show an increase in November when Eh conditions were most favorable for their growth. This leads to the conclusion that temperature had the most influence on these bacterial populations.

High viable counts of SRB and IOB in January, 1988, two months after acid mine drainage flow in the system, even though the soil temperatures were low, would suggest that most of these bacteria were dormant or merely at sustaining levels. However, Eh and pH conditions were favorable for SRB and not for IOB. Assessment of the activity of the bacteria in the substrate is necessary before drawing further conclusions.

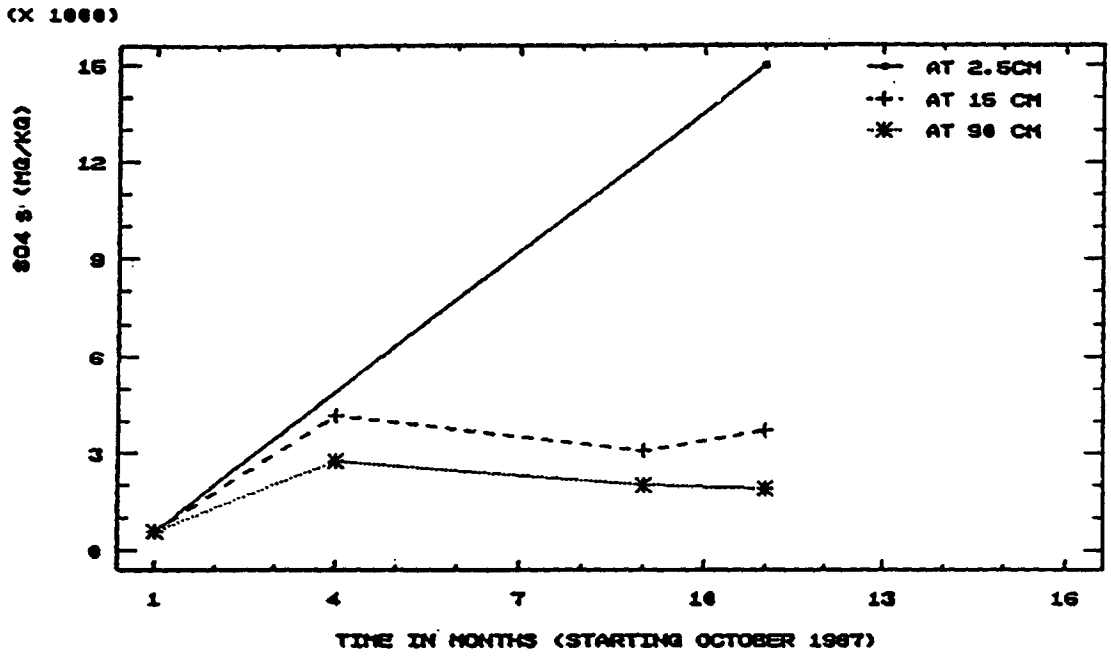
2. Effect of Growth and Multiplication of the Four Bacterial Groups on Their Environment

To determine the activity of the bacteria in the cell substrate, estimates of the in situ rates of iron and manganese oxidation and sulfate reduction are needed. Such data are only obtainable with the use of radioactive isotope tracers such as $Na_2{}^{35}SO_4$. In situ estimates of the rate of sulfate reduction have been obtained by injecting radioactive $Na_2{}^{35}SO_4$ into small samples of sediment and measuring the rate of formation of acid volatile ^{35}S as $H_2{}^{35}S$[76]. Use of radioactive $Na_2{}^{35}SO_4$ was not feasible in our study because the necessary instrumentation was not available. In the case of iron and manganese oxidation, the problem of determining rates of reaction is complicated by the presence of large amounts of colored humic substances which interfere in colorimetric methods for iron and manganese[109]. In addition, chelation of Fe^{3+} and Fe^{2+} by humic substances, and formation of complexes of bicarbonates and sulfates with manganese as well as ligands of the metal with organic compounds complicate the determination of the in situ rate of iron and manganese oxidation. Soil analysis conducted throughout the sampling period can aid in the indirect determination of bacterial activity.

2.1 Iron-Oxidizing Bacteria and Heterotrophic Iron Oxidizers

Summer Eh and pH conditions along with high sulfate concentrations at the surface of the soil (Appendix C and Figures 30, 31, 32) suggest that IOB and HIOB were actively producing sulfate and releasing Fe^{2+} into solution. Ferrous ions would transform, uncatalyzed, into an insoluble ferric sulfate such as jarosite $[HFe_3(SO_4)_2(OH)_6]$[36], ferric hydroxide, or ferric oxyhydroxide. An increase of sulfate in the substrate could be a result of formation of insoluble sulfate salts. Ferrous ions increase cannot be relied upon as an indication of bacterial activity. Sulfate salts of most metals are water soluble, while sulfate salts of Ca, Ba, and Sr tend to be insoluble in water[42]. Analysis of initial substrates demonstrated the presence of high concentrations of Ca (12,850 mg/kg in the compost substrate, and 16,300 mg/kg in the peat, manure and wood products substrate), which would be precipitated and immobilized by sulfate as gypsum ($CaSO_4.H_2O$). This finding was suspected before the chemical analysis was conducted, when white crystals started forming at the surface of the soil and around the base of plants. These crystals were probably gypsum crystals since Ba concentrations were orders of magnitude lower than Ca.

(a)



(b)

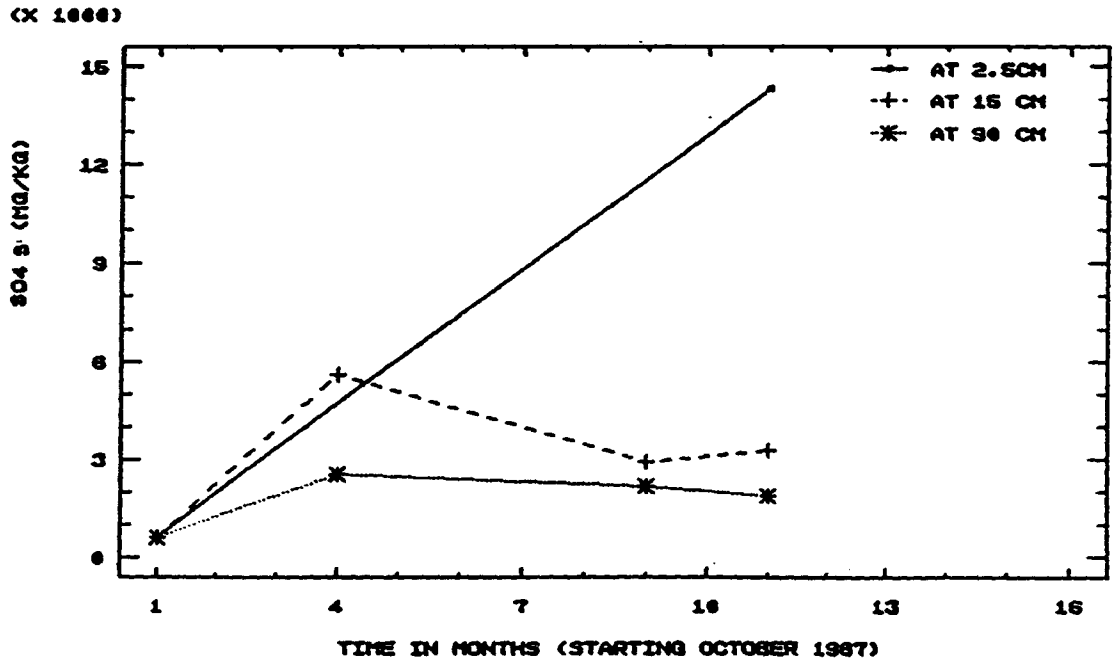


FIGURE 30. Sulfate Sulfur Concentrations in Cell A Substrate, (a) Near the Inlet and (b) Near the Outlet.

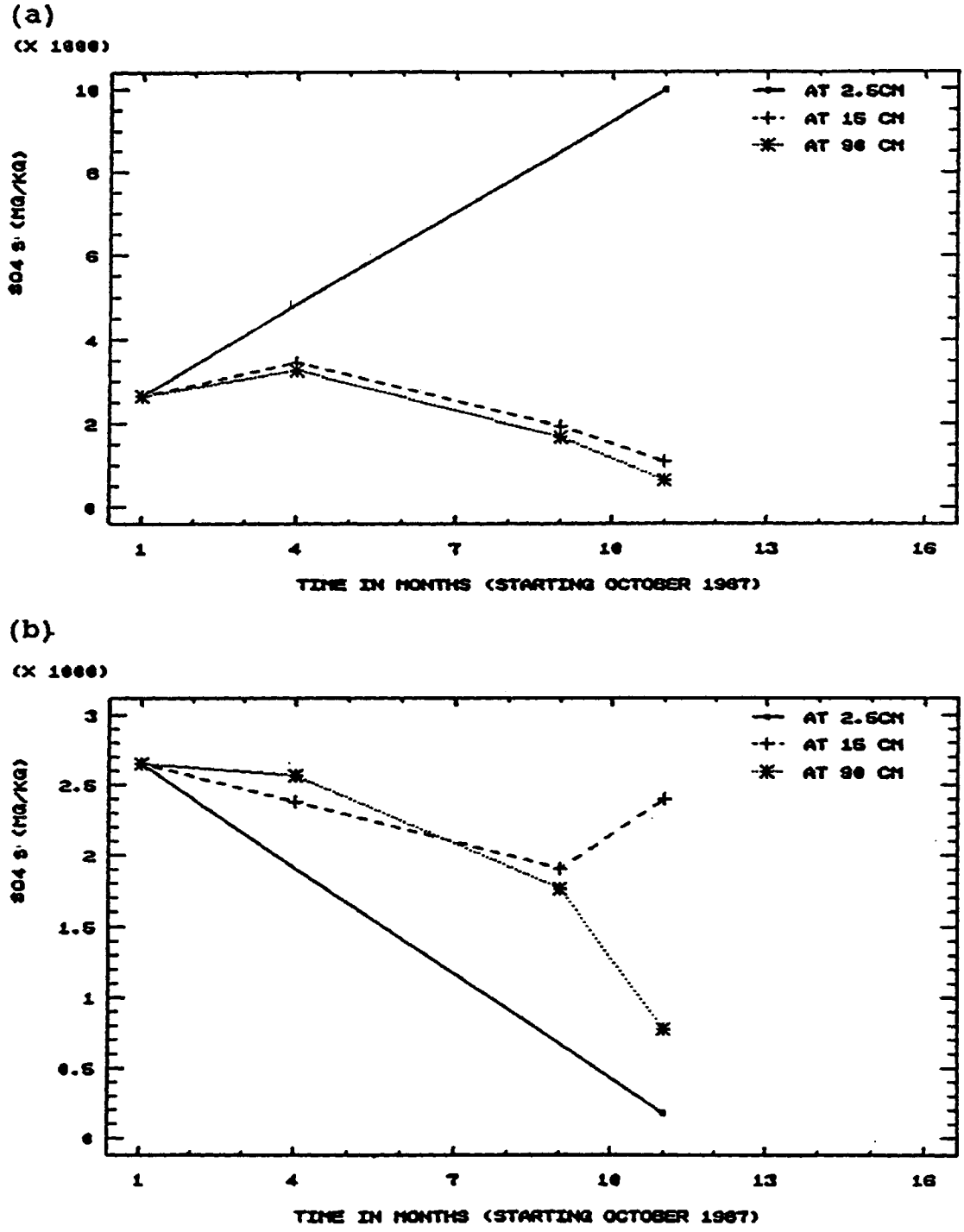


FIGURE 31. Sulfate Sulfur Concentrations in Cell B Substrate, (a) Near the Inlet and (b) Near the Outlet.

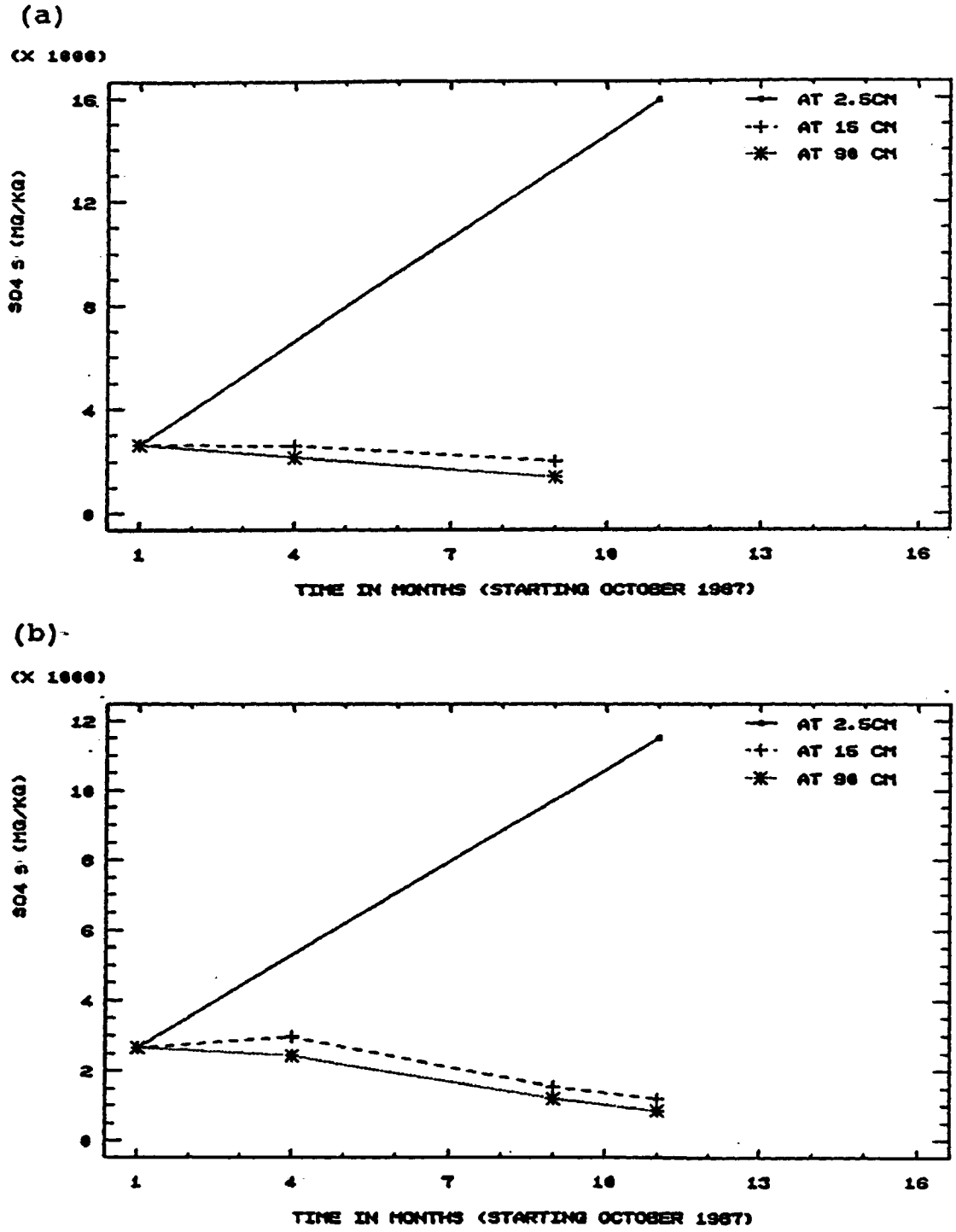


FIGURE 32. Sulfate Sulfur Concentrations in Cell C Substrate, (a) Near the Inlet and (b) Near the Outlet.

Metal concentrations, represented graphically in Appendix D (for Al, Fe, Cu, Pb, Mn, Zn) were very high at the surface of the substrate. Percent increase of these metals over the sampling period from October, 1987 (initial) through August, 1988 were calculated. Percent increase of total iron in the surface soils is greater than at the 15 and 90 cm soil layer (Tables 7, 8, 9). Iron could be present in many different forms, such as chelated iron, or pyrite. More detail is reported in Cohen et. al[110]. The percent increase of pyrite (Tables 10, 11, 12) in the surface substrate was not different from that at depth and, in addition, it did not account for the increase in iron concentration in that soil layer (e.g. iron increased 1,278 percent in cell A near the inlet while pyrite only increased 631 percent). Tables 7, 8, and 9 show that iron was being accumulated in cell A substrate at a higher rate than that in B and C, and concentrations were, in general, larger near the inlet of the three cells. Thiobacillus ferrooxidans capable of oxidizing Fe^{2+}, as well as the heterotrophic iron oxidizers, were probably actively precipitating iron on the surface of the wetland.

The decrease in Thiobacillus ferrooxidans population during the month of June, 1988, was not associated with lower

TABLE 7.
PERCENT INCREASE OF METAL CONCENTRATIONS IN SUBSTRATES OF CELLS A, B, AND C AT 2.5 CM FROM THE SURFACE (NEAR THE INLETS AND OUTLETS), AUGUST, 1988

	Al	Fe	Cu	Pb	Mn	Zn
INLET						
Cell A	135.94	1277.99	1334.00	145.00	32.44	2510.62
Cell B	706.96	617.65	822.58	100.00	108.91	589.76
Cell C	72.60	1185.07	767.74	100.00	231.68	545.67
OUTLET						
Cell A	174.65	277.99	2500.00	325.00	530.03	6546.02
Cell B	50.04	203.17	748.39	118.75	67.82	536.22
Cell C	81.68	803.17	570.97	93.75	103.96	100.79

TABLE 8.
PERCENT INCREASE OF METAL CONCENTRATIONS IN SUBSTRATES OF CELLS A, B, AND C AT 15 CM FROM THE SURFACE (NEAR THE INLETS AND OUTLETS)
NOVEMBER, 1988

	Al	Fe	Cu	Pb	Mn	Zn
INLET						
Cell A	82.49	93.30	638.00	370.50	55.23	3572.57
Cell B	7.19	68.32	83.22	112.50	161.39	156.35
Cell C	30.96	90.04	120.64	48.75	103.46	167.72
OUTLET						
Cell A	81.29	117.22	1008.00	495.00	86.06	4824.78
Cell B	-1.29	14.93	235.48	154.37	150.00	507.87
Cell C	52.91	82.80	200.97	191.25	195.05	470.08

TABLE 9.
PERCENT INCREASE OF METAL CONTENTS IN SUBSTRATES OF CELLS A, B, AND C AT 90 CM FRM THE SURFACE (NEAR THE INLETS AND OUTLETS)
NOVEMBER, 1988

	Al	Fe	Cu	Pb	Mn	Zn
INLET						
Cell A	14.10	58.85	89.20	115.50	259.25	423.89
Cell B	4.01	52.94	122.90	48.12	40.10	100.79
Cell C	12.79	12.67	48.39	36.87	22.28	16.93
OUTLET						
Cell A	28.29	65.55	100.00	93.00	87.13	401.77
Cell B	2.35	9.50	93.87	61.25	69.31	-21.26
Cell C	13.55	18.55	44.19	54.37	13.86	55.12

TABLE 10
PERCENT INCREASE IN CONCENTRATIONS OF
THREE SULFUR FORMS IN SUBSTRATES OF
CELLS A, B, AND C AT 2.5 CM FROM
THE SURFACE (NEAR THE INLETS
AND OUTLETS) AUGUST, 1988

	TOTAL S	PYRITIC S	ORGANIC S
INLET			
CELL A	493.78	630.77	48.15
CELL B	184.68	219.39	-3.23
CELL C	336.99	36.88	312.90
OUTLET			
CELL A	993.15	207.69	766.67
CELL B	37.80	59.70	241.94
CELL C	201.00	-39.16	177.42

TABLE 11
PERCENT INCREASE IN CONCENTRATIONS OF THESE SULFUR FORMS IN SUBSTRATES OF CELLS A, B, AND C AT 15 CM FROM THE SURFACE (NEAR THE INLETS AND OUTLETS) AUGUST, 1988 (EXCEPT AS SPECIFIED)

	TOTAL S	PYRITIC S	ORGANIC S
INLET			
CELL A	378.66**	323.08	259.26
CELL B	49.70***	14.07	48.39*
CELL C	-87.58*	74.90	158.06*
OUTLET			
CELL A	506.87**	361.54	151.85
CELL B	358.26***	59.70	312.90
CELL C	68.03***	227.00	138.71

* June, 1988
** November, 1988
*** January, 1989

TABLE 12
PERCENT INCREASE IN CONCENTRATIONS OF
THREE SULFUR FORMS IN SUBSTRATES OF
CELLS A, B, AND C AT 90 CM FROM
THE SURFACE (NEAR THE INLETS
AND OUTLETS) AUGUST, 1988
(EXCEPT AS SPECIFIED)

	TOTAL S	PYRITIC S	ORGANIC S
INLET			
CELL A	258.66**	515.38	140.74
CELL B	9.25***	52.09	151.61
CELL C	15.39***	-1.14*	93.55*
OUTLET			
CELL A	107.16**	361.54	85.19
CELL B	8.38***	29.28	164.52
CELL C	36.41***	21.67	312.90

* June, 1988
** November, 1988
*** January, 1989

iron concentrations, suggesting that smaller bacterial population did not translate into lower rates of activity, but into more active IOB.

Analysis of the output waters from each cell, compared to the mine drainage over the sampling period, showed that Fe is being removed from the AMD, however, Mn is not removed[111].

2.2 Heterotrophic Manganese-Oxidizing Bacteria

Heterotrophic manganese-oxidizers are expected to be active in the soil as suggested by the environmental conditions. However, manganese was not being removed and was essentially the same in the output waters as in the mine drainage[111]. A grey precipitate along the surface of cell A was sometimes observed, which might have been a manganese hydroxide precipitate, and correlated with variations in the manganese concentration. Results of the soil analyses indicated an accumulation of Mn in the wetland, at the surface and at 15 cm (Appendix C and D). At 15 cm depth, a large number of bacteria can utilize organic complexes with manganese, releasing Mn(II) into solution, which can be oxidized by HMOB. At the surface of the substrate, bicarbonates and sulfates can form complexes with manganese that are more resistant to oxidation than free manganese[45].

Mn^{2+} also sorbs onto $Fe(OH)_3$. Previous research determined that upon acidification of precipitated iron and manganese, Mn^{2+} but not Fe^{2+} was released into solution[31]. Additional interference of high iron concentrations at the surface of the wetland can result from the oxidation of Fe^{2+} to Fe^{3+} by MnO_2 formed by HMOB and the subsequent release of Mn^{2+}[40].

Biochemical reduction of MnO_2 may be catalyzed by Thiobacillus ferrooxidans[40] (present in high counts at 2.5 cm):

$$3MnO_2 + 4H^+ + S \quad 3\ Mn^{2+} + SO_4^{2-} + 2H_2O.$$

Furthermore, rapid water flow observed in the cells, directly across the surface, carrying Mn^{2+} ion present in the AMD as well as Mn^{2+} released into solution by the above-mentioned mechanisms, contributed to the volume of the output waters and their manganese concentrations. Thus, the fact that manganese was not removed from the wetland does not necessarily mean that HMOB were not active. Iron and manganese were also found to be accumulating at 90 cm from the surface (Table 9). At this depth, microbial oxidation of these metals is not likely to occur. Ferrous ions and Mn^{2+} are most probably reacting with H_2S produced by SRB.

2.3 Sulfate-Reducing Bacteria

Based on current literature, it is believed that the most active bacteria in metal removal are the sulfate-reducing bacteria in the anaerobic zone of a wetland.

In an attempt to estimate the activity of SRB in the substrate, thus their ability to treat AMD, soil samples were analyzed for sulfur forms and metal concentrations.

The substrates were rich in organic matter, which implies that cellulolytic organisms could proliferate, producing organic nutrients essential for bacterial growth. Furthermore, another group of bacteria utilizing, but not completely degrading organic matter such as cellulose, might be active, raising the pH, resulting in near neutral or alkaline conditions[71]. Sulfate-reducing bacteria, incapable of tolerating pH <4, may then establish themselves. It was also determined that the compost alone, when mixed with Big Five tunnel water, raised the pH to a level where SRB grow[109].

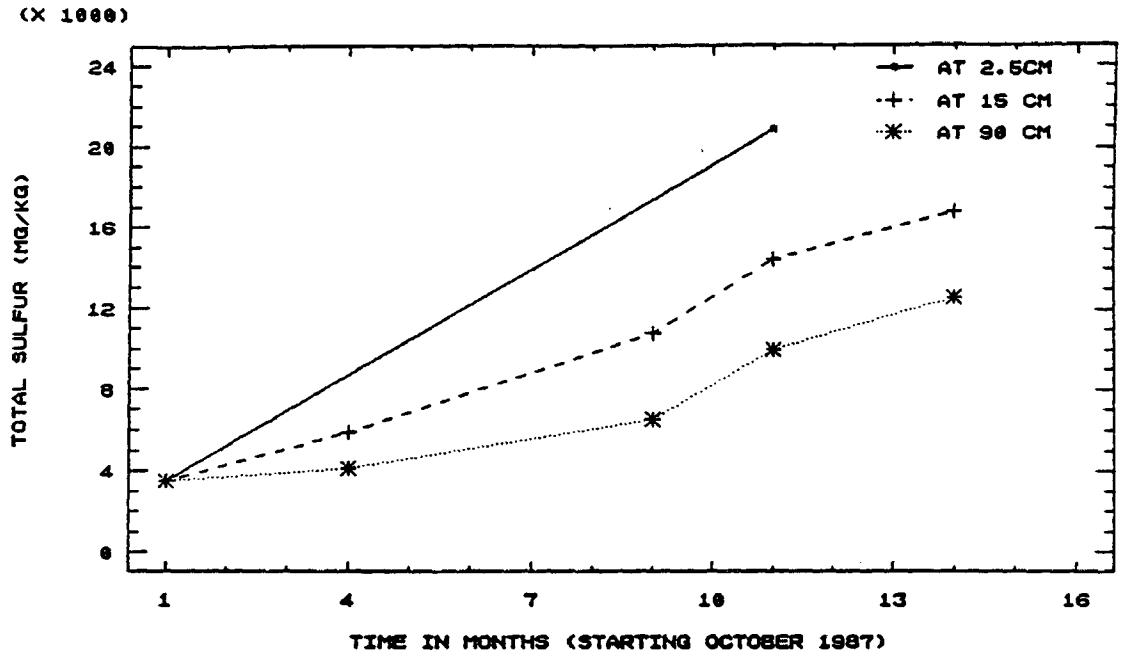
Sulfate-reducing bacteria have been reported to utilize a wide range of carbon sources and not restricted to lactate and pyruvate[58]. They can also utilize molecular hydrogen as an energy source, in the presence of SO_4^{2-}. Nutrient concentrations and SO_4^{2-} were available to the SRB. Soil and

environmental conditions suggest that SRB are actively reducing sulfate and removing metals from the AMD.

Total sulfur concentrations in the substrates were, in general, higher in cell A and exhibited an increase over the sampling period, whereas concentrations in cells B and C were not much different over the same time period (Figures 33, 34, 35 and Tables 10, 11, 12). Some increase in total sulfur was noted in cells B and C in samples collected near the outlets which correlates with SRB populations.

During the summer, data show that sulfur accumulated at the surface of the substrate of all three cells, mainly caused by accumulation of SO_4^{2-} (Figures 30, 31, 32). Sulfur-oxidizing bacteria, such as thiobacilli, were possibly active at this layer, where oxygen was being injected by plants, increasing the redox potential. Low pH at this depth (2.5 cm) was also an indication of the presence of sulfur oxidizers. Percent increase of pyritic sulfur at the surface (Table 10) was very high, especially in cell A. This implies that SRB in that soil layer were active and produced H_2S. Hydrogen sulfide then reacts with Fe^{2+} present in the mine water or released into solution by iron-reducing bacteria found in the aerobic-anaerobic interface[112]. Iron and sulfur oxidizers could be recycling pyrite by oxidizing both the iron and

(a)



(b)

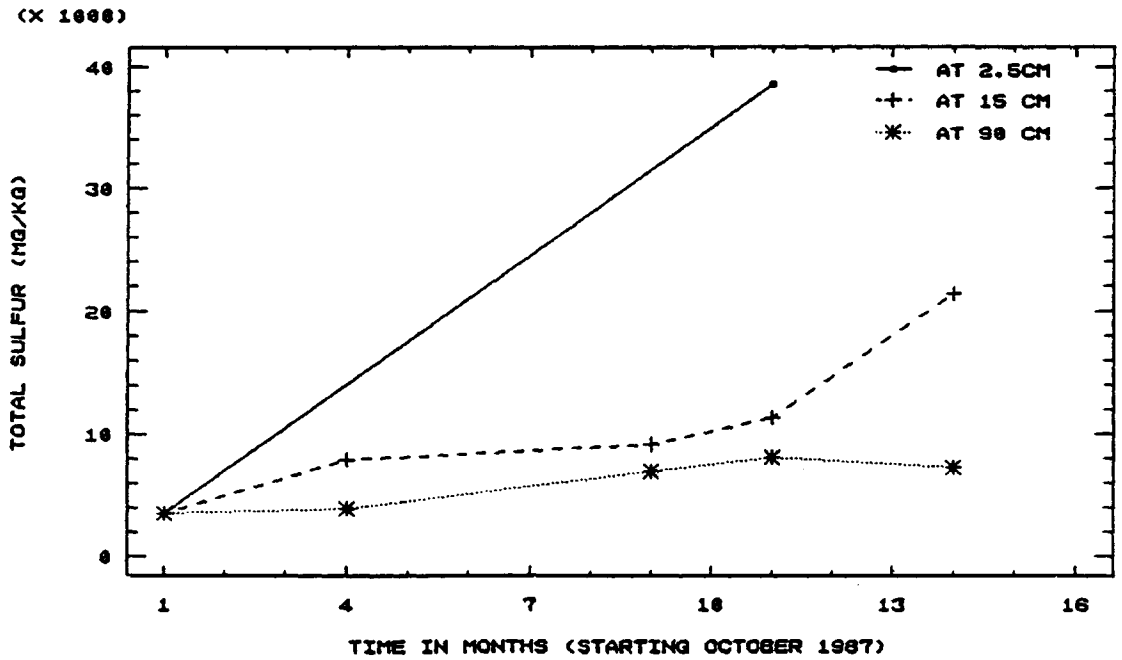


FIGURE 33. Total Sulfur Concentrations in Cell A Substrate, (a) Near the Inlet and (b) Near the Outlet.

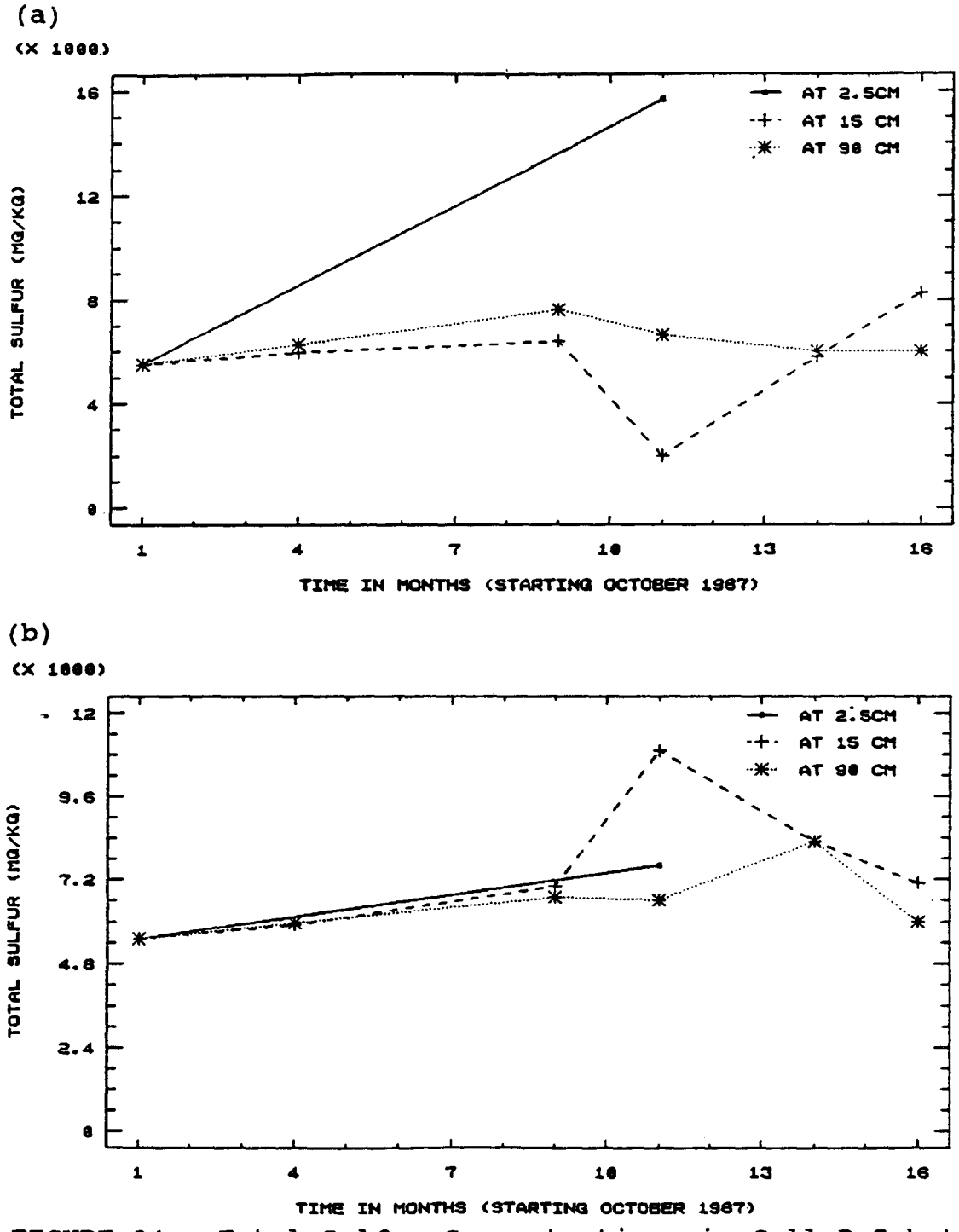
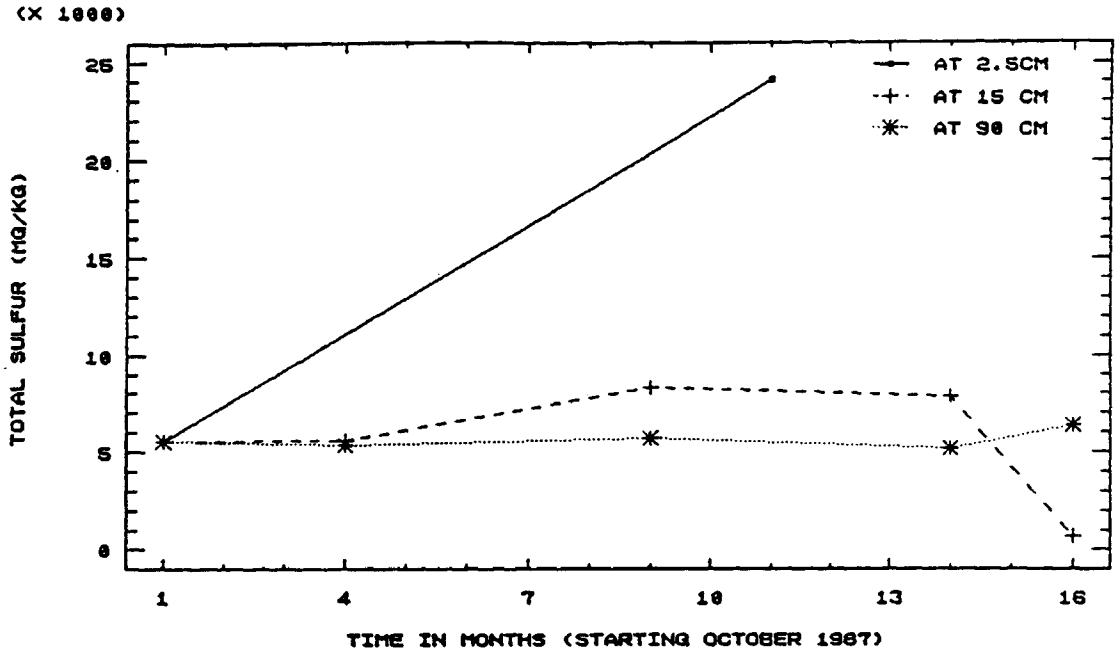


FIGURE 34. Total Sulfur Concentrations in Cell B Substrate, (a) Near the Inlet and (b) Near the Outlet.

(a)



(b)

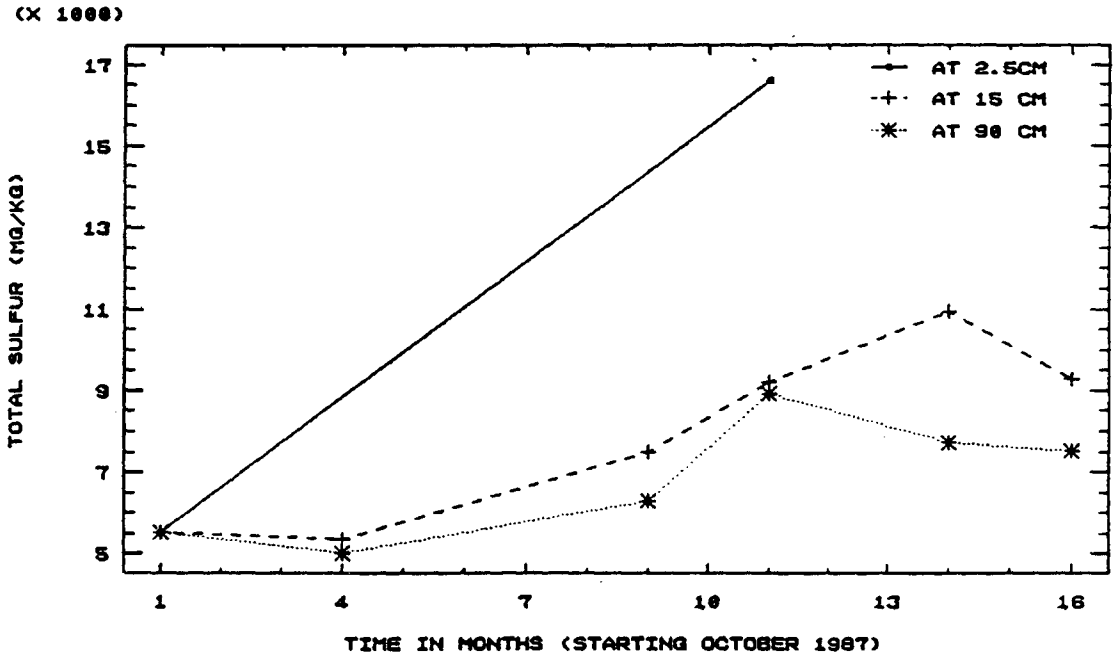


FIGURE 35. Total Sulfur Concentrations in Cell C Substrate, (a) Near the Inlet and (b) Near the Outlet.

sulfur moiety[113]. Sulfate-reducing bacteria populations in January, 1988 were active as suggested by results of pyritic sulfur analysis (Figures 36, 37, 38). Pyritic sulfur concentrations were high in January and showed a decrease in June. The same pattern was observed with SRB populations. This led to the conclusion that SRB activity may have been inhibited during that period. Causes of this smaller size of SRB viable counts were explained earlier in this discussion. Pyritic sulfur concentrations (Figure 36) suggested that SRB were most active in cell A compost.

Tables 7, 8 and 9 show that metals were accumulated in the substrates. At the surface, metal oxides were possibly formed. At 15 and 90 cm, metal sulfides are more likely to be found. Percent increase of metals, especially Cu and Zn, is highest in cell A substrate. Aluminum increased at the lowest rate in all three cells, but cell A was still better at removing it from the mine drainage.

Although the comparison of soil analyses with bacterial populations is not a direct measure of the activity of these bacteria, it gives us a general idea about their ecology in the constructed wetland.

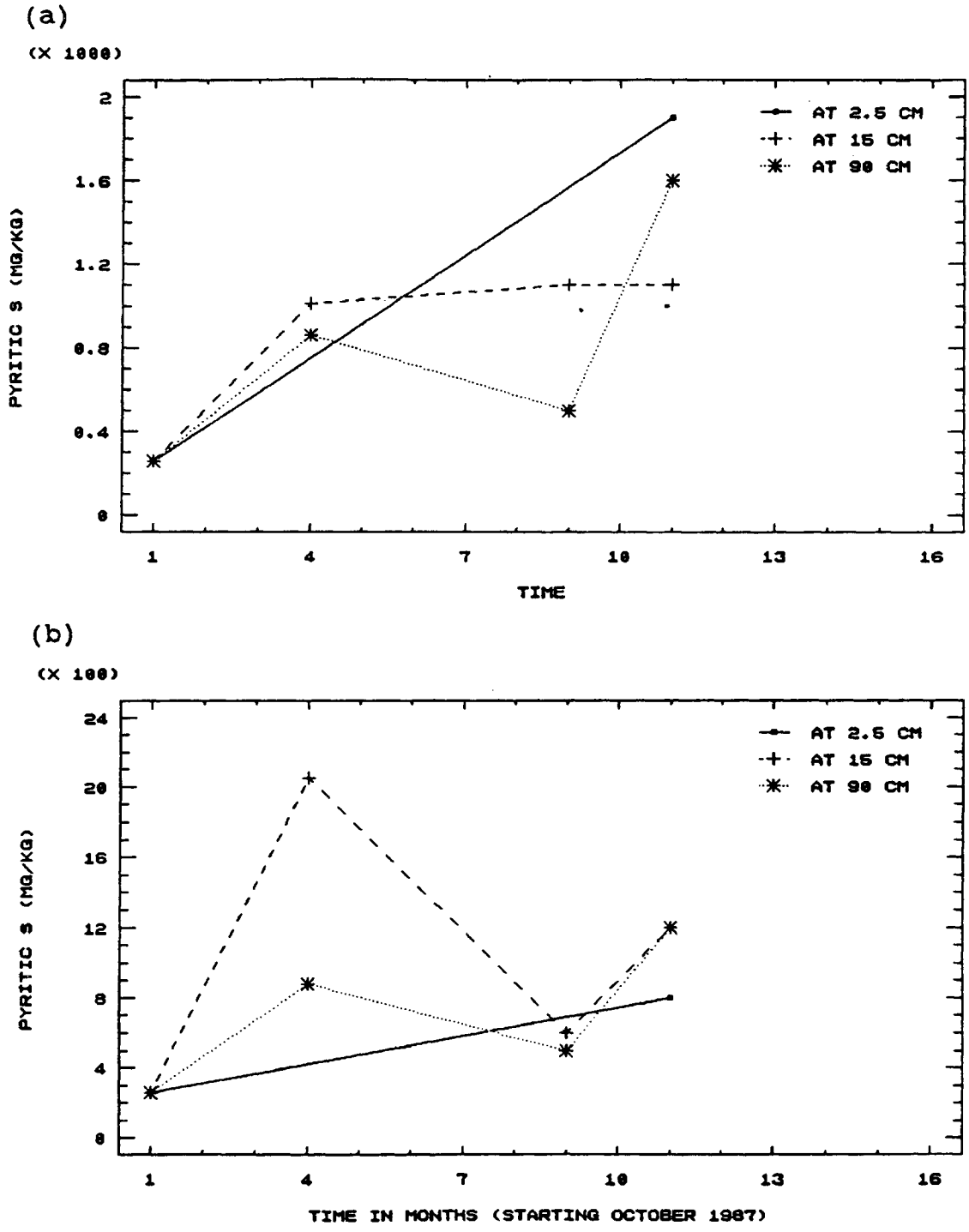
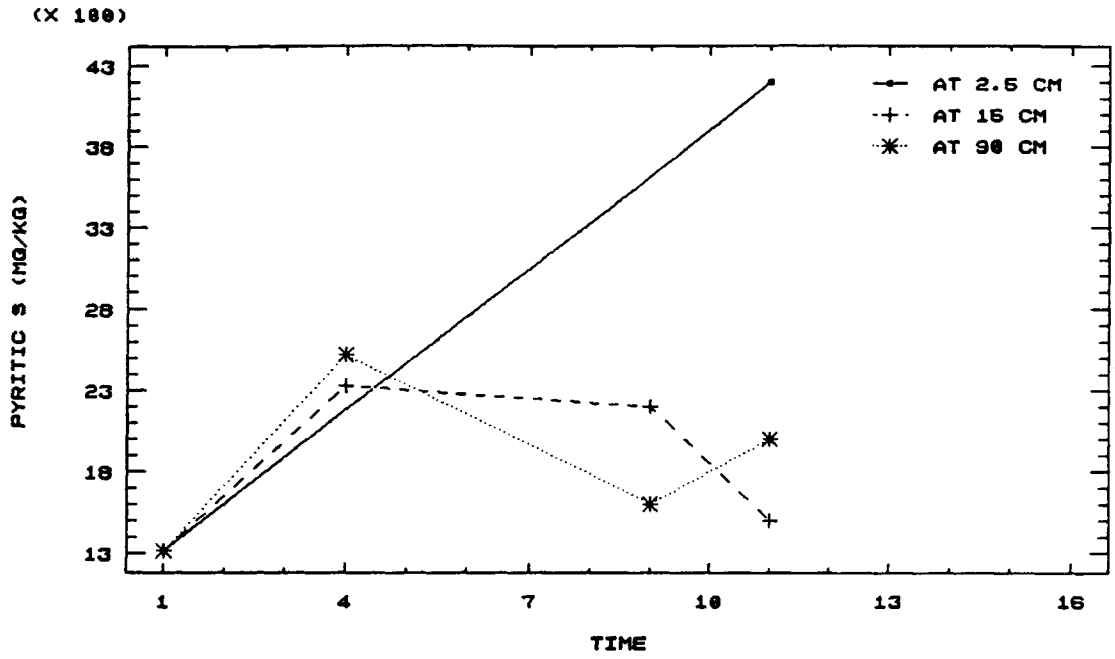


FIGURE 36. Pyritic Sulfur Concentrations in Cell A Substrate, (a) Near the Inlet and (b) Near the Outlet.

(a)



(b)

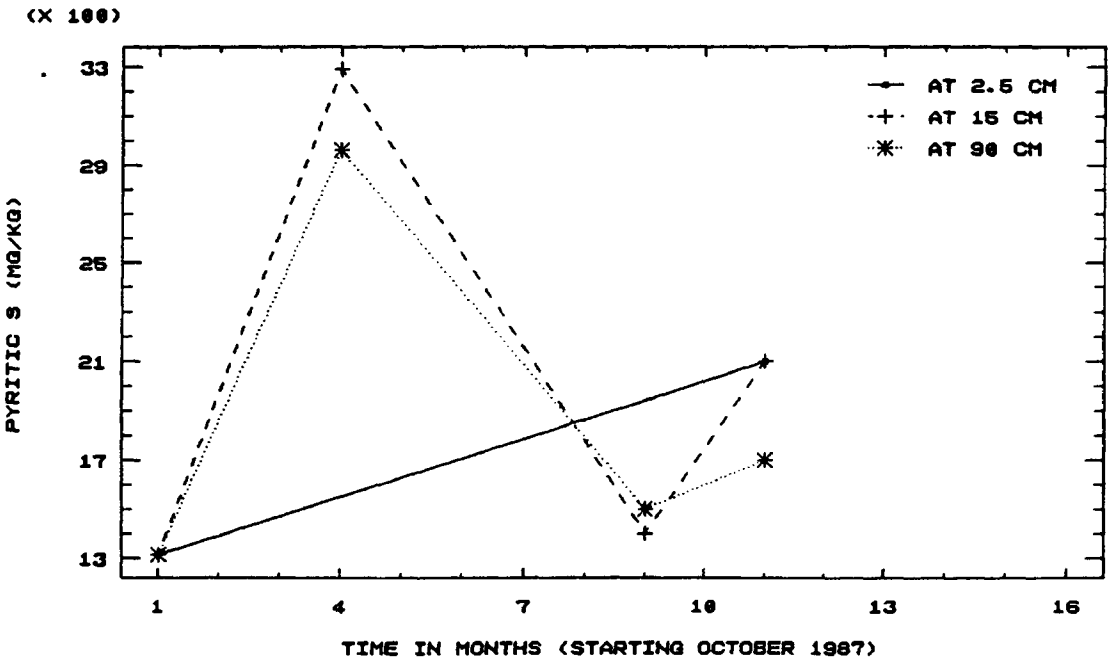


FIGURE 37. Pyritic Sulfur Concentrations in Cell B Substrate, (a) Near the Inlet and (b) Near the Outlet.

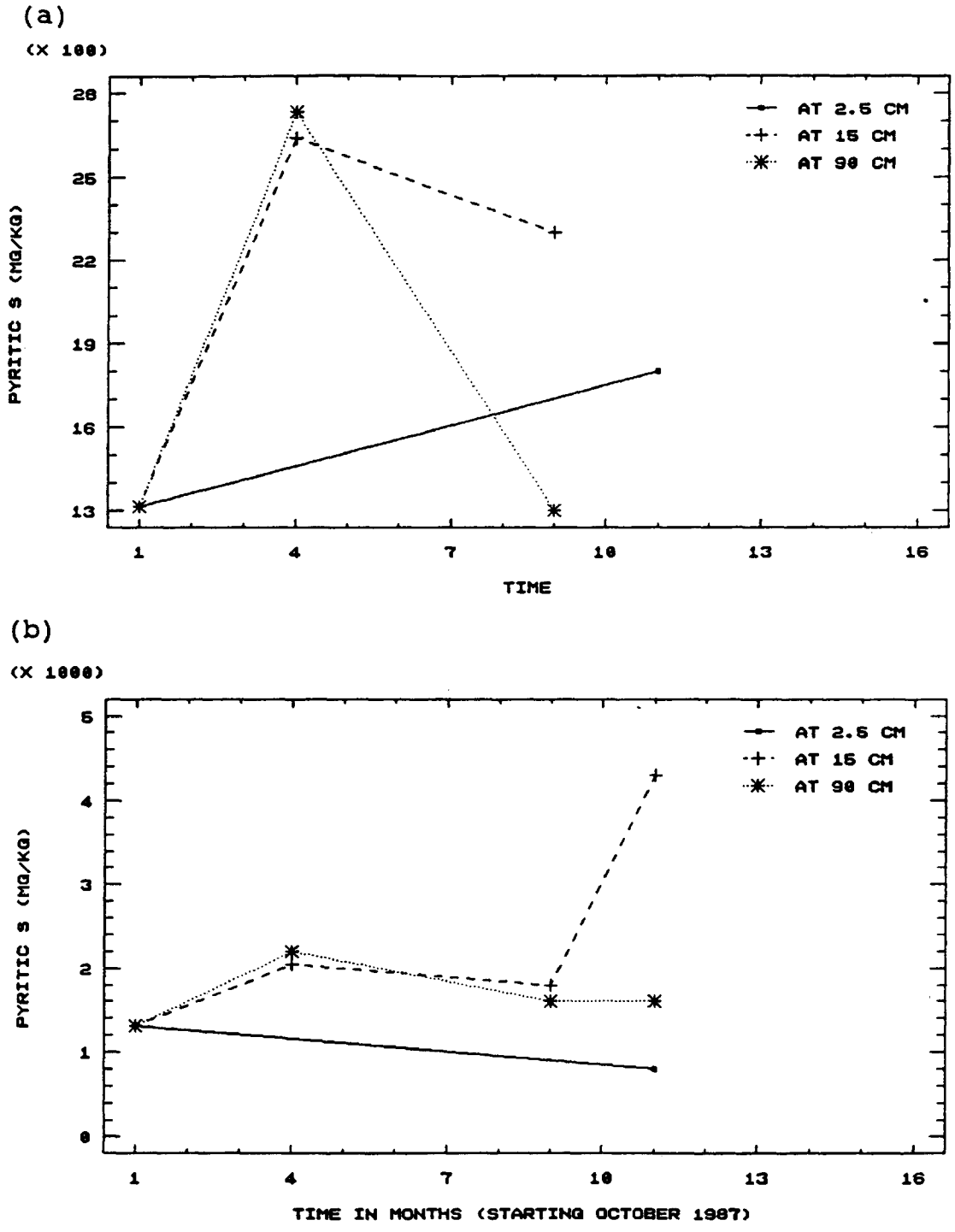


FIGURE 38. Pyritic Sulfur Concentrations in Cell C Substrate, (a) Near the Inlet and (b) Near the Outlet.

CHAPTER V

CONCLUSION

Results of the study suggest that the system was mostly anaerobic with a very thin aerobic layer, although the rise in Eh during the summer months suggests release of oxygen into the sediments by plant roots. Sulfate reducers were dominant in the soil even during the winter, while iron- and manganese-oxidizing bacteria were limited to the surface of the wetland in numbers orders of magnitude lower than SRB. Nutrient concentrations were satisfactory for growth of the intrinsic microflora.

Warmer temperatures of the summer increased rates of sulfate reduction[80,81]. However, results of this study were not in agreement with previous findings. Temperature had an indirect effect on bacteria population size by changing the redox potential of the soil perhaps through plant root activity. Sulfate-reducing bacteria populations decreased with higher temperature, while iron- and manganese-oxidizing bacteria populations increased.

Cell A substrate (compost mixture) was the most effective of the three cells in removing metals from Big Five Tunnel drainage. SRB were actively producing H_2S which reacted with metal ions in the soil and precipitated as metal sulfide. As

long as reducing conditions are maintained, metal sulfides will be stable in the wetland soil and prevented from reaching Clear Creek. Cell B substrate was the least effective, and the addition of limestone to the same substrate placed in cell C enhanced metal removal to a certain extent.

Oxidizing bacteria were also highest in cell A, trapping iron and manganese ions at the soil surface. The aerobic formation of these oxidized ions is initially required for the reduction of Fe^{3+} and Mn^{4+} at the aerobic-anaerobic interface. Such oxidation and reduction provided large quantities of the metals and increased exposure time of metals in the mine drainage to H_2S. Metals are thus precipitated as metal sulfides while metal oxides are only transitory.

Manganese, however, was not removed by the wetland and might require the implementation of a second phase in the system. A shallow aerobic system could be constructed downstream from the anaerobic wetland, to receive waters containing lower iron concentration. Iron usually interferes with Mn stability. Manganese reducing activity might be controlled if the system was oxidized. This could be achieved through terracing of the wetland, ensuring aeration of the water. The research group at the Colorado School of Mines has recently

constructed an aerobic cell for Mn removal on the Big Five tunnel site. Success of this system is yet to be determined.

CHAPTER VI

SUGGESTIONS FOR FURTHER RESEARCH

At this stage of the project, there appears to be several aspects that need to be researched.

1. Since manganese removal in the system was not satisfactory, it is necessary to investigate more efficient means for achieving such goals.

2. Based on the fact that plant roots can inject oxygen in the soil during the growing season, subsequent effects on bacterial population need to be studied in the system, which might lead to a better understanding of the wetland ecosystem.

3. For better evaluation of the role of the groups of bacteria under study in metal removal, direct measurement of their activity is needed.

4. Due to the possible competition between nitrate-reducing bacteria and sulfate reducers, further soil sampling and analysis appear to be necessary. Study of the ecology of the nitrate-reducing bacteria in the wetland and their interactions with the other groups in the system is essential for the assessment of the effectiveness of the constructed wetland.

REFERENCES

1. Dollhopf, D. J., J. D. Goering, R. B. Rennick, R. B. Horton, W. K. Gauger, J. B. Guckert, P. M. Jones, K. C. Cooksey, K. E. Bucklin, R. Weed, and M. M. Lehman, "Hydrochemical, Vegetational and Microbiological Effects of a Natural and a Constructed Wetland on the Control of Acid Mine Drainage," Bozeman, MT, Reclamation Research Unit, Montana State University and MSI Detoxification Inc., RRU 8804, 214 pp, (1988).

2. Emerick, J. C. W. W. Huskie, and D. J. Cooper, "Treatment of Discharge from a High Elevation Metal Mine in the Colorado Rockies Using an Existing Wetland," Golden, CO, Colorado School of Mines (1986).

3. Holm, J. D., "Regulatory Considerations and Passive Mine Drainage Treatment System Performance in Colorado." Denver, CO, Mined Land Reclamation Division, Department of Natural Resources (1987).

4. Vransh, G., "Mine Drainage: The Common Enemy," Denver, CO, Proceeding of the First International Mine Drainage Symposium, p. 54-97 (1979).

5. Pesavent, B. G., "Factors to be Considered in the Use of Wetlands to Treat Acid Mine Drainage," Halifax, Canada, Environmental Canada Acid Mine Drainage Symposium, p. 499 (1987).

6. Silver, M., "Distribution of Iron-Oxidizing Bacteria in the Nordic Uranium Tailings Deposits, Elliot Lake, Ontario, Canada," Applied Environmental Minobiology, 54(4), p. 846-852 (1987).

7. Wieder, R. K., and G. E. Lang, "Modification of Acid Mine Drainage in a Freshwater Wetland," Morgantown, Virginia, West Virginia University, Symposium on Wetlands of the Unglaciated Appalachian Region, B. R. McDonald (ed.), p. 43-53 (1982).

8. Kleinman, R. L. P., "A Biological Alternative to Conventional AMD Treatment," Greenlands, 17(1), p. 30, (1987).

9. Kleinman, R. L. P., "Treatment of Acid Mine Water by Wetlands," U.S. Department of the Interior, Bureau of Mines, Control of Acid Mine Drainage, Prodeeding of a Technology Transfer Seminar, IC 9027, (1989).

10. Wieder, R. K., K. P. Heston, E. M. O'Hara, G. E. Lang, A. E. Whitehouse, and J. Hett, "Aluminum Retention in a Man-Made Sphagnum Wetland," Water, Air and Soil Pollution (37) p. 177-191 (1988).

11. "The Ecological Impacts of Wastewater on Wetlands, An Annotated Bibliography," U.S. Environmental Protection Agency and U.S. Department of the Interior, EPA 905/3-84-002 (1984).

12. Hyde, H. C., R. S. Ross, and F. Demgen, "Technology Assessment of Wetlands for Municipal Wastewater Treatment," U.S. Environmental Protection Agency, EPA 60052-84-154 (1984).

13. Gillette, B., "Revolution in Wastewater Treatment," Biocycle 29(3), p. 48 (1988).

14. "The Effects of Wastewater Treatment Facilities on Wetlands in the Midwest," Environmental Protection Agency, EPA-905/3-83-002 (1988).

15. Hickok, E. A., M. C. Hannaman, and N. C. Wenck, "Urban Runoff Treatment Methods, Volume 1 - Non-Structural Wetland Treatment," Environmental Protection Agency, EPA-600/2-77-217 (1977).

16. Brodie, G. A., D. A. Hammer, and D. A. Tomljanovich, "Constructed Wetlands for Acid Drainage Control in the Tennessee Valley," in: Mine Drainage and Surface Mine Reclamation, U.S. Bureau of Mines, Information Circular 9183, p. 325-331, (1988).

17. Stark, L. R., R. L. Kolbash, H. J. Webster, S. E. Stevens, Jr., K. A. Dianis, and E. R. Murphy," The Simco #4 Wetland: Biological Patterns and Performance of Wet-land Receiving Mine Drainage," in: Mine Drainage and Surface Mine Reclamation, U.S. Bureau of Mines, Information Circular 9183, p. 332-344, (1988).

18. Wieder, R. K., "Determining the Capacity for Metal Retention in Man-Made Wetlands Constructed for Treatment

of Coal Mine Drainage." In: Mine Drainage and Surface Mine Reclamation, U.S. Bureau of Mines, Information Circular 9183, p. 375-381, (1988).

19. Hiel, M. T., and F. J. Kerins, Jr., "The Tracy Wetlands: a Case Study of Two Passive Mine Drainage Treatment Systems in Montana," in: Mine Drainage and Surface Mine Reclamation, U.S. Bureau of Mines, Information Circular 9183, p. 352-358, (1988).

20. Holm, J. D., "Passive Mine Drainage Treatment Selected Case Studies in Medine," Proceedings National Conference on Environmental Engineering, ASCE (1983).

21. "Wetlands: Their Use and Regulation," Washington, D.C., U.S. Congress, Office of Technology Assessment, OTA-0-206, (1984).

22. Guertin, D., J. C. Emerick, and E. A. Howard, "Passive Treatment Systems: Theoretical Assessment and Evalua- tion Colorado School of Mines," Department of Environmental Sciences and Engineering Ecology, Golden, CO (1984).

23. Wildeman, T. R., and L. Laudon, "The Use of Wetlands for Treatment of Environmental Problems in Mining, Non-Coal Mining Applications," Chelsea, MI, Lewis Publishing, Proceedings of the International Conference on Constructed Wetlands for Wastewater Treatments (in review).

24. Stone, W. R., "The Presence of Iron and Manganese-Oxidizing Bacteria in Natural and Simulated Bogs," Treatment of Mine Drainage by Wetlands, p. 30-36 (1986).

25. Kleinman, R. L. P., and P. M. Erickson, "Control of Acid Drainage from Coal Refuse Using Anionic Surfactants," United States Department of the Interior, RI 8847 (1983).

26. Rawat, N. S. and G. Singh, "Chemical, Microbiological and Geological Aspects of Acid Mine Drainage," Journal of Scientific and Industrial Research, 42, p. 448-455 (1983).

27. Kleinman, R. L. P., D. A. Crerar, and R. R. Pacelli, "Biogeochemistry of Acid Mine Drainage and a Method to Control Acid Formation," Mining Engineering, 33(3) (1981).

28. Huskie, W. W., "The Pennsylvania Mine Drainage Diversion Study: Evaluation of an Existing High Mountain Wetland

for Passive Treatment of Metal-Laden Acid Mine Discharge in Colorado," Colorado School of Mines, Golden, CO T-3359 (1987).

29. Singer, P. C., and W. Stumm, "Acidic Mine Drainage. The Rate Determining Step," Science, 167, p. 1121-1123 (1970).

30. Erickson, P. M., R. L. P. Kleinmann, and S. J. Onysko, "Control of Acid Mine Drainage by Application of Bactericidal Materials," U.S. Department of the Interior, Bureau of Mines, Control of Acid Mine Drainage, Proceeding of a Technology Transfer Seminar, IC 9027 (1985).

31. Marshall, K. C., "Biogeochemistry of Manganese Minerals," in: Biogeochemical Cycling of Mineral-Forming Elements, Trudinger, P. A. and D. J. Swaine (eds.), p. 253-292 (1979).

32. Walsh, F., and R. Mitchell, "A pH Dependent Succession of Iron Bacteria," Environmental Science and Technology, 6, p. 809-812 (1972).

33. Nealson, K. H., "The Microbial Iron Cycle," Boston, MA, Blackwell Scientific Publications, Microbial Geochemistry, Krumbein, W. E. (ed.) (1983).

34. Silver, M., H. L. Ehrlich and K. C. Ivarson, "Soil Mineral Transformation Mediated by Soil Microbes," Interactions of Soil Minerals With Natural Organics and Microbes, p. 497-519 (1986).

35. Kelly, L. P., and O. H. Tuovinen, "Recommendation That the Names Ferrobacillus fenooxidans Leathen and Braley and Ferrobacillus sulfooxidans Kinsel, Be Recognized as Synonyms of Thiobacillus ferrooxidans Temple and Colmer," International Journal of Systemestic Bacteriology, 22(3) (1972).

36. Lazaroff, N., W. sigal, and A. Wasserman, "Iron Oxidation and Precipitation of Ferric Hydroxysulfates by Resting Thiobacillus ferrooxidans cells," Applied and Environmental Microbiology, 43, p. 924-938 (1982).

37. Schnaitman, C. A., M. S. Korazynski, and D. G. Landgren, "Kinetics Studies of Iron Oxidation by Whole Cells of Ferrobacillus ferrooxidans, Journal of Bacteriology, 99, p. 552-557 (1969).

38. Alexander, M., Introduction to Soil Microbiology, Second Edition, New York, NY, John Wiley and Sons (1977).

39. Halbach, P., and K. H. Ujma, "Mobilization, Transport and Microbially Assisted Precipitation of Iron in a Bog Creek," Ann Arbor, MI, Ann Arbor Science Publishers Inc., Environmental Biogeochemistry and Geomicrobiology. Volume 2: The Terrestrial Environment, Krumbein, W. E. (ed.), P. 493-502 (1978).

40. Zajic, J. E., Microbial Biogeochemistry, New York, NY, Academic Press (1969).

41. Brierly, J., Personal Communication (1988).

42. Erlich, H. L., Geomicrobiology, New York, NY, Marcel Dekker Inc, (1981).

43. Hem, J. D., "Deposition and Solution of Manganese Oxides," U.S. Geological Survey Water Supply Paper, 1667-B (1964).

44. Nealson, K. H., B. M. Tebo and R. A. Rosson, "Occurrence and Mechanisms of Microbial Oxidation of Manganese," London, England, Academic Press, Inc., Advances in Applied Microbiology, Volume 33, Laskin, A. I., (ed), p. 279-318 (1987).

45. Nealson, K. H., "The Microbial Manganese Cycle," Boston, MA, Blackwell Scientific Publications, Microbial Geochemistry, Krumbein, W. E. (ed.), P. 191-221 (1983).

46. Schweisfurth, R. D. Eleftheriadis, H. Gundlach, M. Jacobs and W. Jung, "Microbiology of the Precipitation of Manganese," Boston, MA, Blackwell Scientific Publications, Microbial Geochemistry, Krumbein, W. E. (ed.), p. 923-928 (1983).

47. Nealson, K. H., "The Isolation and Characterization of Marine Bacteria Which Catalyzes Manganese Oxidation," Ann Arbor, MI, Ann Arbor Science Publishers, Inc., Environmental Biogeochemistry and Geomicrobiology. Volume 3: Methods, Metals and Assessment, Drumbein, W. E. (ed.), P. 847-858 (1978).

48. Muir, M. D., Microenvironments of Some Modern and Fossil Iron-and Manganese-Oxidizing Bacteria," Ann Arbor, MI,

Ann Arbor Science Publishers, Inc., Environmental Biogeochemistry and Geomicrobiology. Volume 3: Methods, Metals, and Assessment, Krumbein, W. E. (ed.), p. 937-944 (1978).

49. Ghiorse, W. C. and P. Hirsch, "Iron and Manganese Deposition by Budding Bacteria," Ann Arbor, MI, Ann Arbor Science Publishers, Inc., Biogeochemistry and Geomicrobiology. Volume 3: Methods, Metals and Assessment, Krumbein, W. E. (ed.) p. 897-909, (1978).

50. Bartlett, D. S., "Global Biology Research Program, Biochemical Processes in Wetlands," Springfield, VA, National Technical Information Service, NASA Conference Publication 2316 (1984).

51. Nriagu, I. O. and G. D. Hem, "Chemistry of Pollutant Sulfur in Natural Waters," New York, NY, John Wiley and Sons, Inc., Sulfur in the Environment. Part II: Ecological Impacts, Nriagu, J. O. (ed.), p. 211-270 (1978).

52. Jorgensen, B. B., "The Microbial Sulphur Cycle," Boston, MA, Blackwell Scientific Publications, Microbial Geochemistry, Krumbein, W. E. (ed.), p. 91-124 (1983).

53. Zinder, S. H. and T. D. Brock, "Microbial Transformation of Sulfur in the Environment," New York, NY, John Wiley and Sons, Inc., Sulfur in the Environment. Part II: Ecological Impacts, Nrigan, J. O. (ed.), p. 445-466 (1978).

54. Spirakis, C. S., "Kinetics of Sulfate Reduction, Metastable Sulfur, and the Genesis of Hydrothermal Uraninitc Deposits, Mississippi Valley Lead-Zinc Deposits, Barite Deposits and Epithermal Base and Precious Metal Deposits," Boulder, CO, Colorado University at Boulder, Department of Geology (1983).

55. Tuttle, J. H., P. R. Dugan and C. I. Randles, "Microbial Sulfate Reduction and its Potential Utility as an Acid Mine Water Pollution Abatement Procedure," Applied Microbiology, 17(2), p. 297-302 (1969).

56. Hedin, R. S., D. M. Hyman and R. W. Hammack, "Implications of Sulfate Reduction and Pyrite Formation Processes for Water Quality in a constructed Wetland, Preliminary Observations," U.S. Bureau of Mines, Mine Drainage and

Surface Mine Reclamation, Information Circular 9183 (1988).

57. Postgate, J. R., The Sulphate-Reducing Bacteria, New York, NY, Cambridge University press (1979).

58. Pfenning, N. and F. Widdel, "Ecology and Physiology of Some Anaerobic Bacteria from the Microbial Sulfur Cycle," Berlin-Springer-Verlag, Biology of Inorganic Nitrogen and Sulfur, Bothe, H. and A. Tebst (eds.), p. 169-177 (1981).

59. Peck, H. D. and J. Legall, "Biochemistry of Dissimilating Sulphate Reduction," London, England, Phil. Trans. R. Sol Land B298, p. 443-466 (1982).

60. Jorgensen, B. B., "Ecology of the Bacteria of the Sulphur Cycle with Special Reference to Anoxic-Oxic Interface Environments," London, England, Phil. Trans. R. Sol. Lond., B298, p. 543-561 (1982).

61. Iverson, W. P., "Microbial Corrosion of Metals," London, England, Academic Press, Inc., Advance in Applied Microbiology, Volume 32, Laskin, A. I. (ed.) p. 1-36 (1987).

62. Phelps, T. J., R. Conrad and J. G. Zeikus, "Sulfate-Dependent Interspecies H_2 Transfer Between Methanosarcia barkeci and Desulfovibria vulgaris during Coculture Metabolism of Acetate on Methanol," Applied and Environmental Microbiology, 50(3), p. 589-594 (1985).

63. Smith, R. L. and M. J. Klug, "Electron Donors Utilized by Sulfate-Reducing Bacteria in Futrophic Lake Sediments," Applied and Environmental Microbiology, 42(1), p. 116-121 (1981).

64. Lein, A. Y., "Formation of Carbonate and Sulfide Minerals during Diagenesis of Reduced Sediments," Ann Arbor, MI, Ann Arbor Science Publishers, Environmental Biogeochemistry and Geomicrobiology Volume 1, Krumbein, W. E. (ed.), P. 339-354 (1978).

65. Postgate, J. R., The Sulphate-Reducing Bacteria, 2nd Edition, London, England, Cambridge University Press, Inc. (1984).

66. Gottschal, J. C., "Occurrence and Fuctioning of Anaerobic Bacteria in Oxidized Environments," London, England,

Elsevier Applied Science Publishers, Microbial Communities in Soils, Jensen, V., A. Kjoller, and L. H. Sorensen (eds.), p. 263-273 (1986).

67. Jorgensen, B. B., M. H. Hansen and K. Ingvorsen, "Sulfate Reduction in Coastal Sediments and the Release of H_2S to the Atmosphere," Ann Arbor, MI, Ann Arbor Science Publishers, Environmental Biogeochemistry and Geomicrobiology, Volume 1, Krumbein, W. E. (ed.), p. 245-253 (1978).

68. Smith, R. L. and M. J. Klug, "Reduction of Sulfur Compounds in the Sediments of a Eutrophic Lake Basin," Applied and Environmental Microbiology, 41(5), p. 1230-1237 (1981).

69. Spratt, H. G., Jr., M. D. Morgan and R. E. Good, "Sulfate Reduction in Peat From a New Jersey Pinelands Cedar Swamp," Applied and Environmental Microbiology, 53(7), p. 1406-1411 (1987).

70. Wakao, N., T. Takahashi, Y. Sakurai, and H. Shiota, "A Treatment of Acid Mine Water Using Sulfate-Reducing Bacteria," J. Ferment Technol. 57(5), p. 445-452 (1979).

71. Jorgejan, A., "Observations on a Microbial Cellulose Degradation Process That Decreases Water Acidity," International Biodeterioration, 22(3), p. 207-211 (1986).

72. Sorenson, J., B. B. Jorgensen, and N. P. Revsbech, "A Comparison of Oxygen, Nitrate and Sulfate Respiration in Coastal Marine Sediments," Microbial Ecology, 5, p. 105-115 (1979).

73. Grimm, D. T., D. J. Cork, and R. A. Uphaus, "Optimization of Desulfabacter for Sulfate Reduction," Memphis, Tennessee Department of Biology, Memphis State University (1983).

74. Ingvorsen, K., A. J. B. Zehnder, and B. B. Jorgensen, "Kinetics of Sulfate and Acetate Uptake by Desulfobacter Postgatei," Applied and Environmental Microbiology, 47(2), p. 403-408 (1984).

75. Banat, I. M., E. B. Lindstrom, D. B. Nedwell, and M. T. Balba, "Evidence for Coexistence of Two Distinct Functional Groups of Sulfate-Reducing Bacteria in

Saltmarsh Sediment," Applied and Environmental Microbiology, 42(6), p. 985-992 (1981).

76. Westermann, P. and B. K. Ahring, "Dynamics of Methane Production, Sulfate Reduction, and Denitrification in a Permanently Waterlogged Alder Swamp," Applied and Environmental Microbiology, 53(10), p. 2554-2559 (1987).

77. Senior, E., E. B. Lindstrom, I. M. Banat, and D. B. Nedwell, "Sulfate Reduction and Methanogenesis in the Sediment of a Saltmarsh on the East Coast of the United Kingdom," Applied and Environmental Microbiology, 43(5), p. 987-997 (1982).

78. Lovely, D. R., D. F. Dwyer, and M. J. Klug, "Kinetic Analysis of Competition Between Sulfate Reducers and Methanogens for Hydrogen in Sediments," Applied and Environmental Microbiology, 43(6), p. 1373-1379 (1982).

79. Sarner, E., B. G. Hultman, and A. E. Berglund, "Anaerobic Treatment Using New Technology for Controlling H_2S Toxicity," Tappi Journal, 71(2), p. 41-45 (1978).

80. Nedwell, D. B., and J. W. Abram, "Relative Influence of Temperature and Electron Donor and Electron Acceptor Concentration on Bacterial Sulfate Reduction in Saltmarsh Sediment," Microbial Ecology, 5, p. 67-72 (1979).

81. Jorgensen, B. B., "The Sulfur Cycle of a Coastal Marine Sediment (Limfjorden, Denmark)," Limnology and Oceanography, 22(5), p. 814-832 (1977).

82. Abdollahi, H., and D. B. Nedwell, "Seasonal Temperature as a Factor Influencing Bacterial Sulfate Reduction in a Saltmarsh Sediment, Microbial Ecology, 5, p. 73-79 (1979).

83. Cutter, G. A., and D. J. Velinsky, "Temporal Variations of Sedimentary Sulfur in a Delaware Saltmarsh," Marine Chemistry, 23, p. 311-327 (1988).

84. McGill, W. B., and C. V. Cole, "Comparative Aspects of Cycling of Organic C, N, S and P Through Soil Organic Matter,," Geochema, 26, p. 67-186 (1981).

85. Ingvorsen, K., J. G. Zeikus, and T. D. Brock, "Dynamics of Bacterial Sulfate Reduction in a Eutrophic Lake,"

Applied and Environmental Microbiology, 42(6), p. 1029-1036 (1981).

86. Herlihy, A. T. and A. L. Mills, "Sulfate Reduction in Freshwater Sediments Receiving Acid Mine Drainage," Applied and Environmental Microbiology, 49(1), p. 179-186 (1985).

87. Jorgensen, B. B. and T. Fenchel, "The Sulfur Cycle of a Marine Sediment Model System," Marine Biology, 24, p. 188-201 (1976).

88. Davison, W., J. P. Lishman, and J. Hilton, "Formation of Pyrite in Freshwater Sediments: Implication for C/S ratios," Geochimica et Cosmochimica Acta, 49, p. 1615-1620 (1985).

89. Howarth, R. W., and B. B. Jorgensen, "Formation of ^{35}S-Labelled Elemental Sulfur and Pyrite in Coastal Marine Sediments (Limfjorden and Kysing Fjord, Denmark) During Short-Term $^{35}SO_4^{2-}$ Reduction Measurements," Geochimica et Cosmochimica Acta, 48, p. 1807, 1818 (1984).

90. Howarth, R. W., and S. Merkel, "Pyrite Formation and the Measurement of Sulfate Reduction in Salt Marsh Sediments," Limnology and Oceanography, 29(3), p. 598-608 (1984).

91. Laudon, L., Thesis Proposal. Department of Geochemistry, Colorado School of Mines, Golden, CO (1988).

92. Olsen, R. L., E. Hinzel, and J. S. Paquett, "Performance of Remedial Response Activities at Controlled Hazardous Waste Sites (Rem II)," Feasibility Study Operable Unite No. One, Clear Creek/Central City, Colorado, REM II, Document No. 202-FS1-RT-FLAQ (1987)

93. Howard, E. A., J. C. Emerick, and T. R. Wildeman, "The Design, Construction and Initial Operation of a Research Site for Passive Mine Drainage Treatment in Idaho Springs, Colorado," Chelsea, MI, Lewis Publishing, Proceedings of the International Conference on Constructed Wetlands for Wastewater Treatment, (in review).

94. Batal, W., L. S. Laudon, T. R. Wildeman, and N. Mohdnovrdin, "Bacteriological Tests From the Constructed Wetland of the Big Five Tunnel, Idaho Springs, Colorado," Chelsea, MI, Lewis Publishing, Proceedings of the Inter-

national Conference on Constructed Wetlands for Wastewater Treatment, (in review).

95. Silverman, M. P., and D. G. Lundgren, "Studies on the Chemoautotrophic Iron Bacterium Ferrobacillus ferro-oxidans. I. An Improved Medium and a Harvesting Procedure for Securing High Cell Yields," Journal of Bacteriology, 77, p. 642-647 (1959).

96. Postgate, J. R. "Media for Sulphur Bacteria," LABP 5-74 Article, p. 1239-1244 (1985).

97. Page, A., R. Miller, and D. Keeney, Methods for Soil Analysis Part II. Second Edition, Madison, WI, American Society of Agronomy, Inc., Soil Society of America, Inc., Chapter 39 (1982).

98. United States Geological Survey, "Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples, USGS Technique of Water Resources Investigations, Book 5, Chapter A4, p. 67-72 (1980).

99. Koch, A. L., "Growth Measurement," Manual of Methods for General Bacteriology, Gerhardt, P., R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg and G. B. Phillips (eds.), Washington DC, American Soceity for Microbiology, p. 179-207 (1981).

100. Bradshaw, L. J., Laboratory Microbiology, 2nd Edition, Philadelphia, PA, W. B. Saunders Company (1973).

101. Silver, M., Personal Communication.

102. American Society for Testing and Materials, Annual Book of ASTM Standards, Volume 5, p. 418-422, (1986).

103. Sokal, R. R. and F. J. Rohlf, Biometry, Second Edition, San Francisco, CA, W. H. Freeman and Company, (1981).

104. Davis, J. C., Statistics and Data Analysis in Geology, London, England, John Wiley and Sons, Inc. (1973).

105. Gottschal, J. C., "Occurence and Functioning of Anaerobic Bacteria in Oxidized Environments," Microbial Communities in Soils, Jensen, V. A., A. Kjoller and L. H. Sorensen (eds.) London, England, Elsevier Applied Science Publishers, p. 263-773 (1986).

106. Jorgensen, B. B., "Bacterial Sulfate Reduction Within Reduced Microniches of Oxidized Marine Sediments," Marine Biology, 41, p. 7-17 (1977).

107. Etherington, J. R., Environment and Plant Ecology, London, England, John Wiley and Sons (1975).

108. Westermann, P., and B. K. Ahring, "Terminal Anaerobic Carbon Mineralization in a Alder Swamp," Microbial Communities in Soils, Jensen, V. A., Kjoller, and L. H. Sorensen (eds.), London, England, Elsevier Applied Science Publishers, p. 305-314 (1986).

109. Updegraff, D. Personal Communication (1989).

110. Cohen, R. R. H., P. Lemke, W. Batal, S. Machemer, and D. Updegraff, "Year End Report for the Big Five Tunnel Constructed Wetland Treatment System," Cincinnati, OH, U.S. Environmental Protection Agency, Office of Research and Development, Risk Reduction Engineering Laboratory.

111. Laudon, L., "Sulfur Mineralization in a Wetland Constructed to Treat Acid Mine Drainage," Golden, CO Colorado School of Mines, T-3660 (1988).

112. Tiedje, J. H., A. J. Sextone, T. B. Parkin, N. P. Revsbech, and D. R. Shelton, "Anaerobic Processes in Soil," Plant Soil, 72, p. 197-212 (1984).

113. Bos, P., and J. G. Kuenen, Microbial Corrosion, London, England, Metals Soceity (1983).

APPENDIX A

PROCEDURES USED FOR CULTURING THE FOUR BACTERIAL GROUPS

APPENDIX A

PROCEDURES USED FOR CULTURING THE FOUR BACTERIAL GROUPS.

1. Preparing the sample dilutions: if the sample is a slurry, mix thoroughly for 20mn and allow large debris to settle. Extract the supernatant with a sterilized glass pipette the tip of which has been cut off for easier extraction of the sample. Gentle grinding using a sterilized mortar and pestle may be necessary. Using the autoclaved glass pipette, take out 10mL of the sample and dilute with 90mL of a sterilized 0.2% NaCl solution obtaining a dilution of about 1:100. If the sample is not fluid, weigh 4g of the sample (using a sterilized spatula) in a tared sterilized petri dish and transfer them into a sterilized mortar. Mix with 6-7 mL of the 0.2% NaCl solution. Gently grind, making a slurry. Extract 5 mL and dilute with 45 mL of 0.2% NaCl solution in autoclaved dilution bottles. The dilution would then be 1:100. Prepare at least four serial dilutions by adding 10 mL sample to 90 mL 0.2% NaCl solution.

2. The Autotrophic Iron-Oxidizing Bacteria Test

A. Method: After Silver (1987)[6], using the Most Probable Number (MPN) method for the enumeration of the bacteria[97,98].

Medium: 9K and ferrous sulfate solution of Silverman and Lundgren (1959)[95] consisting of: 3.0 g of $(NH_4)_2SO_4$, 0.10 g KCl, 0.50 g K_2HPO_4, 0.5 g MgSO4.$7H_2O$, 0.01 g $Ca(NO_3)_2$, 1.0 mL of 10N H_2SO_4, and 700 ml deionized water. 300 ml of 14.74% (w/v) $FeSO_4.7H_2O$ solution using sterilized deionized water.

Autoclave at 250°F for 20 min: deionized water, 600 mL beaker, spatulas, 14 mL test tubes and caps (five tubes for each dilution), screw-cap Nalgene(R) jars, glass pipettes (previously prepared), pipette tips, 0.2% NaCl solution, mortars and pestles, dilution bottles, and small Petri dishes.

B. In a 1L beaker, mix the ingredients of the 9K solution, except for the $FeSo_4 \cdot 7H_2O$, with 600 mL deionized water. Adjust the volume to 700mL using deionized water and make sure that all the chemicals have dissolved. The solution autoclave in a polypropelene bottle at 250°F for 20 min. In the sterilized 600 mL beaker containing 200 mL cool sterilized deionized water, $FeSO_4.7H_2O$ and mix well. The volume with sterilized deionized water to 300 mL making a 14.74% (w/v) solution . Mix both solutions in the polypropelene bottle and shaken well. The medium had a pH of 3.0 to 3.6.

C. Add 1 mL of each dilution to 4 mL and 9K ferrous sulfate solution in the sterilized test tubes and cap them with plastic caps permitting exchange of O_2. Use four consecutive

dilutions, five tubes per dilution, for a total of 20 tubes per sample. Incubate at 30°C for three weeks. Check every week for positive results to determine the vigor of the bacteria. The formation of the orange ferric hydroxide along with microscopic verification (presence of rod-shaped cells) indicate the growth of the bacteria. The MPN method is then used to enumerate the bacteria. Obtain the number of bacteria per gram of dry soil.

3. The Sulfate-Reducing Bacteria Test

A. Method: After Postgate (1979)[57,96], with the use of the MPN method for the enumeration of the bacteria.

Medium: Modified medium B (Postgate 1979). Add 1.0ml of 0.1% resazurin to 1L of modified medium B consisting of: 0.5 g/l K_2HPO_4, 1 g/l NH_4Cl, 2 g/l $MgSO_4.7H_2O$, 1 g/l yeast extract, 0.1 g/l ascorbic acid, 0.5 g/l $FeSO_4.7H_2O$, 1 g/l $CaSO_4$ (used 1.26 g of $CaSO_4.2H_2O$), 3.5 g/l sodium lactate (used 2.8 g/l or 2.4 cc lactic acid), and 0.1 g/l thioglycollic acid (used 0.075 ml thioglycollic acid).

Autoclave: Deionized water, 0.2% NaCl solution, dilution bottles, mortars and pestles, pipette tips, pipettes (the tip of which has been cut off), spatula, screw-cap Nalgene jars.

B. Prepare the medium by dissolving all the ingredients in 800 mL of nitrogen bubbled distilled water under low heat. Adjust the pH with 1M NaOH to 7.0-7.5 (this takes about 5 mL of NaOH). Pour the solution in a 1L volumetric flask and adjust the volume to 1L. The solution will have a grayish-white precipitate. Prepare another liter of the same solution and autoclave it in a polypropelene bottle at 250°F for 20 mn.
C. Prepare the serial dilutions using the procedure in Part 1. Determine the dry weight. Place about 8 mL of the modified medium B in 9 mL screw-cap test tubes. Autoclave for 20 min at 250°C F. Close the tubes tightly and allow them to cool. Add 1 mL of each dilution to the autoclaved test tubes (containing the medium), fill them to the top with autoclaved medium B (previously prepared) allowing no air space. Close the tubes tightly and incubate them at 30°C for three weeks. Check every three to four days for positive results, indicated by a black precipitate in the tubes and the odor of H_2S. Use the MPN method to enumerate the bacteria and obtain the number of bacteria in 1g of dry soil.

4. The Heterotrophic Iron Oxidizing Bacteria Test
A. Method used: The spread plate method[99,100,101]. Ingredients for the medium: 1. Yeast extract (0.2g/L); 2. Peptone

(0.2g/L); 3. Agar (15 g/l); and tap water (1L). Autoclave: 0.2% NaCl solution, deionized water, dilution bottles, screw-cap Nalgene jars, pipette (the tip of which has been cut off), pipette tips, spatula, mortar and pestle.

B. Mix the ingredients in a beaker and dissolve with heating (on a hot plate) constantly stirring with a glass rod. Bring the solution to a boil for 1 min or so. Cover the beaker with aluminum foil and autoclave at 121°C for 10 min. Cool the agar medium to 45°C (in a water bath for 10 min). Prepare 1 g/l $FeSO_4.7H_2O$ solution using sterilized distilled water. Clean the desk top with alcohol before placing the sterilized plastic petri dishes. Mix the 1 g/L $FeSO_4.7H_2O$ solution with the warm (45°C) liquid agar then pour it into the small section of the plates. Immediately cover the plates. Bend down and look at the edges of the medium in the plates, gently agitate them without lifting the dishes. When no shifting in the medium occurs, flip the plates over and leave them overnight.

C. Prepare serial dilutions within 24 hours using sterilized instruments and distilled water or 0.2 percent NaCl solution, following the procedure in Part 1.

D. Inoculation of the plates is done by the bent glass rod method. Dip the short side of the rod in 70% alcohol and

insert it into a Bunsun burner flame until all the alcohol has burned from the rod, and allow it to cool. With a sterilized pipette add 0.1 mL of the sample (dilution) to each of the petri dishes and spread with the bent glass rod (washing with alcohol and flaming the rod for each plate). Cover the plates and incubate at 30°C (for five-seven days). With a colony counter (or a microscope when necessary) count the bacterial colonies on the agar. The number obtained is the Total Heterotrophic Bacterial Count.

E. In a brown bottle prepare a 0.2 percent tetramethylbenzidine powder in a glove box. Flood the plates with 3-4 mL of the solution and count the colonies that develop a deep blue color within an hour. The number obtained is the Heterotrophic Iron Oxidizing Bacterial Count.

5. Heterotrophic Manganese Oxidizers Bacterial Tests

Repeat procedure III using 1 g/mL $MnSO_4$ solution in Section B. instead of 1 g/L $FeSO_4$ solution.

APPENDIX B

SAMPLE WORKSHEET USED FOR ASSESSMENT OF THE MOST PROBABLE NUMBER OF IRON-OXIDIZING AND SULFATE-REDUCING BACTERIA PER GRAM OF DRY SOIL

Sample No_____ Date Collected_____ Time Collected_____
Sample Location_____ Depth_____ Collected By_____
Temperature_____ pH_____ Eh_____ Flow Rate_____
Field Measurement Sheet No_____
Date of Dilutions_____ Time_____ Prepared By_____

First Dilution

1 Tare (g)	2 Wet Weight (+Dish)(g)	3 0.2% NaCl (ml)	4 Wet Weight (g)	5 Calc. Dry Weight (g)	6 Tot. Volume (3+4)

Determination of the Dilution Factor Correction (Dil. Fac. Cor.)

Tare (g)	Wet Weight (g)	Dry Weight + Dish (g)	Dry Weight (g)	% Solid	Average %	Dil. Fac. Cor. (5/6) x10

Preparation of Dilutions

ml Sample	ml 0.2% NaCl	Dilution Factor	Corrected Dilution Factor

Checking for Bacterial Indication

Sulfate Reducing Bacteria

Date_____ By_____

Dilution	---	---	---	---
No of +tive Tubes	---	---	---	---

Date_____ By_____

Dilution	---	---	---	---
No of +tive Tubes	---	---	---	---

Date_____ By_____

Dilution	---	---	---	---
No of +tive Tubes	---	---	---	---

Date_____ By_____

Dilution	---	---	---	---
No of +tive Tubes	---	---	---	---

Iron Oxidizing Bacteria

Date_____ By_____

	---	---	---	---
	---	---	---	---

Date_____ By_____

	---	---	---	---
	---	---	---	---

Date_____ By_____

	---	---	---	---
	---	---	---	---

Date_____ By_____

	---	---	---	---
	---	---	---	---

Number of SRB per g of Soil

P1	P2	P3	MPN	Bact/g
---	---	---		

Number of IOB per g of Soil

P1	P2	P3	MPN	Bact/g
---	---	---		

APPENDIX C

RESULTS OF SOIL ANALYSES

KEY TO ABBREVIATIONS

The first letter in the sample number refers to the depth at which the sample was collected. S for Surface (2.5 cm), T for top (15 cm), B for Bottom (90 cm).

The second letter in the sample number refers to the cell the soil sample was taken from (A, B, or C).

The number in the sample number indicates the location of the sample: 1, 2, 3 refers to the wells close to the inlet near which the soil composite was collected,4, 5, 6 refer to the wells close to the outlet, e.g. SA3 is a soil sample taken at 2.5 cm, from cell A near well 3 which is close to the inlet.

The last part of the sample number refers to the date at the time the sample was collected.

SAMPLE-DATE		Compost Mixture-10-87	Peat,Manure,Wood Products-10-87	SA3-08-88
TOTAL S	mg/kg	3520	5515	20900
PYRITIC S	mg/kg	605	2650	1900
SO4 S	mg/kg	260	1315	15000
ORGANIC S	mg/kg	2700	1550	4000
TOTAL C	mg/kg	185000	127000	1330
NH4-N	mg/kg	2694	609	0
TOTAL N	mg/kg	15213	5592	2030
TOTAL P	mg/kg	5371	2895	191
Al	mg/kg	5425	6605	12800
Cu	mg/kg	50	31	717
Fe	mg/kg	10450	11050	144000
Pb	mg/kg	20	16	49
Mn	mg/kg	373	202	494
Zn	mg/kg	113	127	2950
SRB	bact/g	500000	20000	1900
Fe-OXID	bact/g	0	0	230000
HFe-OXID	bact/g			283000
HMn-OXID	bact/g			283000
EH	mV			60
PH				5.5
TEMP	C			16

SAMPLE-DATE		SA3-11-88	TA3-01-88	TA3-06-88	TA3-08-88
TOTAL S	mg/kg		5910	10800	14500
PYRITIC S	mg/kg		1010	1100	1100
SO4 S	mg/kg		4160	3060	3700
ORGANIC S	mg/kg		740	6600	9700
TOTAL C	mg/kg		90400	2050	5800
NH4-N	mg/kg		396	297	264
TOTAL N	mg/kg		9700	2860	1910
TOTAL P	mg/kg		4350	2290	2810
Al	mg/kg		5200	9780	5650
Cu	mg/kg		85	178	156
Fe	mg/kg		18200	24600	20200
Pb	mg/kg		36	27	24
Mn	mg/kg		500	1440	1310
Zn	mg/kg		305	1280	1060
SRB	bact/g	2900000	14000000	1100000	130000
Fe-OXID	bact/g	190000	18000	1300	7500
HFe-OXID	bact/g	180000		19000	70000
HMn-OXID	bact/g	66000		19000	73000
EH	mV	135	30	-60	-44
PH		3.5	6.5	6.8	6.7
TEMP	C	0	6	9.9	17

SAMPLE-DATE		TA3-11-88	BA3-01-88	BA3-06-88	BA3-08-88
TOTAL S	mg/kg	16849	4110	6500	10000
PYRITIC S	mg/kg		860	500	1600
SO4 S	mg/kg		2770	2020	1900
ORGANIC S	mg/kg		480	4000	6500
TOTAL C	mg/kg		132000	679	2880
NH4-N	mg/kg	118	697	447	505
TOTAL N	mg/kg	12529	12900	4340	1590
TOTAL P	mg/kg	2971	5050	2900	3150
Al	mg/kg	9900	5040	786	5550
Cu	mg/kg	369	112	102	153
Fe	mg/kg	20200	18400	21700	21900
Pb	mg/kg	94.1	60	18	44
Mn	mg/kg	579	541	773	713
Zn	mg/kg	4150	466	441	612
SRB	bact/g	270000	11000000	1600000	2200000
Fe-OXID	bact/g	2700	30000	1400	760
HFe-OXID	bact/g	5600			2800
HMn-OXID	bact/g	26000			2800
EH	mV	-25	-60	-25	45
PH		4	7.6	7.3	7.2
TEMP	C	3.8	7	10.8	16.5

SAMPLE-DATE		BA3-11-88	SA6-08-88	SA6-11-88	TA5-01-88
TOTAL S	mg/kg	12608	38500		7930
PYRITIC S	mg/kg		800		2050
SO4 S	mg/kg		14300		5610
ORGANIC S	mg/kg		23400		270
TOTAL C	mg/kg		760		123000
NH4-N	mg/kg	57	21		66.9
TOTAL N	mg/kg	15156	2740		9250
TOTAL P	mg/kg	3044	6170		3750
Al	mg/kg	6190	14900		4250
Cu	mg/kg	94.6	1300		167
Fe	mg/kg	16600	39500		16000
Pb	mg/kg	43.1	85		33
Mn	mg/kg	1340	2350		999
Zn	mg/kg	592	7510		667
SRB	bact/g	15000000	4200000	880000	28000000
Fe-OXID	bact/g	380	28000	200000	950000
HFe-OXID	bact/g		361000	54000	
HMn-OXID	bact/g		361000	37000	
EH	mV	-109	-20	205	-40
PH		5.9	6.2	3	7.1
TEMP	C	8.2	16	0	6.8

SAMPLE-DATE		TA6-06-88	TA6-08-88	TA6-11-88	BA5-01-88
TOTAL S	mg/kg	9100	11300	21362	3960
PYRITIC S	mg/kg	600	1200		880
SO4 S	mg/kg	2940	3300		2540
ORGANIC S	mg/kg	5600	6800		540
TOTAL C	mg/kg	1440	4100		138000
NH4-N	mg/kg	527	616	135	807
TOTAL N	mg/kg	3150	1890	20494	13200
TOTAL P	mg/kg	4020	3400	4158	4460
Al	mg/kg	10400	5570	9835	4190
Cu	mg/kg	209	196	554	115
Fe	mg/kg	26600	19600	22700	14200
Pb	mg/kg	43	38	119	31
Mn	mg/kg	1070	1000	694	562
Zn	mg/kg	1010	966	5565	333
SRB	bact/g	510000	510000	710000	10000000
Fe-OXID	bact/g	1900	1900	13000	580000
HFe-OXID	bact/g	31000	33000	5600	
HMn-OXID	bact/g	31000	340000	19000	
EH	mV	51	25	120	-60
PH		6.7	6.2	4	7.6
TEMP	C	10.5	14.6	4.1	6

SAMPLE-DATE		BA6-06-88	BA6-08-88	BA6-11-88	SB1-08-88
TOTAL S	mg/kg	7000	8100	7292	15700
PYRITIC S	mg/kg	500	1200		4200
SO4 S	mg/kg	2190	1900		9960
ORGANIC S	mg/kg	4300	5000		1500
TOTAL C	mg/kg	520	4780		1490
NH4-N	mg/kg	740	721	1046	10
TOTAL N	mg/kg	1960	2160	14727	1330
TOTAL P	mg/kg	3310	4610	4697	877
Al	mg/kg	8700	4830	6960	53300
Cu	mg/kg	139	150	100	286
Fe	mg/kg	25500	22400	17300	79300
Pb	mg/kg	22	24	38.6	32
Mn	mg/kg	685	740	698	422
Zn	mg/kg	554	787	567	876
SRB	bact/g	2100000	7000000	5200000	49000
Fe-OXID	bact/g	1900	1200	2000	49000
HFe-OXID	bact/g				297000
HMn-OXID	bact/g				297000
EH	mV	29	-45	-63	195
PH		7.4	7.3	6.7	3.9
TEMP	C	11	14	7.3	17

SAMPLE-DATE		SB1-11-88	SB1-01-89	TB3-01-88	TB1-06-88
TOTAL S	mg/kg			5990	6400
PYRITIC S	mg/kg			2330	2200
SO4 S	mg/kg			3440	1940
ORGANIC S	mg/kg			220	2300
TOTAL C	mg/kg			120000	1250
NH4-N	mg/kg			6.9	131
TOTAL N	mg/kg			3530	1340
TOTAL P	mg/kg			1690	359
Al	mg/kg			7030	12600
Cu	mg/kg			60	96
Fe	mg/kg			13500	24000
Pb	mg/kg			21	19
Mn	mg/kg			508	450
Zn	mg/kg			463	376
SRB	bact/g	3500000	2300000	10000000	370000
Fe-OXID	bact/g	190000	86000	5100	4100
HFe-OXID	bact/g	14000	5300		2200
HMn-OXID	bact/g	2200	5300		7200
EH	mV	225	388	170	185
PH		3.9	5.3	5.8	6.5
TEMP	C	4	6	8	10.9

SAMPLE-DATE		TB1-08-88	TB1-11-88	TB1-01-89	BB1-01-88
TOTAL S	mg/kg	2000	5791	8256	6260
PYRITIC S	mg/kg	1500			2520
SO4 S	mg/kg	1100			3260
ORGANIC S	mg/kg				480
TOTAL C	mg/kg	6080			92600
NH4-N	mg/kg	103	71		290
TOTAL N	mg/kg	419	3421		4330
TOTAL P	mg/kg	1070	1695		2500
Al	mg/kg	9240	7080		5790
Cu	mg/kg	71	56.8		39
Fe	mg/kg	21500	18600		12400
Pb	mg/kg	16	34		18
Mn	mg/kg	357	528		248
Zn	mg/kg	189	337		194
SRB	bact/g	570000	760000	77000	4700000
Fe-OXID	bact/g	1400	430	500	12000
HFe-OXID	bact/g	14000	4300	1400	
HMn-OXID	bact/g	36000	5600	2300	
EH	mV	175	-85	-40	130
PH		6.3	5.9	5.9	6.8
TEMP	C	13.6	6.8	5.8	4

SAMPLE-DATE		BB1-06-88	BB1-08-88	BB1-11-88	BB1-01-89
TOTAL S	mg/kg	7600	6600	5998	6025
PYRITIC S	mg/kg	1600	2000		
SO4 S	mg/kg	1660	660		
ORGANIC S	mg/kg	4300	3900		
TOTAL C	mg/kg	1260	4500		
NH4-N	mg/kg	270	213	340	
TOTAL N	mg/kg	2030	504	2429	
TOTAL P	mg/kg	93.8	998	3524	
Al	mg/kg	9660	6340	6870	
Cu	mg/kg	35	73	69.1	
Fe	mg/kg	14000	15800	16900	
Pb	mg/kg	13	23	23.7	
Mn	mg/kg	227	264	283	
Zn	mg/kg	148	222	255	
SRB	bact/g	400000	1100000	4000000	360000
Fe-OXID	bact/g	920	190	400	220
HFe-OXID	bact/g		1900		
HMn-OXID	bact/g		12000		
EH	mV	329	40	-75	93
PH		7.4	7.6	6.9	6.8
TEMP	C	13.5	14.2	7.3	4.9

SAMPLE-DATE		SB6-08-88	SB6-11-88	SB6-01-89	TB5-01-88
TOTAL S	mg/kg	7600			5900
PYRITIC S	mg/kg	2100			3290
SO4 S	mg/kg	180			2380
ORGANIC S	mg/kg	5300			230
TOTAL C	mg/kg	1730			84500
NH4-N	mg/kg	128			110
TOTAL N	mg/kg	5400			3840
TOTAL P	mg/kg	679			2010
Al	mg/kg	9910			6850
Cu	mg/kg	263			61
Fe	mg/kg	33500			14300
Pb	mg/kg	35			30
Mn	mg/kg	339			328
Zn	mg/kg	808			211
SRB	bact/g	62000	830000	1800000	9300000
Fe-OXID	bact/g	1700	1700	6600	7600
HFe-OXID	bact/g	103000	2700	1500	
HMn-OXID	bact/g	103000	1500	1200	
EH	mV	580	105	160	140
PH		3.4	4.1	4.7	6.2
TEMP	C	16	4	-.5	3

SAMPLE-DATE		TB6-06-88	TB6-08-88	TB6-11-88	TB6-01-89
TOTAL S	mg/kg	7000	10900	8281	7103
PYRITIC S	mg/kg	1400	2100		
SO4 S	mg/kg	1910	2400		
ORGANIC S	mg/kg	3700	6400		
TOTAL C	mg/kg	1940	5200		
NH4-N	mg/kg	90	69	118	
TOTAL N	mg/kg	4620	294	4065	
TOTAL P	mg/kg	702	1070	2713	
Al	mg/kg	11400	7360	6520	
Cu	mg/kg	264	141	104	
Fe	mg/kg	14700	17800	12700	
Pb	mg/kg	29	34	40.7	
Mn	mg/kg	374	593	505	
Zn	mg/kg	1030	873	772	
SRB	bact/g	180000	8400000	5500000	89000
Fe-OXID	bact/g	1100	450	390	130
HFe-OXID	bact/g	3200	36000	1800	1200
HMn-OXID	bact/g	9700	190000	24000	2300
EH	mV	319	130	-33	-70
PH		6.7	7	5.9	5.7
TEMP	C	12.5	13.8	1	5

SAMPLE-DATE		BB5-01-88	BB6-06-88	BB6-08-88	BB6-11-88
TOTAL S	mg/kg	5970	6700	6600	8283
PYRITIC S	mg/kg	2960	1500	1700	
SO4 S	mg/kg	2560	1770	780	
ORGANIC S	mg/kg	450	3400	4100	
TOTAL C	mg/kg	116000	1110	6220	
NH4-N	mg/kg	288	326	209	290
TOTAL N	mg/kg	3980	1470	419	3852
TOTAL P	mg/kg	2440	273	1260	2422
Al	mg/kg	5920	9630	6450	6760
Cu	mg/kg	27	46	74	60.1
Fe	mg/kg	11300	13700	11800	12100
Pb	mg/kg	14	15	26	25.8
Mn	mg/kg	187	294	279	342
Zn	mg/kg	105	189	296	100
SRB	bact/g	2500000	1100000	4500000	3300000
Fe-OXID	bact/g	1500	820	1500	30
HFe-OXID	bact/g				
HMn-OXID	bact/g				
EH	mV	-160	285	45	-130
PH		7.1	6.2	7.3	6.9
TEMP	C	4.5	12.8	14.2	7.2

SAMPLE-DATE		BB6-01-89	SC1-08-88	SC1-11-88	SC1-01-89
TOTAL S	mg/kg	5977	24100		
PYRITIC S	mg/kg		1800		
SO4 S	mg/kg		15900		
ORGANIC S	mg/kg		6400		
TOTAL C	mg/kg		305		
NH4-N	mg/kg				
TOTAL N	mg/kg		2320		
TOTAL P	mg/kg		1170		
Al	mg/kg		11400		
Cu	mg/kg		269		
Fe	mg/kg		142000		
Pb	mg/kg		32		
Mn	mg/kg		670		
Zn	mg/kg		820		
SRB	bact/g	140000	2500000	660000	20000
Fe-OXID	bact/g	100	62000	6600	740
HFe-OXID	bact/g		566000	5300	450
HMn-OXID	bact/g		566000	5100	1200
EH	mV	-65	350	180	168
PH		7	4.9	3.9	4.8
TEMP	C	5	17	1.5	6.8

SAMPLE-DATE		TC1-01-88	TC1-06-88	TC1-08-88	TC1-11-88
TOTAL S	mg/kg	5610	8300		7865
PYRITIC S	mg/kg	2640	2300		
SO4 S	mg/kg	2610	1990		
ORGANIC S	mg/kg	360	4000		
TOTAL C	mg/kg	68000	960	5110	
NH4-N	mg/kg	45.6	134	85	40
TOTAL N	mg/kg	4610	3440	184	1863
TOTAL P	mg/kg	1860	779		1349
Al	mg/kg	6840	10600	9340	8650
Cu	mg/kg	34	50	69	68.4
Fe	mg/kg	13100	18500	23800	21000
Pb	mg/kg	18	13	18	23.8
Mn	mg/kg	320	449	454	411
Zn	mg/kg	178	227	164	340
SRB	bact/g	12000000	760000	3200000	2000000
Fe-OXID	bact/g	38000	2600	1100	1400
HFe-OXID	bact/g		16000	27000	4700
HMn-OXID	bact/g		17000	27000	18000
EH	mV	-10	340	339	-80
PH		7	6.9	5.8	5.6
TEMP	C	7	15.5	17.3	5.3

SAMPLE-DATE		TC1-01-89	BC1-01-88	BC1-06-88	BC1-08-88
TOTAL S	mg/kg	685	5360	5700	
PYRITIC S	mg/kg		2730	1300	
SO4 S	mg/kg		2160	1400	
ORGANIC S	mg/kg		470	3000	
TOTAL C	mg/kg		123000	520	3600
NH4-N	mg/kg		237	241	245
TOTAL N	mg/kg		4760	3450	752
TOTAL P	mg/kg		2240	525	
Al	mg/kg		6770	11100	6950
Cu	mg/kg		27	50	69
Fe	mg/kg		12700	15600	14500
Pb	mg/kg		18	13	15
Mn	mg/kg		238	242	210
Zn	mg/kg		137	177	191
SRB	bact/g	21000	8300000	1100000	7700000
Fe-OXID	bact/g	1000	4100	870	2000
HFe-OXID	bact/g	910			1800
HMn-OXID	bact/g	1400			14000
EH	mV	-18	85	159	315
PH		5.4	5.7	6.3	5.8
TEMP	C	7.2	5	13.1	16

SAMPLE-DATE		BC1-11-88	BC1-01-89	SC5-08-88	SC5-11-88
TOTAL S	mg/kg	5213	6364	16600	
PYRITIC S	mg/kg			800	
SO4 S	mg/kg			11500	
ORGANIC S	mg/kg			4300	
TOTAL C	mg/kg			1050	
NH4-N	mg/kg	267		48	
TOTAL N	mg/kg	3014		2030	
TOTAL P	mg/kg	2822		521	
Al	mg/kg	7450		12000	
Cu	mg/kg	46		208	
Fe	mg/kg	12450		99800	
Pb	mg/kg	21.9		31	
Mn	mg/kg	247		412	
Zn	mg/kg	148.5		255	
SRB	bact/g	11000000	220000	2100000	1800000
Fe-OXID	bact/g	200	5900	21000	24000
HFe-OXID	bact/g			484000	8500
HMn-OXID	bact/g			484000	7300
EH	mV	-103	0	500	0
PH		7	6.7	3.9	5.1
TEMP	C	5.9	4.7	17	1

SAMPLE-DATE		SC5-01-89	TC4-01-88	TC5-06-88	TC5-08-88
TOTAL S	mg/kg		5360	7500	9200
PYRITIC S	mg/kg		2040	1800	4300
SO4 S	mg/kg		2980	1550	1200
ORGANIC S	mg/kg		340	4100	3700
TOTAL C	mg/kg		76400	1850	6500
NH4-N	mg/kg		321	155	126
TOTAL N	mg/kg		3060	1060	1370
TOTAL P	mg/kg		1680	791	1030
Al	mg/kg		7590	13700	7780
Cu	mg/kg		40	77	89
Fe	mg/kg		16500	19100	16400
Pb	mg/kg		18	30	23
Mn	mg/kg		369	444	432
Zn	mg/kg		197	344	354
SRB	bact/g	740000	10000000	180000	17000000
Fe-OXID	bact/g	14000	5100	990	160
HFe-OXID	bact/g	1300		1700	44000
HMn-OXID	bact/g	11000		4900	35000
EH	mV	550	80	370	210
PH		3.2	5.8	5.8	6.3
TEMP	C	5	4	16	16.3

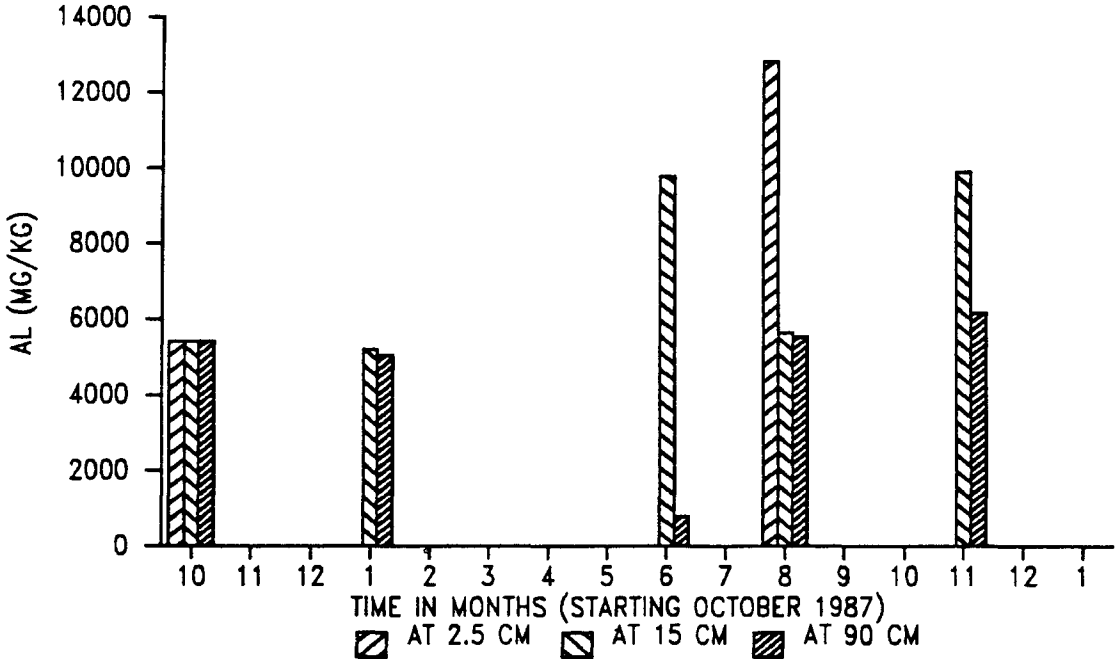
SAMPLE-DATE		TC5-11-88	TC5-01-89	BC4-01-88	BC5-06-88
TOTAL S	mg/kg	10925	9267	5000	6300
PYRITIC S	mg/kg			2200	1600
SO4 S	mg/kg			2420	1220
ORGANIC S	mg/kg			380	3500
TOTAL C	mg/kg			91600	686
NH4-N	mg/kg	109		284	311
TOTAL N	mg/kg	3483		3540	1570
TOTAL P	mg/kg	2069		2400	935
Al	mg/kg	10100		6870	10300
Cu	mg/kg	93.3		33	33
Fe	mg/kg	20200		13300	14200
Pb	mg/kg	46.6		19	17
Mn	mg/kg	596		228	224
Zn	mg/kg	724		155	144
SRB	bact/g	6100000	460000	8600000	1200000
Fe-OXID	bact/g	370	1100	18000	960
HFe-OXID	bact/g	3100	1800		
HMn-OXID	bact/g	3000	18000		
EH	mV	-63	-110	155	302
PH		5.7	6.5	5.6	6.3
TEMP	C	5.4	4.5	6	12.5

SAMPLE-DATE		BC5-08-88	BC5-11-88	BC5-01-89
TOTAL S	mg/kg	8900	7711	7523
PYRITIC S	mg/kg	1600		
SO4 S	mg/kg	870		
ORGANIC S	mg/kg	6400		
TOTAL C	mg/kg	5020		
NH4-N	mg/kg	203	295	
TOTAL N	mg/kg	555	4758	
TOTAL P	mg/kg	1150	3061	
Al	mg/kg	7060	7500	
Cu	mg/kg	49	44.7	
Fe	mg/kg	12000	13100	
Pb	mg/kg	20	24.7	
Mn	mg/kg	214	230	
Zn	mg/kg	160	197	
SRB	bact/g	4600000	9700000	120000.
Fe-OXID	bact/g	1300	370	650
HFe-OXID	bact/g			
HMn-OXID	bact/g			
EH	mV	140	-103	-100
PH		6.3	6.9	7
TEMP	C	16.3	4.2	4

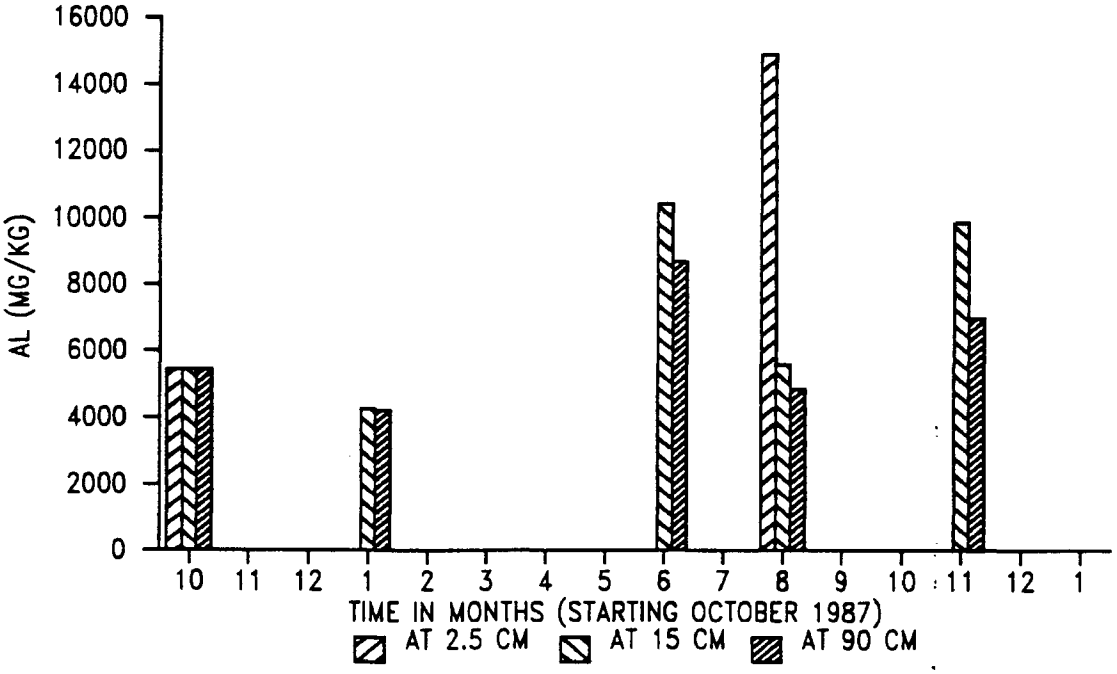
APPENDIX D

GRAPHICAL REPRESENTATION OF IRON, MANGANESE, ZINC, ALUMINUM, LEAD AND COPPER

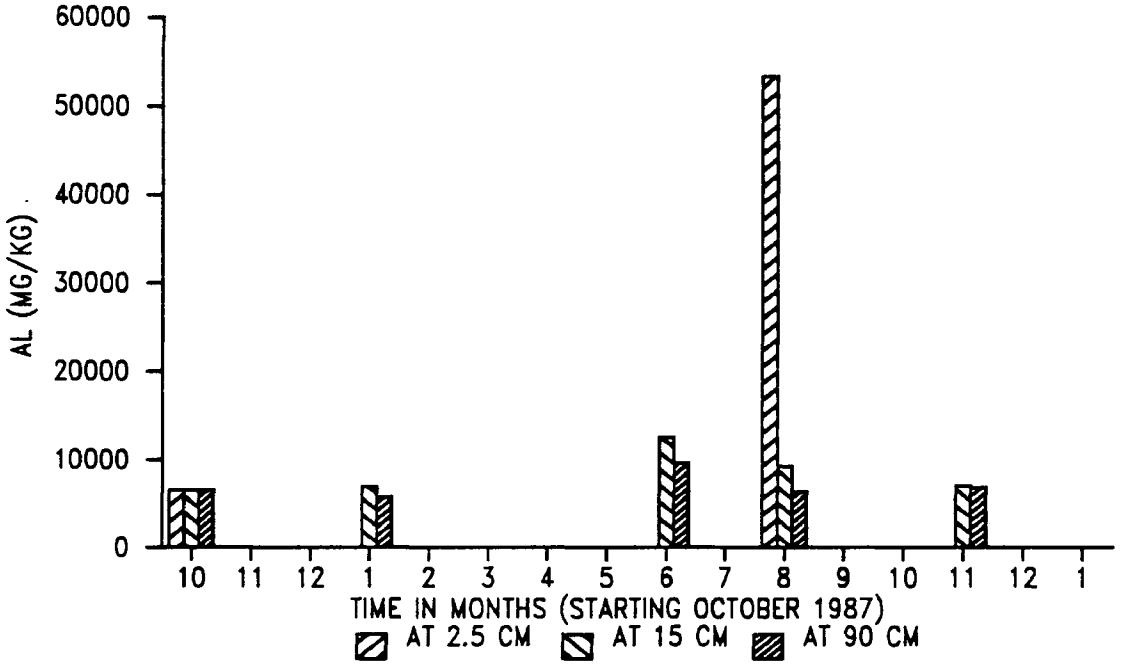
AL CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



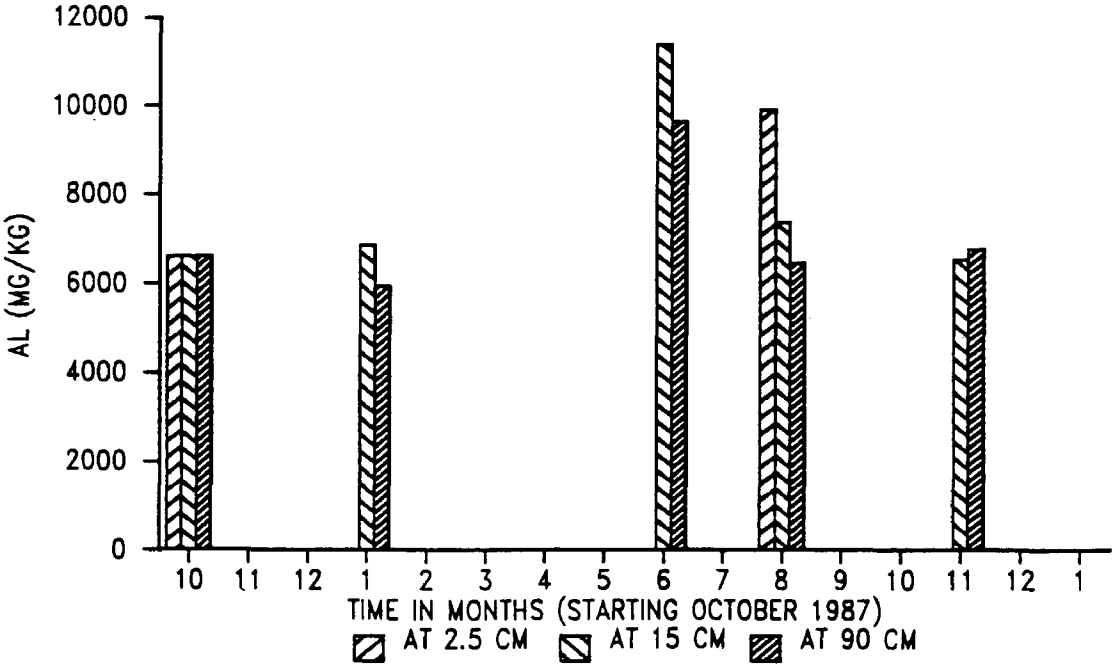
AL CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



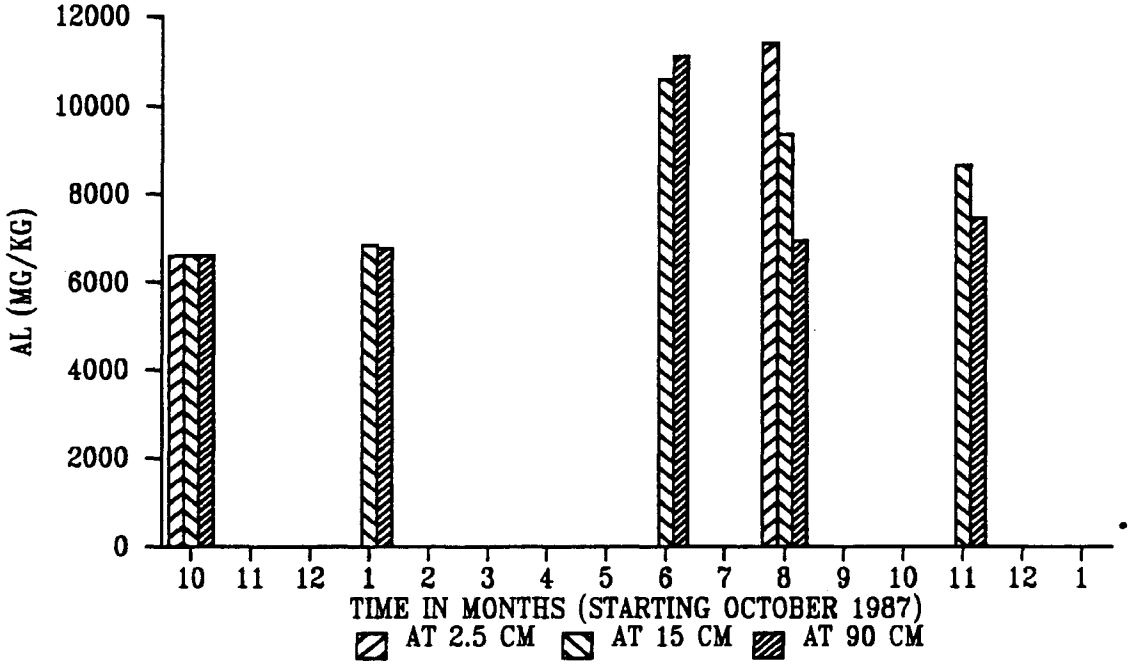
AL CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



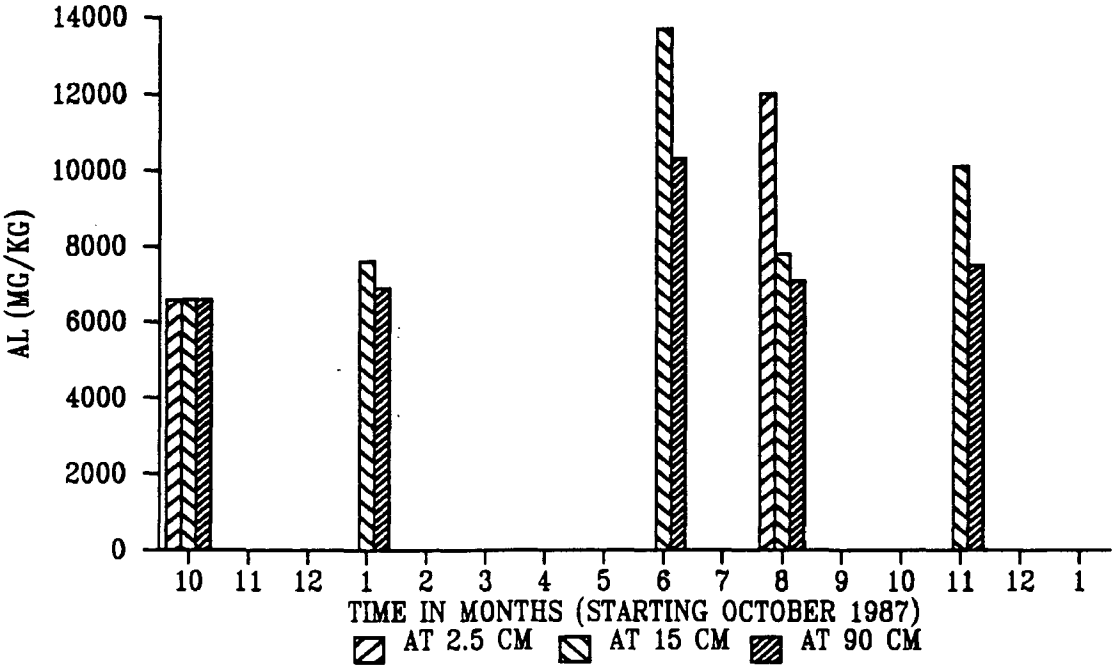
AL CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



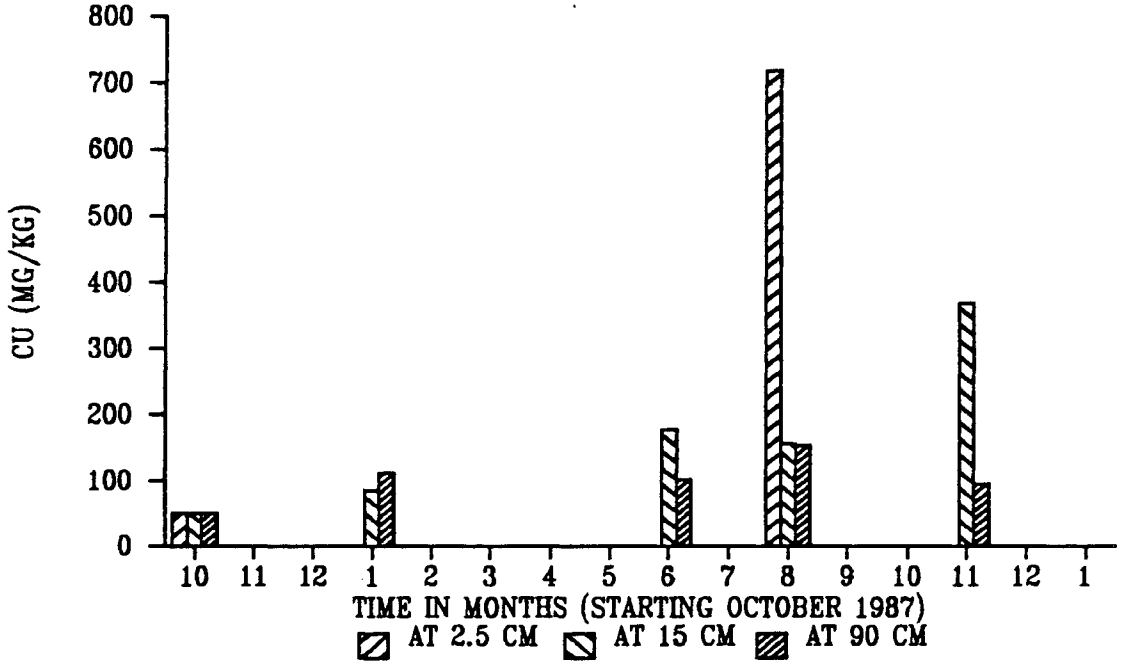
AL CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



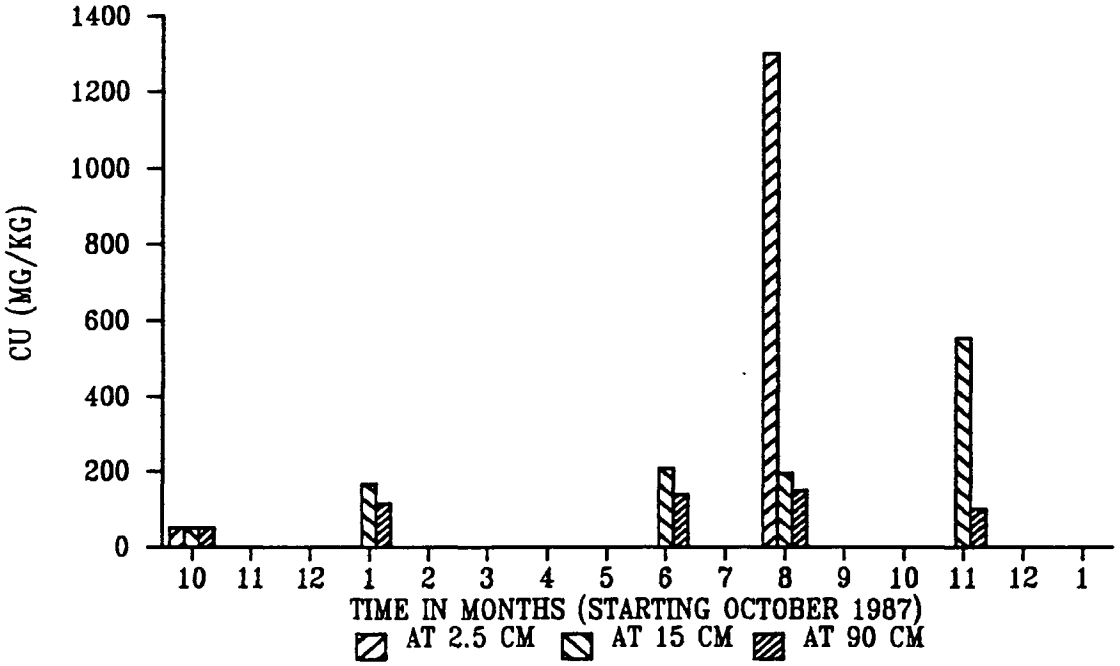
AL CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



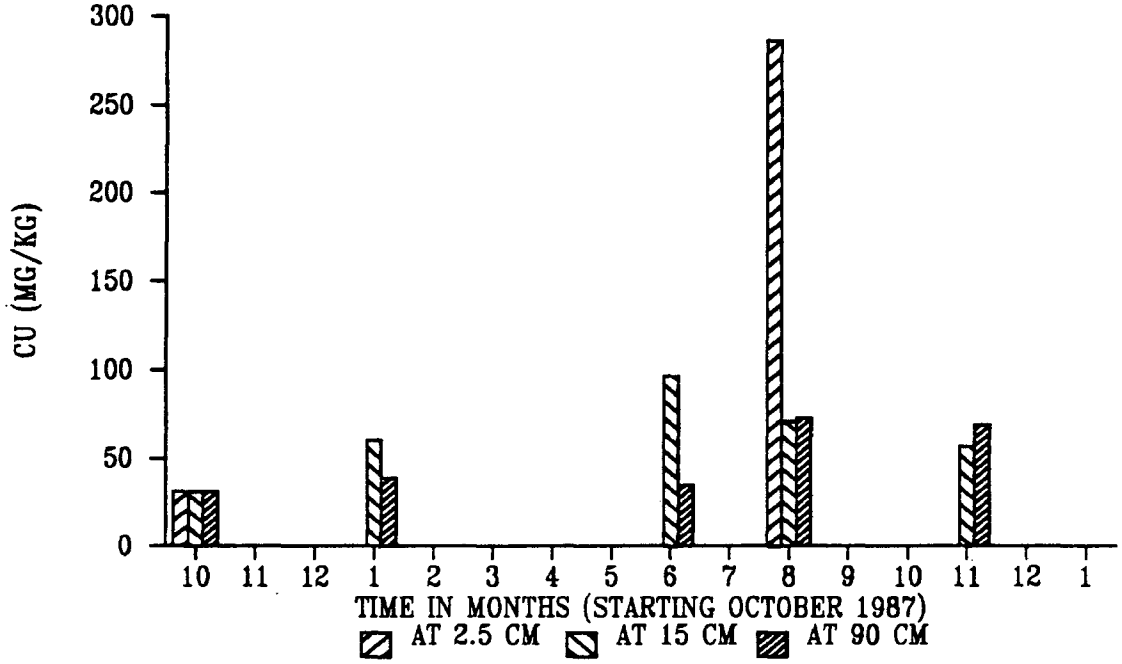
CU CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



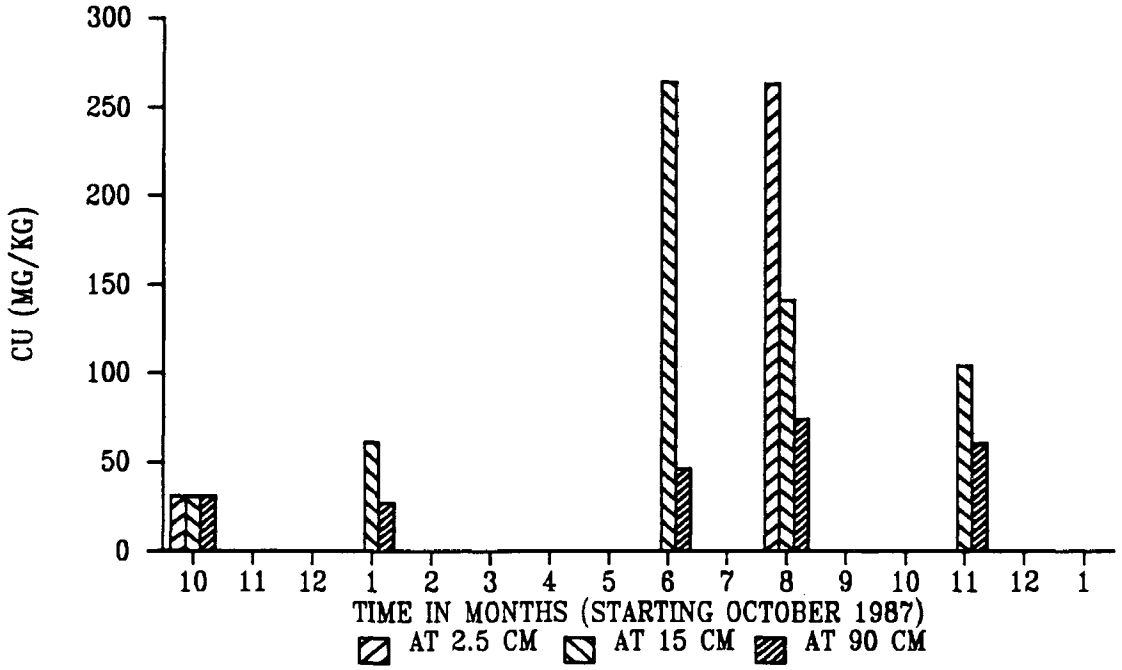
CU CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



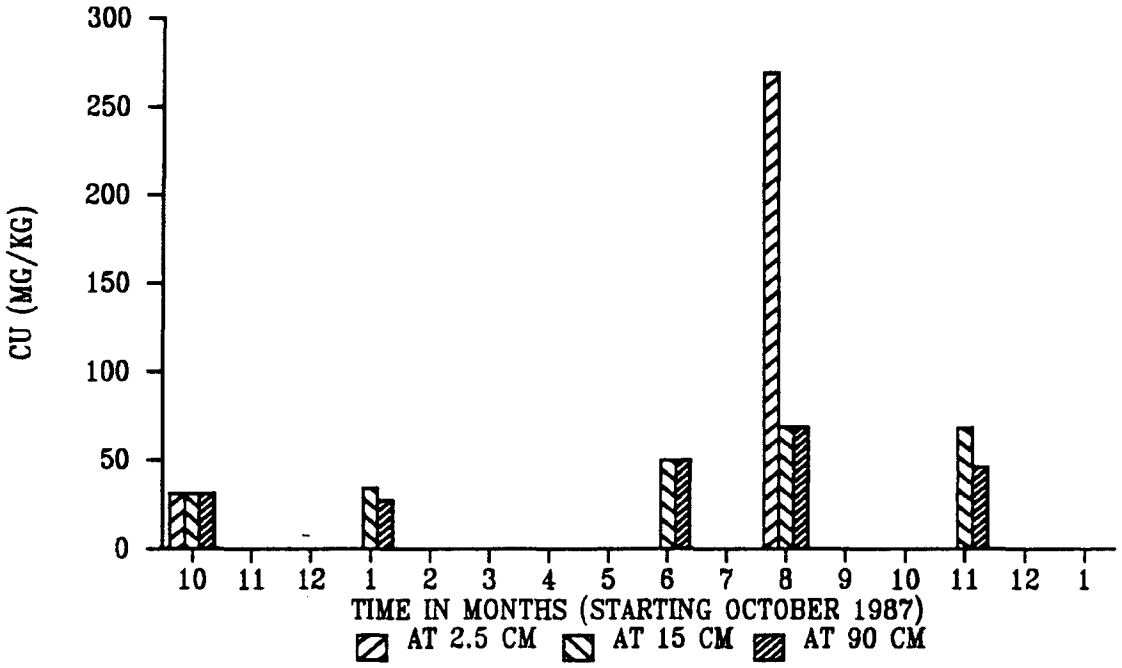
CU CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



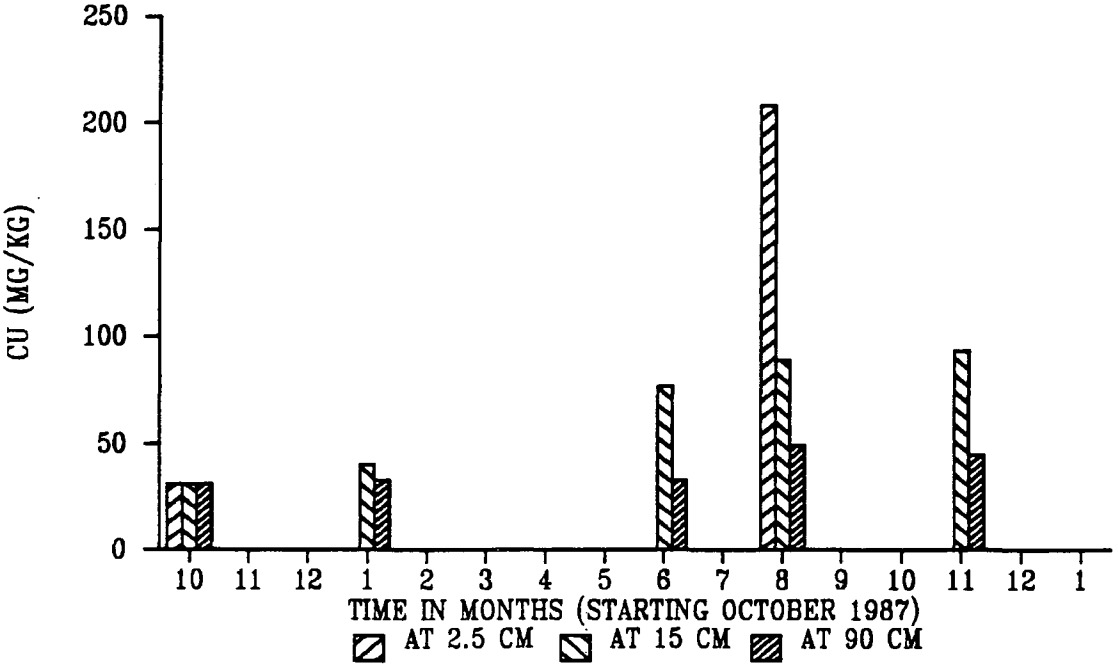
CU CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



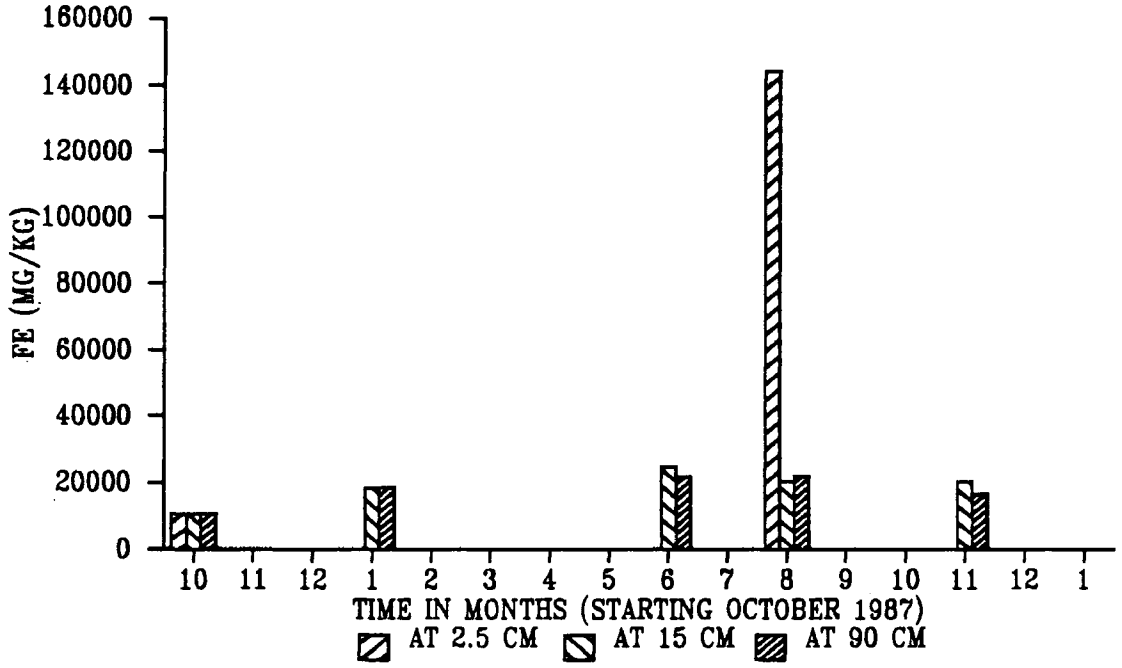
CU CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



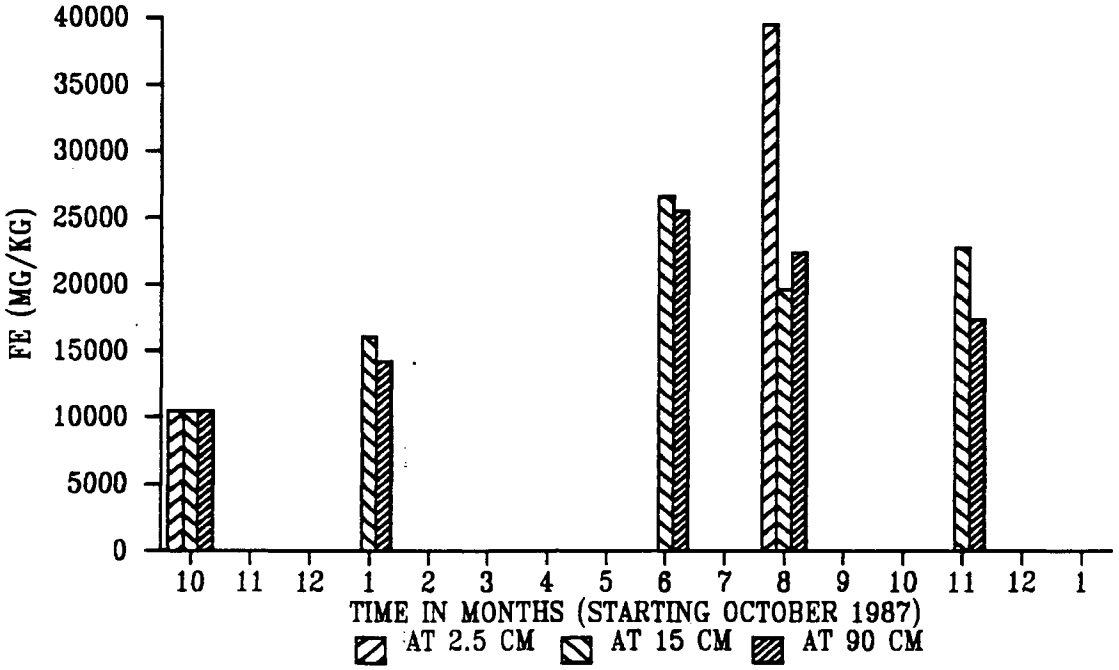
CU CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



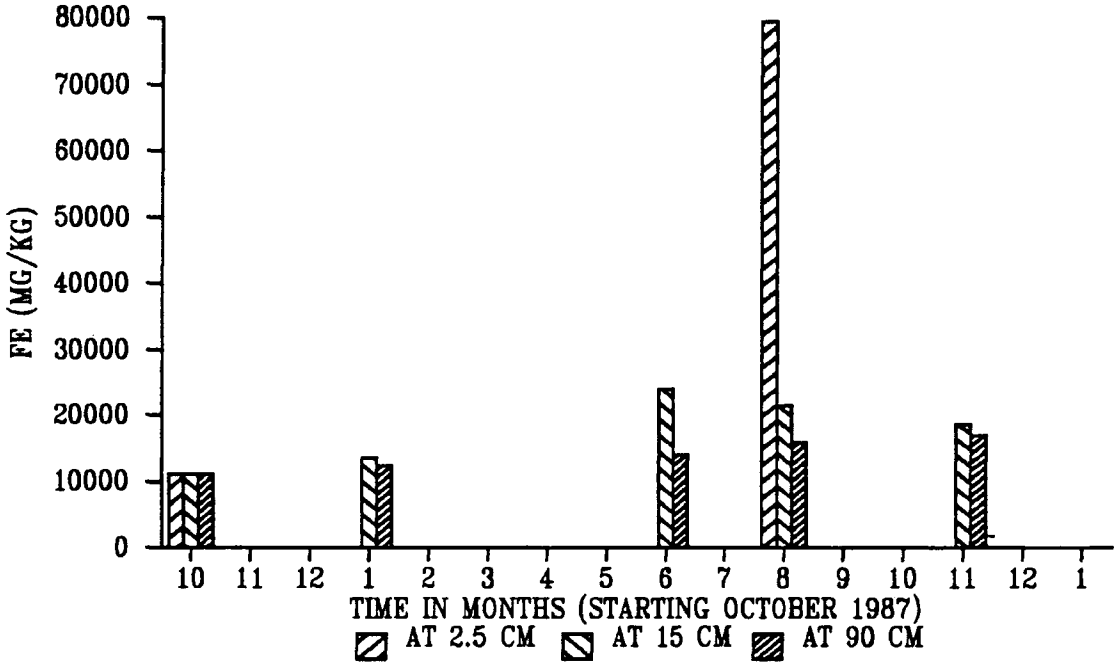
FE CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



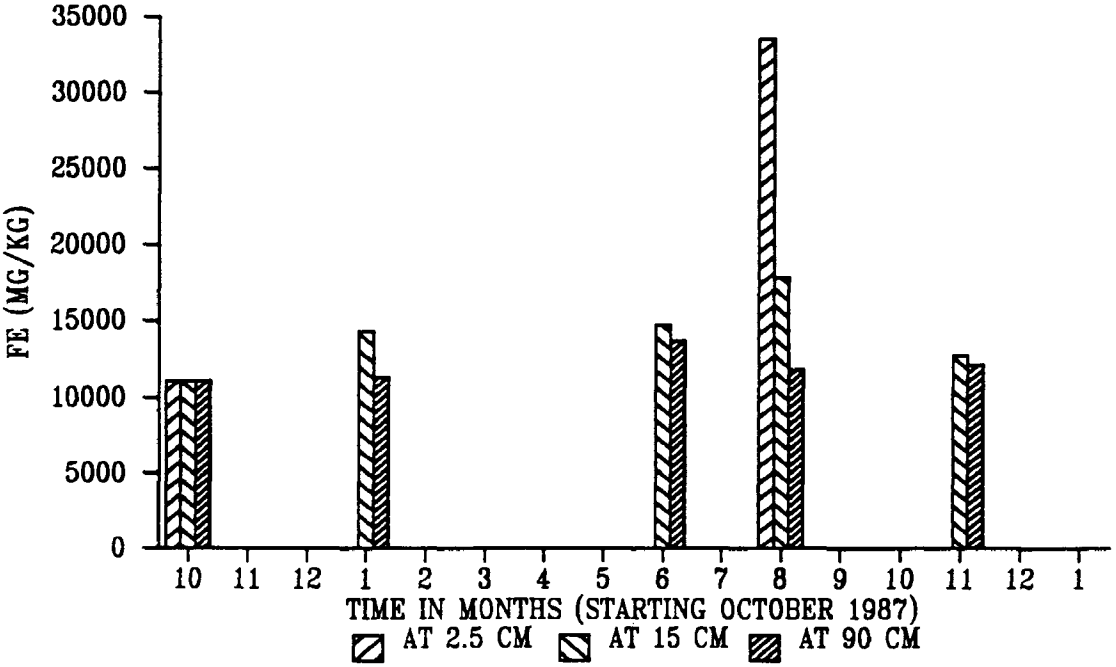
FE CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



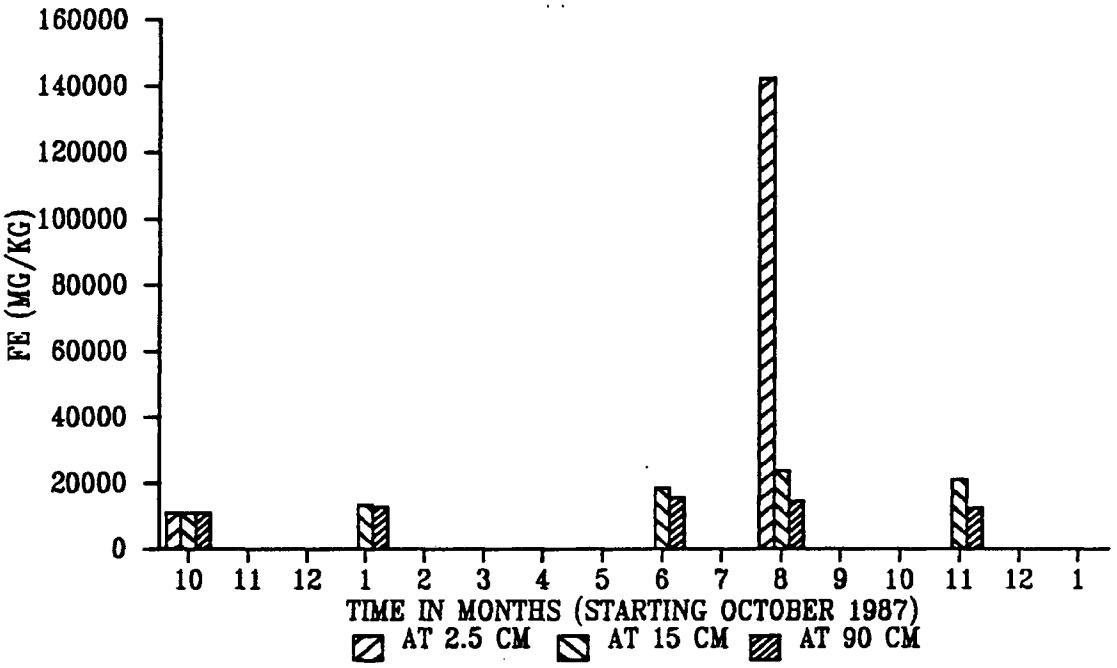
FE CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



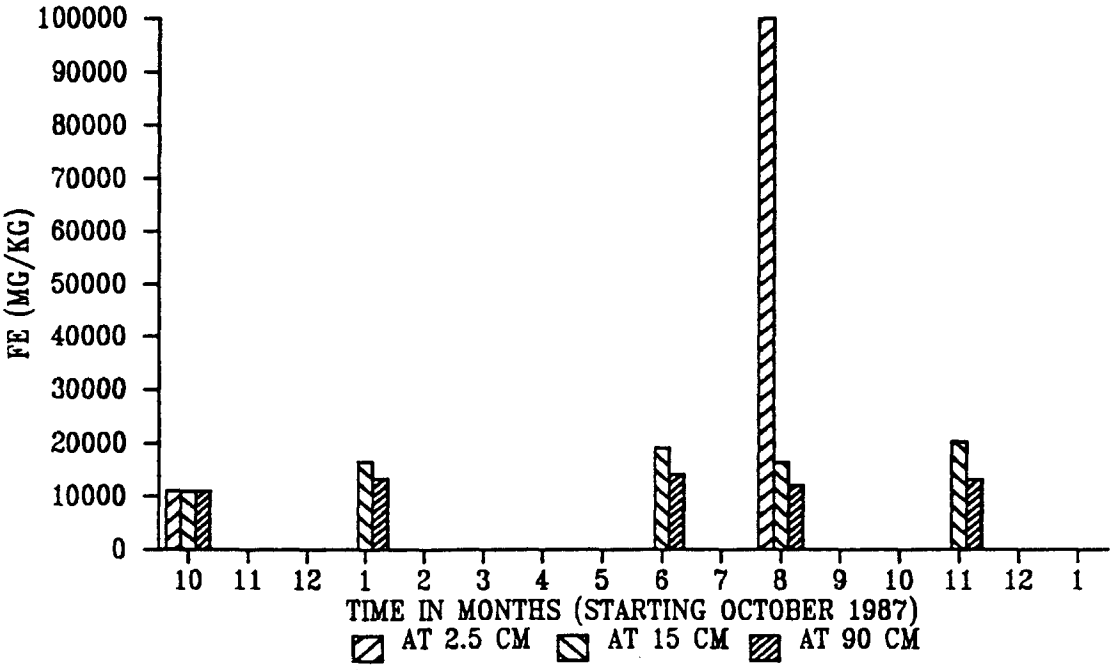
FE CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



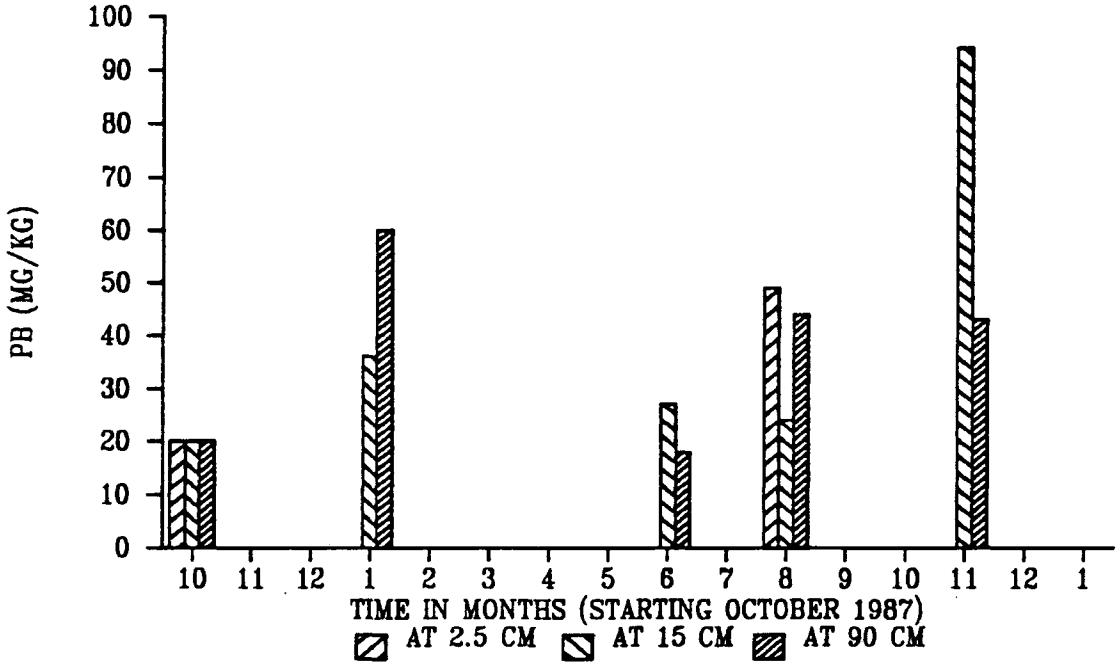
FE CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



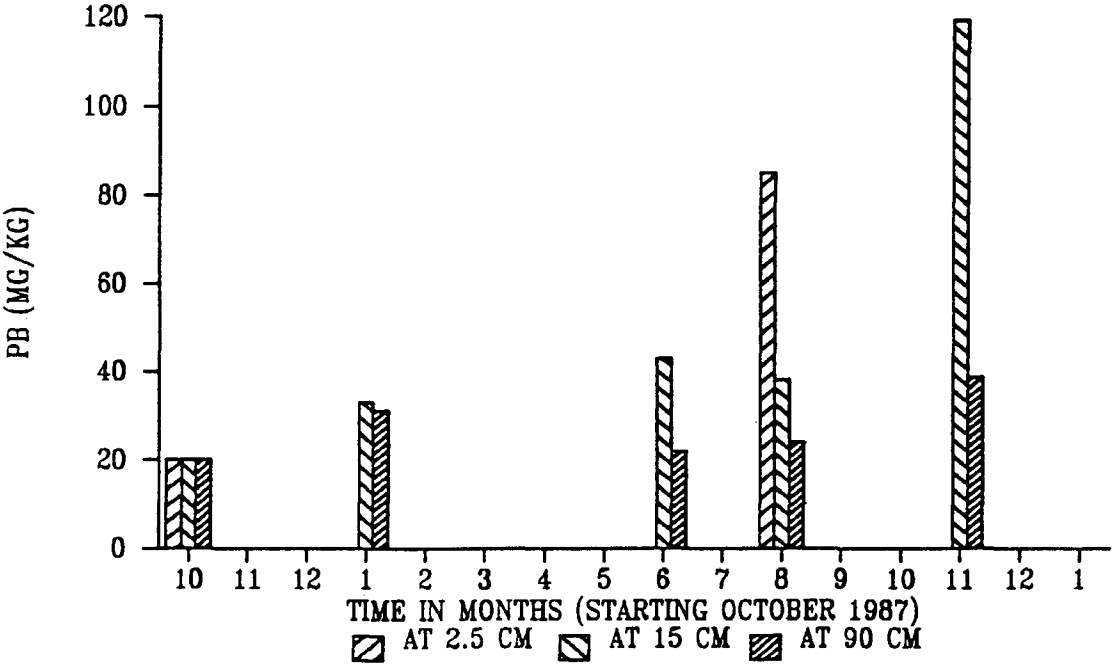
FE CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



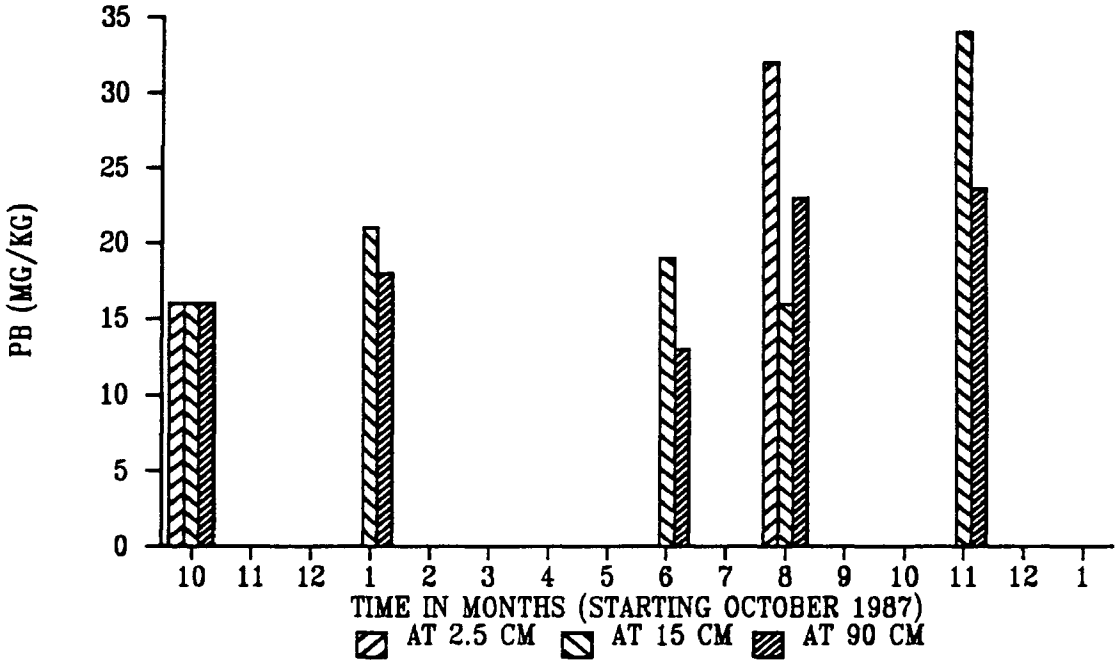
PB CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



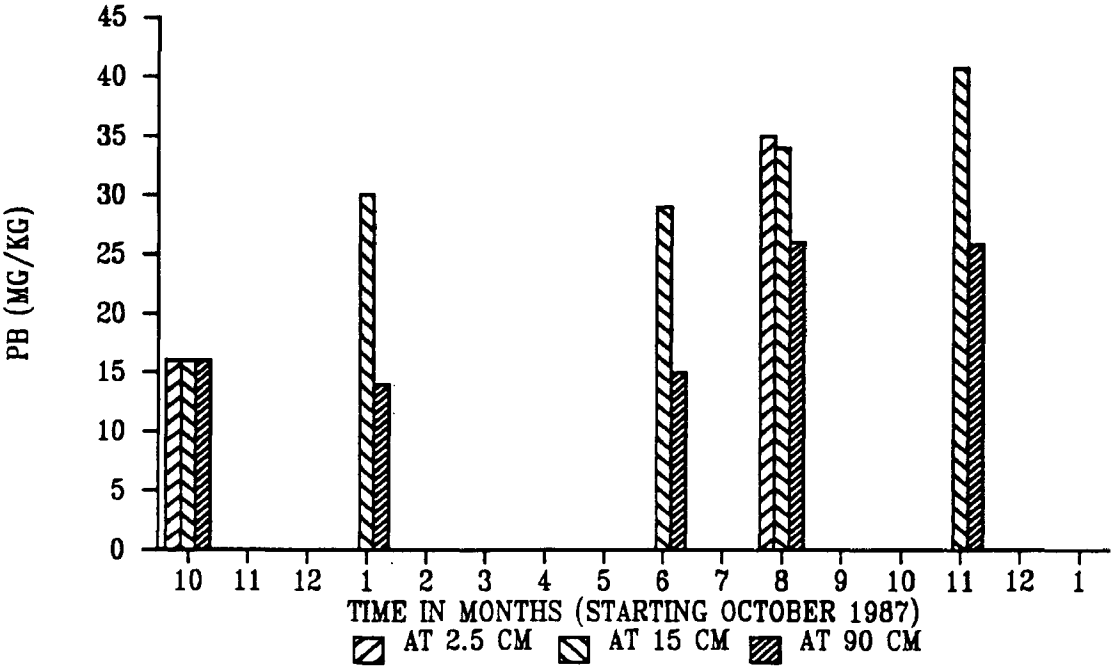
PB CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



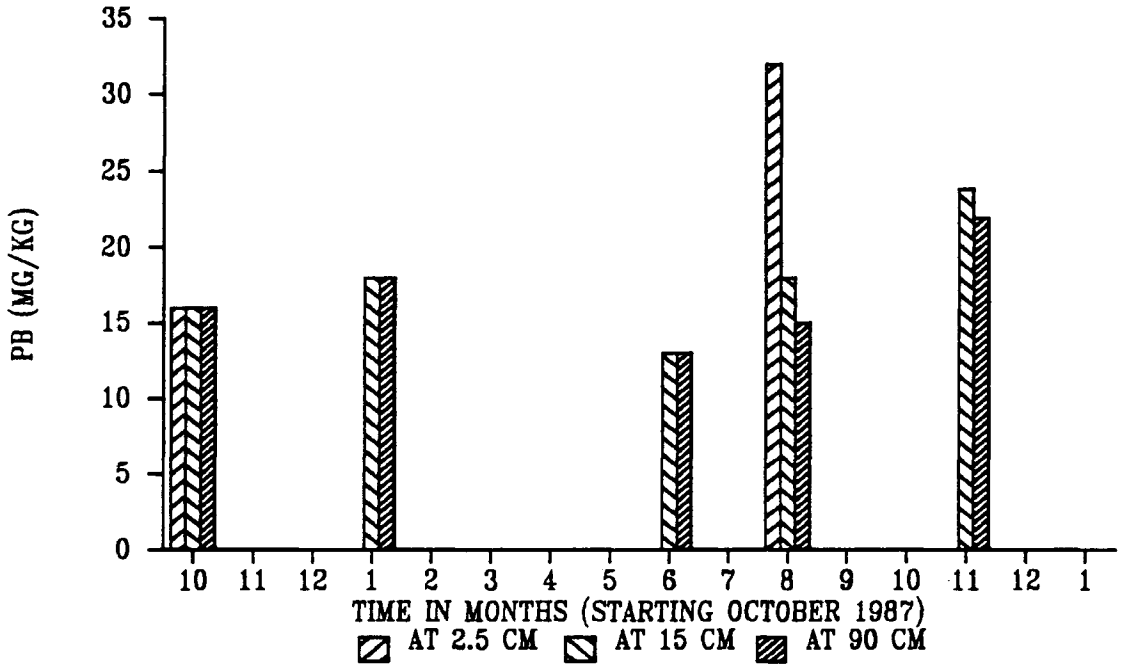
PB CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



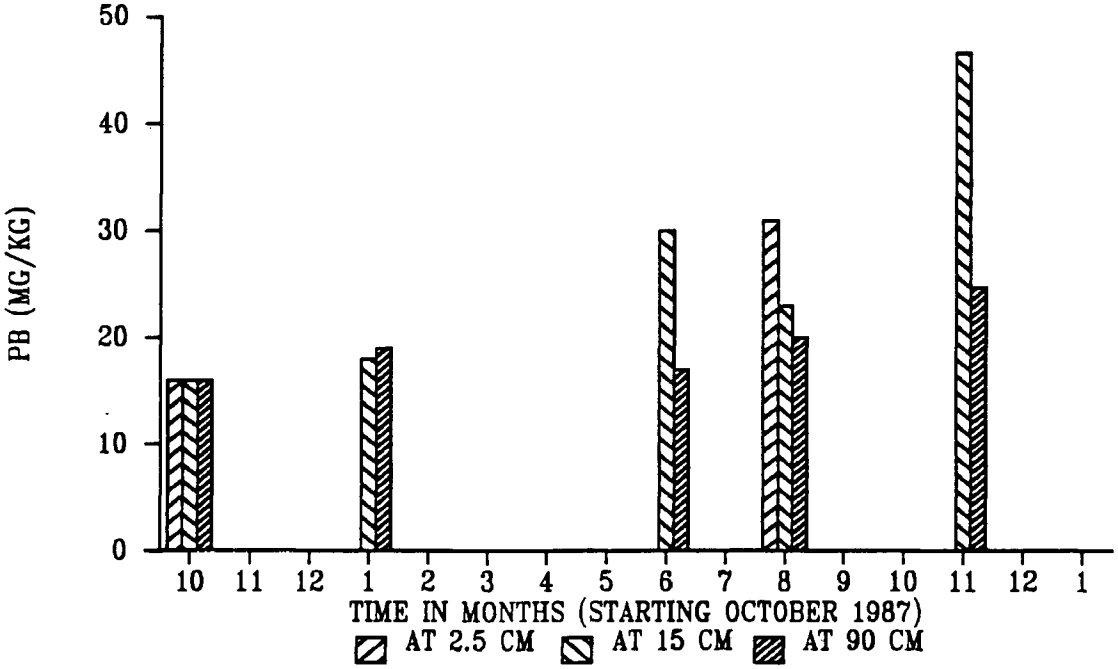
PB CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



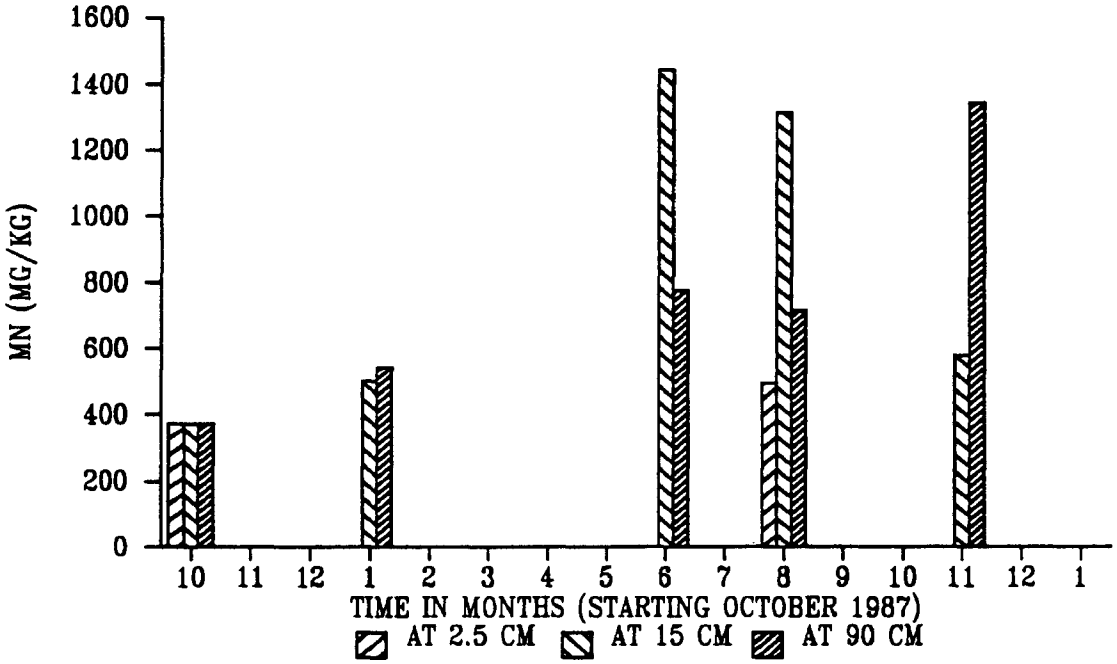
PB CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



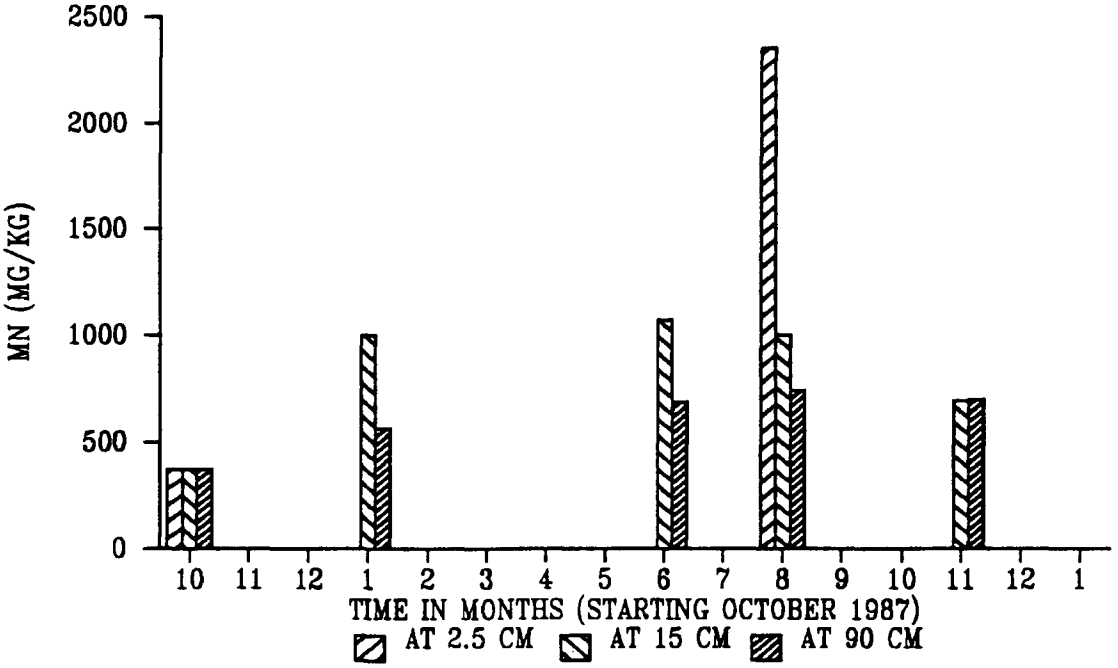
PB CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



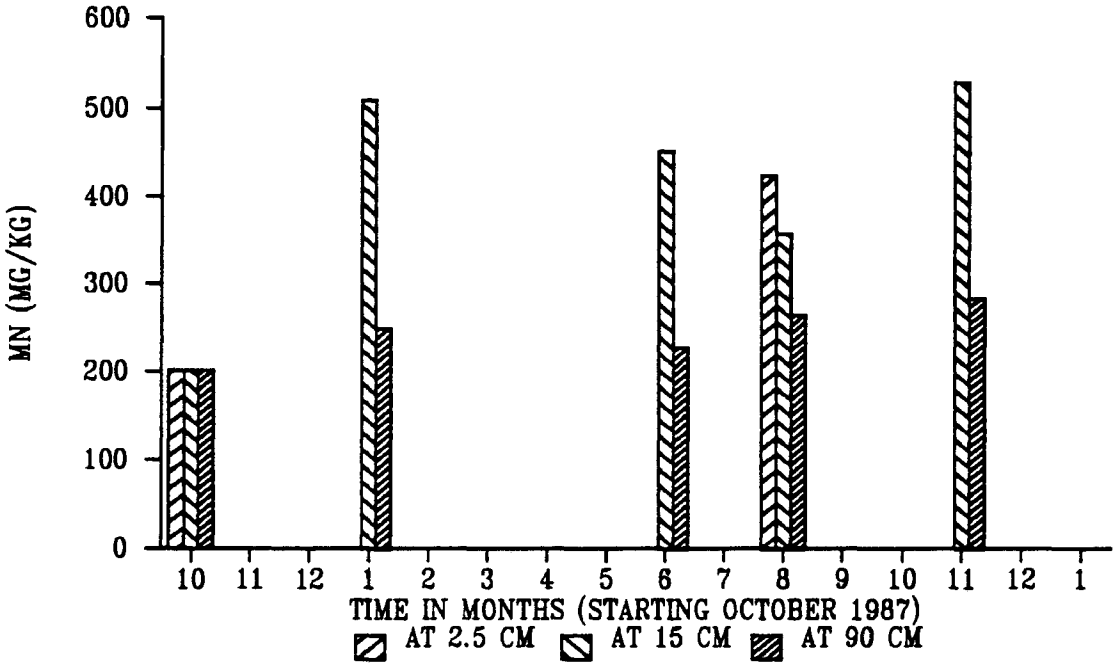
MN CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



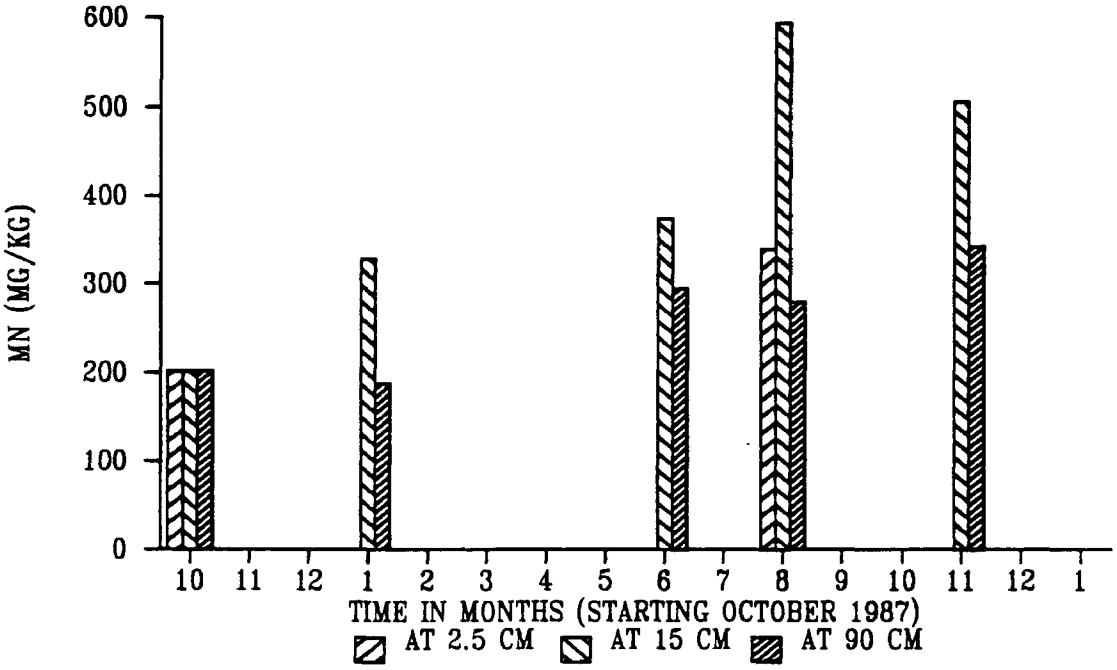
MN CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM



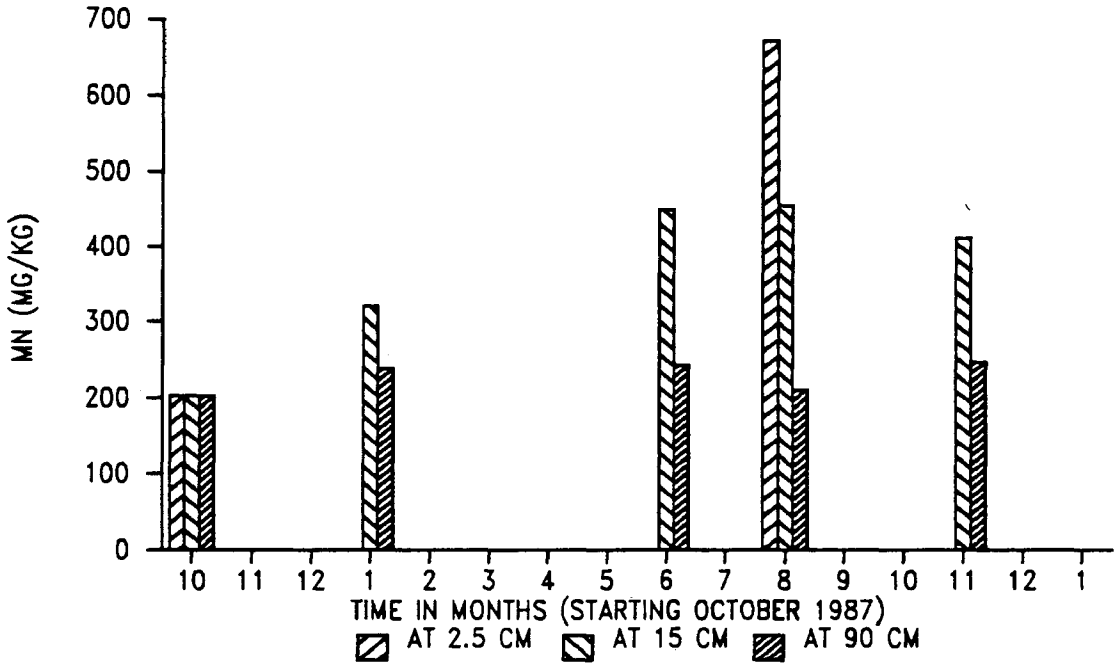
MN CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM



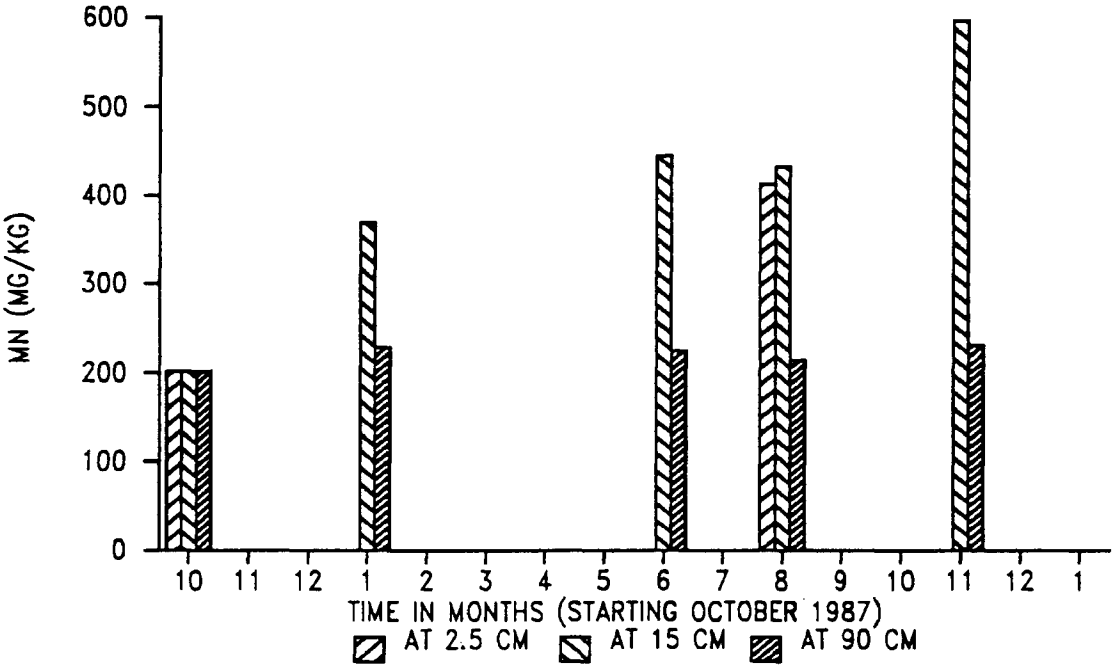
MN CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM

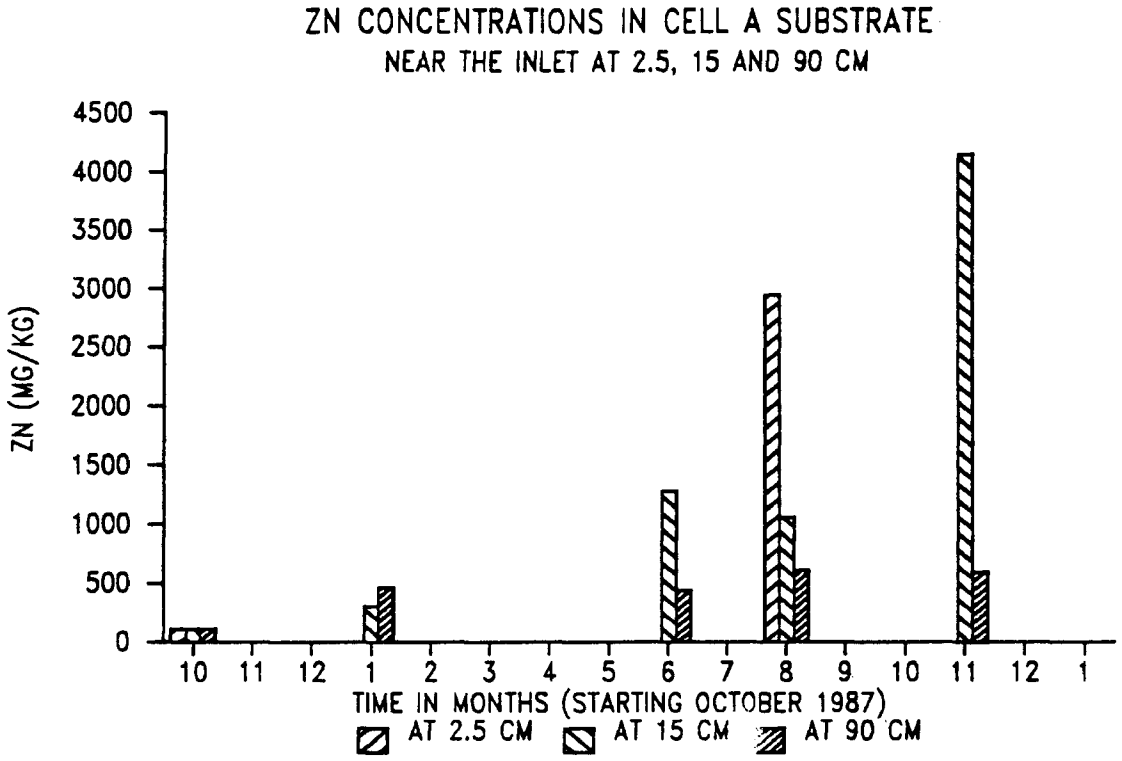


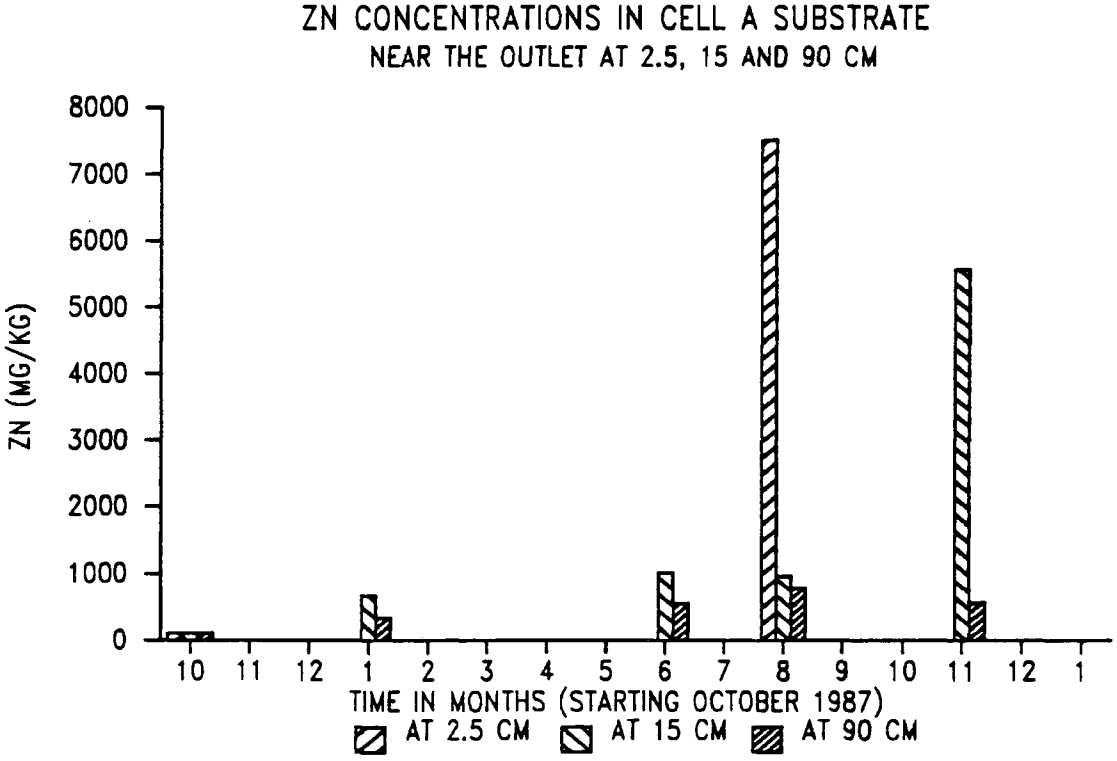
MN CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM

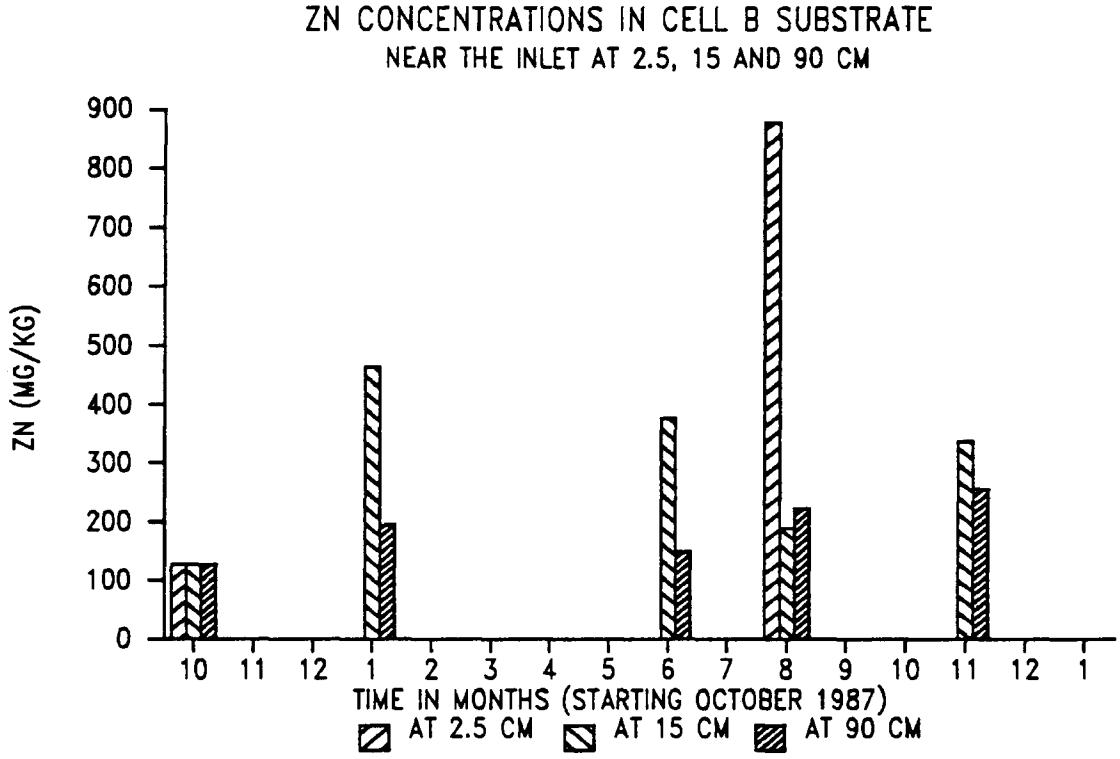


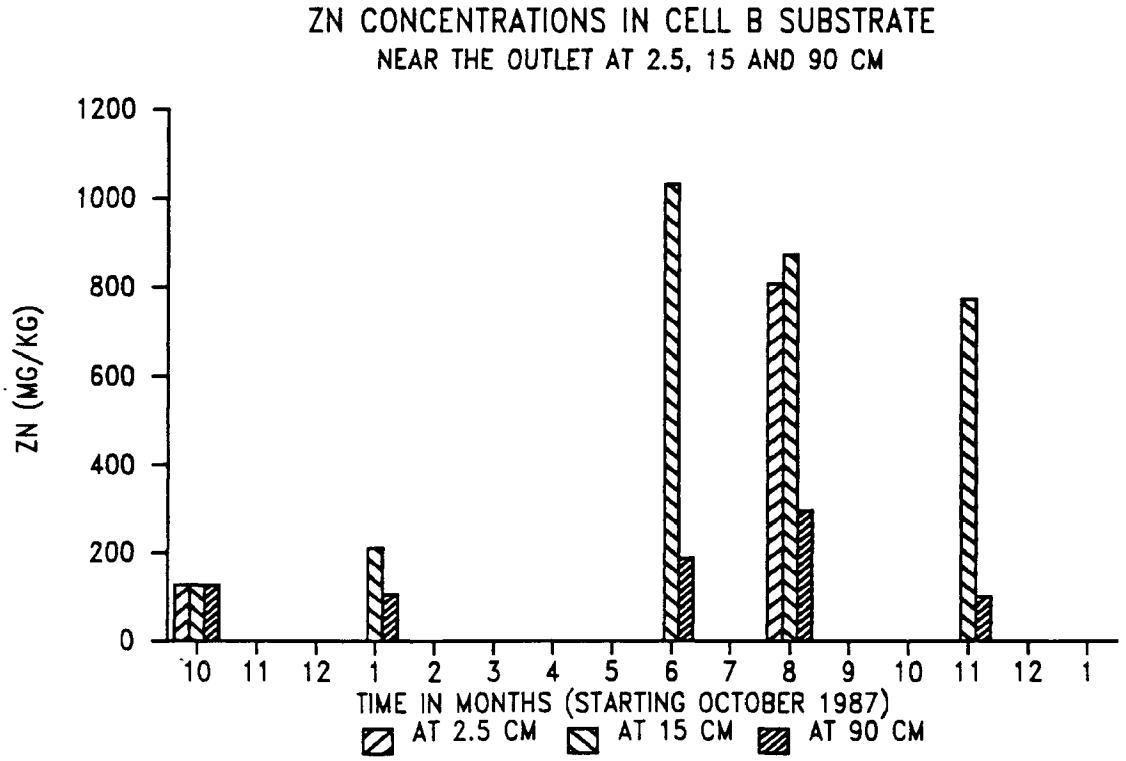
MN CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM

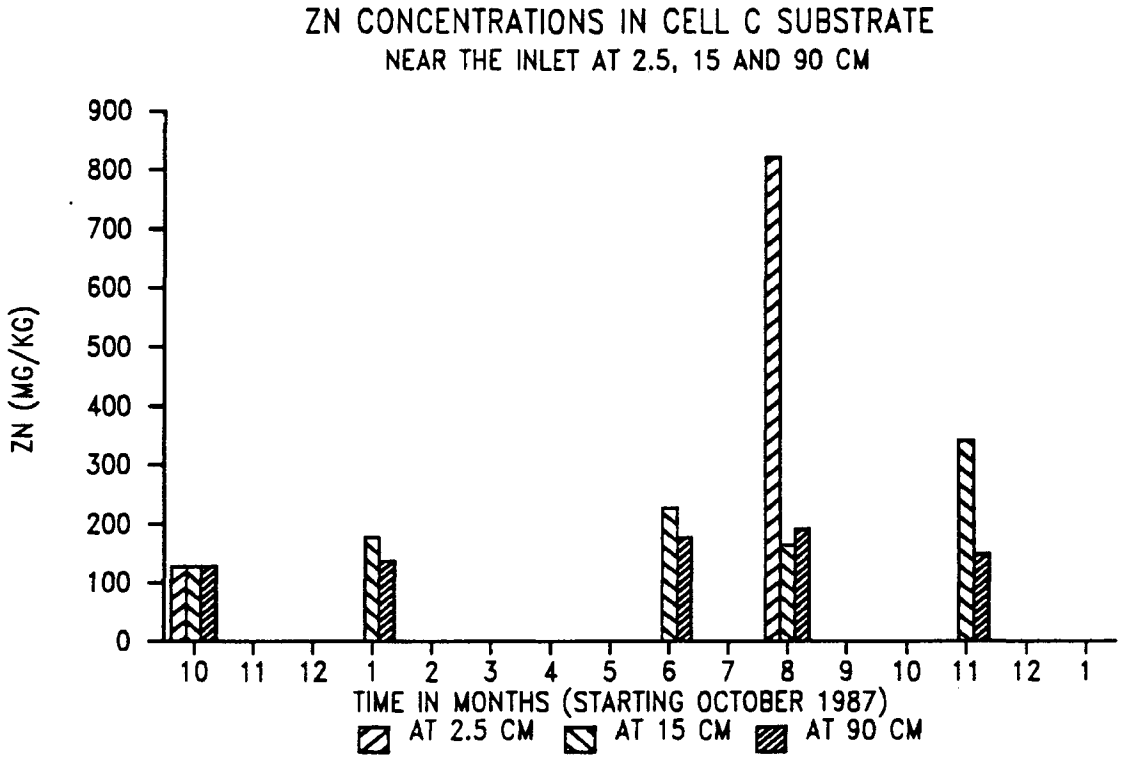


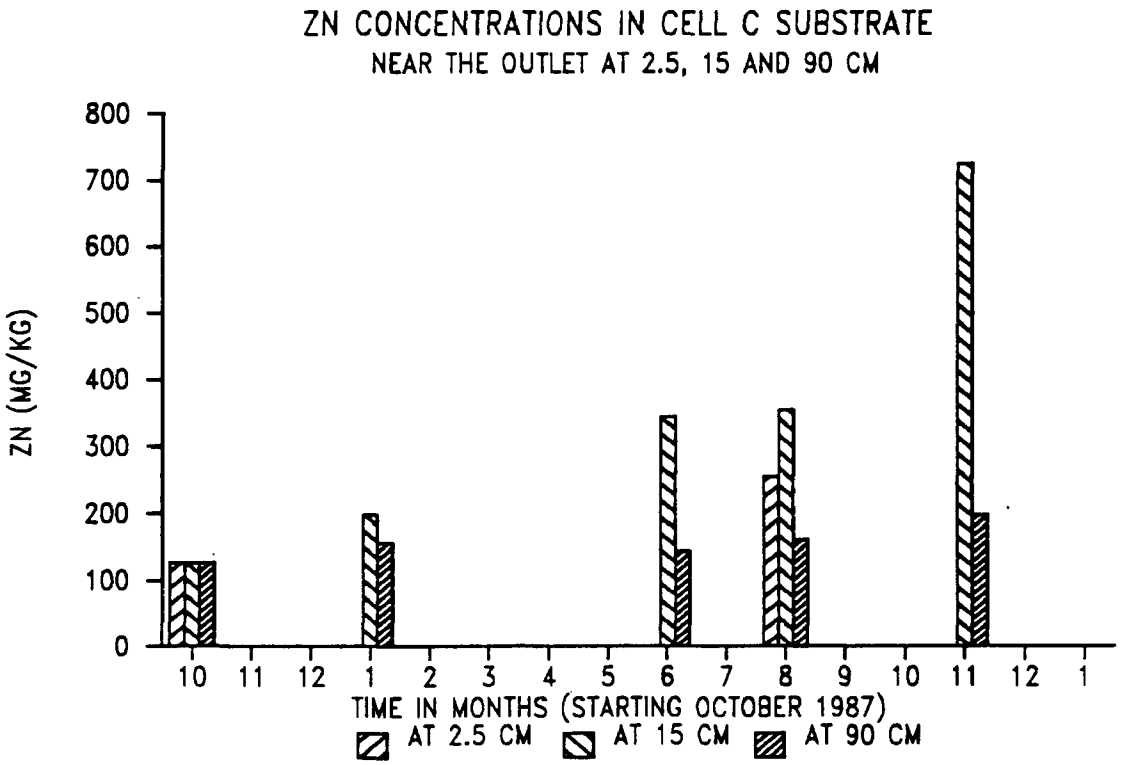

ZN CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM
ZN (MG/KG)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1
TIME IN MONTHS (STARTING OCTOBER 1987)
AT 2.5 CM
AT 15 CM
AT 90 CM

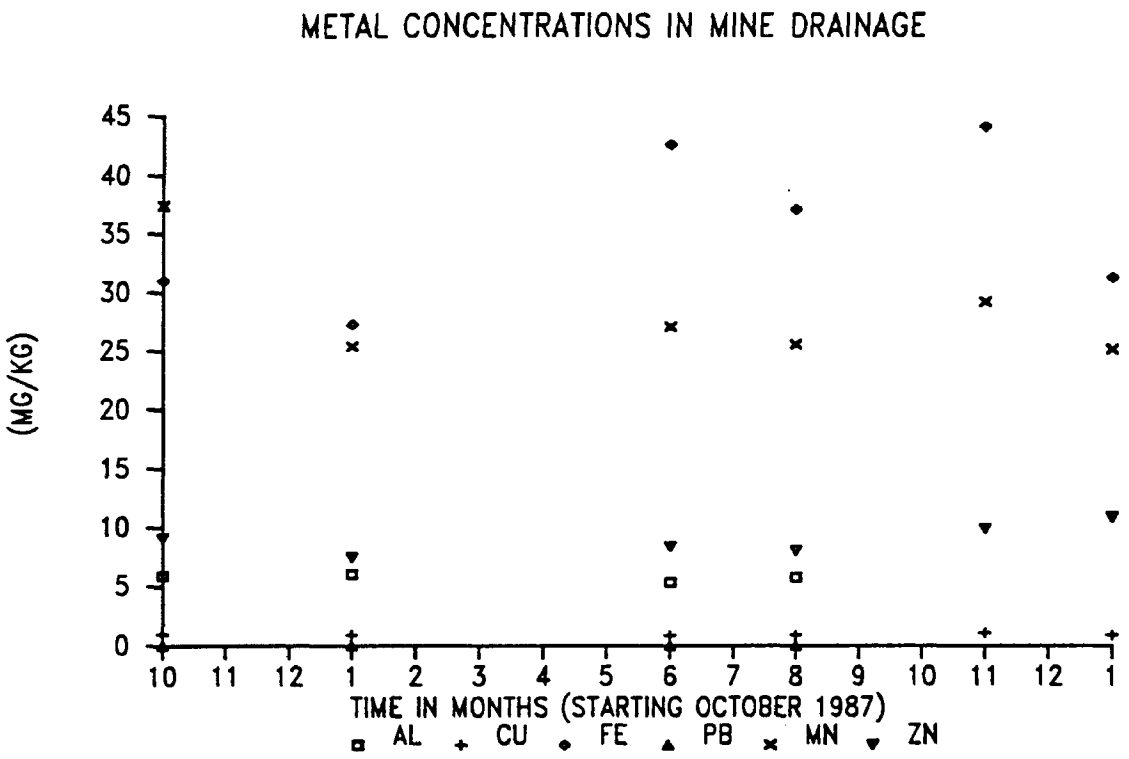

ZN CONCENTRATIONS IN CELL A SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM
ZN (MG/KG)
8000
7000
6000
5000
4000
3000
2000
1000
0
10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1
TIME IN MONTHS (STARTING OCTOBER 1987)
AT 2.5 CM
AT 15 CM
AT 90 CM


ZN CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM
ZN (MG/KG)
900
800
700
600
500
400
300
200
100
0
10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1
TIME IN MONTHS (STARTING OCTOBER 1987)
AT 2.5 CM
AT 15 CM
AT 90 CM


ZN CONCENTRATIONS IN CELL B SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM
ZN (MG/KG)
1200
1000
800
600
400
200
0
10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1
TIME IN MONTHS (STARTING OCTOBER 1987)
AT 2.5 CM
AT 15 CM
AT 90 CM


ZN CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE INLET AT 2.5, 15 AND 90 CM
ZN (MG/KG)
900
800
700
600
500
400
300
200
100
0
10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1
TIME IN MONTHS (STARTING OCTOBER 1987)
AT 2.5 CM
AT 15 CM
AT 90 CM


ZN CONCENTRATIONS IN CELL C SUBSTRATE
NEAR THE OUTLET AT 2.5, 15 AND 90 CM
ZN (MG/KG)
800
700
600
500
400
300
200
100
0
10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1
TIME IN MONTHS (STARTING OCTOBER 1987)
AT 2.5 CM
AT 15 CM
AT 90 CM


METAL CONCENTRATIONS IN MINE DRAINAGE
(MG/KG)
45
40
35
30
25
20
15
10
5
0
10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1
TIME IN MONTHS (STARTING OCTOBER 1987)
AL CU FE PB MN ZN