STUDIES INTO THE EFFECTS OF CADMIUM AND LOW pH ON METHANE PRODUCTION FROM ANAEROBIC LAKE SEDIMENTS

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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TO MY FAMILY

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ABSTRACT

Investigations into the effects of the heavy metal cadmium (Cd) and low pH upon methanogenesis from anaerobic lake sediments were conducted over the period summer 1978 to early spring 1981 at the Freshwater Institute, Winnipeg, and at the Experimental Lakes Area (ELA), northwestern Ontario. Experiments conducted at ELA consisted of both laboratory and field studies, while those at the Institute consisted of developmental and laboratory studies

The laboratory studies indicated that lowering the pH of the test system below approximately 5.5 would inhibit methanogenesis. Lowering the pH to approximately 3.5 would result in total inhibition of methane production and visible changes in the sediments studies.

The use of Cd water column concentrations as low as 3 ug/l was found sufficient to cause total inhibition of methanogenesis in the laboratory studies.

In the field studies acidification of one test system to a pH of approximately 4.8 resulted in incomplete inhibition of methanogenesis, reducing the rate of production to approximately 50% of the controls. Using Cd and Cd plus low pH seemed to have little or no effect on the rate of methanogenesis in the field test systems as compared to the control systems.

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INTRODUCTION

INTRODUCTION

Virtually all nutrient recycling on the earth occurs as the result of bacterial decomposition. Indeed, it has been said that "if all decomposing bacteria ceased their activities for a period of only three months, then nearly all green plant production on this planet would cease almost immediately and that no higher animal (including mankind) could survive this temporary interruption of bacterial recycling" (Russell-Hunter, 1970).

In the freshwater environment much of the decomposing activity takes place in anaerobic sediments, where one finds a wide variety of bacterial metabolic groups. Included among these groups are the sulfate reducers (Postgate; 1974; Pfennig et al, 1981), denitrifying bacteria (Jeter & Ingraham 1981), and the methane producers (Bryant, 1974; Mah & Smith; 1981), all of which mediate the terminal processes of anaerobic decomposition (Wetzel, 1975; Stanier et al., 1976; Zeikus, 1977). This mediation involves the utilization of potentiably autoinhibitory metabolic byproducts (for example; H_2, long chain fatty acids, aromatic acids, etc.) of bacteria that function earlier in the decomposition pathway. The endproducts (such as H_2S, N_2 and CH_4) of the terminal organisms are nearly inert under anaerobic conditions and escape easily from lake sediments, and so pose few toxicity problems (Zeikus, 1977; Rudd & Taylor, 1980). Without the action of the terminal organisms toxic compounds would build up in lake sediments, all anaerobic decomposition would cease, and an important source of nutrients would be lost (Zeikus, 1977). Methane production has been suggested as

a measure of heterotrophic activity in lake sediments (Robertson, 1979; Kelly & Chynoweth, 1979). As methane is relatively easy to detect in sediments and in the water column (Rudd et al, 1974; Kelly & Chynoweth, 1979, 1980), and as it is an important source of recycled carbon (Rudd & Hamilton, 1978, 1979), it was chosen as the indicator for this study.

Cadmium (Cd) is recognized as a potent toxic agent which is entering the environment at an ever increasing rate, primarily because of anthropogenic activities (Fulkerson et al., 1973; Nriagu, 1980a, 1980b). This metal is so universally toxic that at no level of intake or in any compound does it serve any useful physiological function (Fulkerson et al., 1973; Nordberg, 1974; Fleischer et al., 1974, Babich & Stotzky, 1978). Many of the acute and chronic toxic effects of Cd upon humans (Babich & Stotzky, 1978, Yosumura et al., 1980), animals (Babich & Stotzky, 1978), and plants (Jastrow & Koepp, 1980; Rai et al., 1981b) have been documented but there seems to have been little emphasis placed on the interaction of this metal with bacteria (Wong et al., 1980). This lack of interest in the effect(s) of Cd on microbial ecology is surprising in view of the extreme toxicity of the metal and its potential for disturbing microbial activities such as decomposition.

Acid precipitation is an environmental problem which has recently been gaining world-wide notoriety because of its international nature. For example, the acid rains which have caused so much damage in the Scandinavian countries are believed to originate over the heavily industrialized portions of continental Europe and England (Oden,

1976; Likens et al., 1979; Babich et al., 1980). The environmental damage caused by acid precipitation is both widespread and severe. Literally thousands of lakes in both Europe and North America have been rendered lifeless due to acidification by acid precipitation (Likens et al., 1979; Hendrey, 1981; Babich et al., 1980). Much work has been done to document the effects of acid precipitation on forests (Evans et al., 1978; Abrahamsen, 1980; Johnson et al., 1981) and fisheries (Schofield, 1976; Beamish & Van Loon, 1977; Wright & Snekvik, 1978), but, beyond noting that rates of decomposition appear to be retarded under acidic conditions (Baath et al., 1979; Traaen, 1980; Friberg et al., 1980), little work has been done with regards to the decomposer bacteria. In view of the importance of these organisms to nutrient recycling this lack of knowledge can be quite serious.

Increases in the heavy metal contents of the waters are known to accompany the acidification of freshwater ecosystems by acid precipitation (Wright & Gessing, 1976; Wright et al., 1976; Dillon et al., 1979; Schindler et al., 1980a). In addition, increased acidity has also been implicated in potentiating the toxicity of metals in aquatic environments. The few studies that have been conducted on the combined effects of these pollutants (heavy metals and acid precipitation) have shown that for the most part their combination is extremely toxic to aquatic life (Schofield, 1976; Dickson, 1978; Rai et al., 1980a; Baker & Schofield, 1982). The presence of increased metal levels in acidified lakes has been blamed for the failure of fish stocking efforts in lakes with reduced although still 'non-toxic' pH levels

(Dickson, 1978; Hendrey, 1981). In the microscopic world, studies over the entire spectrum of microbial life from algae (Rai et al., 1981a) to yeasts (Avakyan, 1971) have shown that acidic pH levels increase the toxicity of metals to all microbial life forms. The interaction of metals and low pH is one of the more poorly studied aspects of the acid precipitation phenomenon, especially in the field. The possibility of synergistic interactions between these pollutants makes the need for more such studies clear.

The purpose of this research project was to obtain information about the effects of cadmium and low pH, acting either singly or in concert, upon methane producing bacteria. The project was conducted in three stages, the first being in the laboratory and designed to determine if methanogenic bacteria were sensitive to Cd and also for the purpose of becoming familiar with the techniques required in the study. The second stage was also conducted in the laboratory, in larger systems than the first. The effects of lower Cd levels than could be utilized in the first stage and also of acidic pH levels were examined in this stage. The third stage of the study was conducted in enclosures located in a freshwater eutrophic lake, Lake 227, of the Experimental Lakes Area, located in the Canadian Shield near Kenora, Ontario. In this stage the effects on methane production of Cd, low pH, and their combination, were examined under almost natural field conditions. Throughout the project specific emphasis was placed on those bacteria found in the anaerobic sediments of Lakes 227.

HISTORICAL REVIEW

HISTORICAL REVIEW

The Methanogens

The formation of methane (methanogenesis) in nature is a uniquely biological event carried out by a small group of microorganisms known as the methanogenic bacteria, or methanogens. These bacteria are a morphologically diverse group of very strict anaerobes whose culture and manipulation require highly specialized techniques (Wolfe, 1971; Zeikus, 1977; Balch et al., 1979; Mah & Smith, 1981). Nutritionally, the methanogens have simple requirements with none of the known pure cultures of these organisms forming methane from compounds more complex than acetate (Bryant, 1974; Zeikus, 1977; Mah & Smith, 1981). In all methanogens tested ammonia is used as the source of nitrogen, phosphate the source of phosphorous and sulfide or cysteine the source of sulfur (Bryant, 1974; Zeikus, 1977; Mah et al., 1977; Mah and Smith, 1981).

Few taxonomic groupings of bacteria show the large morphological diversity that the methanogens do. There is some controversy as to their classification, but there are currently three taxonomic orders, containing 13 species, that are generally acknowledged (Bach et al., 1979; Mah & Smith, 1981). As shown in table 1, in these three orders can be found all of the major cell morphological types (rods, sarcinal, spherical and spirillae). Considerable variation exists in cell dimensions, organizations, shpaes, DNA ratios, motility and temperature optima within both individual species and genera (Bryant, 1974, Zeikus, 1977; Balch et al., 1979; Mah & Smith, 1981).

Table 1. Characteristics of methanogenic species in pure culture (after Mah & Smith, 1981)

Order	Species	Morphology	Gram stain	Motility	Temp. optimum (C^{o})	pH optimum	DNA G+C moles %
Methano-bacteriales	Methanobacterium formicicum	long rod to filament	var.	-	37-45	6.6-7.8	40.7-42
	bryantii	long rod	var.	-	37-39	6.9-7.2	32.7-38
	thermoautotrophicum	long rod to filament	+	-	65-70	7.2-7.6	49.7-52
	Methanobreyibacter ruminantium	lancet-shaped cocci	+	±	37-39	6.3-6.8	30.6
	smithii	lancet-shaped cocci	+	-	37-39	6.9-7.4	31-32
	arboriphilus	short rods	+	-	37-39	7.5-8.0	27.5-31.6
Methano-microbiales	Methanomicrobium mobile	short rods	-	+	40	6.1-6.9	48.8
	Methanogenium cariaci	irregular, small cocci	-	+	20-25	6.8-7.3	51.6
	marisnigri	irregular, small cocci	-	+	20-25	6.2-6.6	61.2
	Methanospirillum hungatei	short to long wavy spirillum	-	+	30-40	6.8-7.5	45-46.5
	Methanosarcina barkeri	pseudosarcina	+	-	35-40	6.7-7.2	38.8-51
Methano-coccales	Methanococcus vannielii	small cocci	-	+	36-40	7.0-9.0	31.1
	voltae	small cocci	-	+	36-40	6.7-7.4	30.7

Nonetheless, all methanogenic species identified to date share certain unique and unifying properties which serve to bind them into a distinct taxonomic group. Some of these properties are set out below.

1) All methanogens are strict anaerobes which obtain energy for growth from the formation of methane by the reduction of carbon dioxide (Wolfe, 1971; Bryant, 1974; Zeikus, 1977; Bryant, 1979; Mah & Smith, 1981).

2) Analyses of nucleotide sequences of 16S ribosomal RNA[1] indicate a common early evolutionary divergence with the archaebacteria[2] from all other forms of life studied thus far (Balch et al., 1977; Fox et al., 1977; Woese et al., 1978; Balch et al., 1979; Woese, 1981).

3) All methanogens examined to date lack the characteristic cell wall polymer component muramic acid, which, with the exception only of the archaebacteria, is found in all other bacteria and cyanobacteria (Zeikus, 1977; Kandler & Hippe, 1977; Kandler & Konig, 1978; Woese et al., 1978; Bryant, 1979; Balch et al., 1979; Woese, 1981).

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1 - Geneological relationships between various bacterial species can be determined by comparative analyses of macromolecular nucleotide sequences: the closer the sequences are to one another the more closely related are the organisms. The 16S ribosomal RNA molecule (the S stands for Svedberg unit, a measure of the rate of sedimentation in an ultra-centrifuge) is the molecule of choice because the ribosome is of ancient origin, is universally distributed and is apparently functionally equivalent in all bacteria. The RNA molecule itself is 1,540 nucleotides long, sufficient for sequence differences to be statistically significant and yet relatively easy to sequence (Balch et al., 1977; Balch et al., 1979; Fox et al., 1980; Woese, 1981).

2 - The Archaebacteriae are a proposed separate kingdom of procaryotes which possess a number of characteristics distinguishing them from the eubacteria (or 'true bacteria') among these characteristics being the lack of muramic acid in the cell walls and unique 16S ribosomal RNA sequences. Included in the Archaebacteriae are the methanogens, the extreme halophiles and the thermoacidophiles (Woese et al., 1978; Fox et al., 1980; Woese, 1981).

4) With one exception (Methanobrevibacter ruminantium) all methanogens contain three unique coenzymes; coenzyme 420 (also known as F-420) which is involved in electron transfer in place of ferredoxin, coenzyme M (2-mercaptoethanesulfonic acid or CoM) which is involved in methyl transfer (M. ruminantium requires CoM to be supplied as a growth factor) and factor B which is involved in the enzymatic formation of methane from methyl-coenzyme M (Gunsalus & Wolfe, 1976; Zeikus, 1977; Mat et al., 1977; Bryant, 1979; Balch et al., 1979).

5) Finally, with only one exception (Methanosarcina TM-1) all methanogens are able to grow on carbon dioxide and hydrogen as the sole sources of carbon and energy (Bryant, 1974; Zeikus, 1977; Mah et al., 1977; Zinder & Mah, 1979; Mah & Smith, 1981).

Environmental role of the Methanogens

The methanogens are ubiquitous in most anaerobic environments. They have been isolated from sewage sludge digestors, the rumen and intestinal tracts of animals, various marine and freshwater sediments and even from the trunks of living trees (Zeikus, 1977; Balch et al., 1979). In such environments these bacteria are the terminal organisms in the anaerobic food chain and utilize potentially toxic byproducts e.g., acetate, hydrogen and carbon dioxide, of the anaerobic decomposition of organic materials (Zeikus, 1977; Mah et al., 1977; Bryant, 1979; Mah & Smith, 1981). The end product of their metabolism, methane (CH_4), is poorly soluble, volatile and essentially inert under anaerobic conditions. Thus, it is non-toxic and escapes easily from most anaerobic environments (Wolfe, 1971; Zeikus, 1977; Mah et al., 1977;

Bryant, 1979; Rudd & Taylor, 1980). In addition, CH_4 is an important part of the carbon cycle in such ecosystems with most being ultimately recycled and only a small fraction escaping to the atmosphere (Rudd and Taylor, 1980).

Recent studies have shown that methanogenic bacteria can strongly influence the flow of carbon and electrons in anaerobic habitats by a process termed interspecies hydrogen transfer (Zeikus, 1977; Mah et al., 1977; Winfrey et al., 1977; Mah & Smith, 1981). The regeneration of NAD (nicotinamide adenine dinucleotide, an oxidation-reduction coenzyme) from NADH (reduced NAD) by the disposal of electrons is accomplished in all fermentative bacteria primarily by the production of various reduced end products such as hydrogen (H_2), ethanol, lactate, formate, or propionate. Pure culture studies have shown that growth of carbohydrate fermenters in the absence of methanogens results in the formation mainly of H_2, carbon dioxide (CO_2), ethanol, formate, acetate, succinate and lactate. Such growth in the presence of methane producing bacteria results in the production mainly of H_2, CO_2, and acetate, with the other products listed above present in only negligible amounts (Wolin, 1974; Zeikus, 1977; Latham & Wolin, 1977; Weimer & Zeikus, 1977; Laube & Martin, 1981). It is believed that with their high affinity for H_2 the methanogens may act as 'electron sinks' during anaerobic decomposition by altering the flow of electrons in the direction of H_2 production. Theoretically this process of interspecies hydrogen transfer, or altered electron flow, which occurs in symbiotic growth of methanogens and non-methanogens has the following results: 1) increased substrate utilization, 2) different proportions

of reduced end products, 3) more adenosine triphosphate (ATP) synthesis by non-methanogens, 4) increased growth of both types of organisms, and 5) displacement of unfavorable reaction equilibria (Zeikus, 1977; Mah et al., 1977; Winfrey et al., 1977; Bryant, 1979; Rudd & Taylor, 1980; Laube & Martin, 1981; Mah & Smith, 1981).

On a larger scale the production and utilization of methane can have a very important impact upon the cycling of carbon in, for example, freshwater ecosystems. Rudd and Hamilton (1978, 1979) found that while the oxidation of CH_4 was not an important source of carbon for either the primary producers or the secondary grazers, when compared to the total carbon input into the lake, CH_4 cycling was very important indeed (Rudd & Hamilton, 1978, 1979; Rudd & Taylor, 1980). In one study (Rudd & Hamilton, 1978, 1979), an amount equal to 36% of the total carbon input into the study lake was found to be recycled by methane oxidation. It was concluded that rapid aerobic carbon recycling keeps CH_4 from being an important carbon source of the primary producers and secondary grazers, but that longer term (at least annual) whole lake carbon cycling of CH_4 does indeed play a major role (Rudd and Hamilton, 1978, 1979; Rudd & Taylor, 1980).

Methane production in the natural environment can be inhibited in a number of ways. For example, oxygenation of the habitat will cause an immediate halt in CH_4 production by methanogens,both by the directly toxic effects of oxygen on them and by the preferential use of oxidative decomposition over fermentation. Under anaerobic conditions sulfate reduction will occur preferentially over the methane fermentation and cause inhibition by substrate competition (Cappenberg and Prins, 1974; Claypool & Kaplan, 1974; Winfrey & Zeikus, 1977; Abram and Newell, 1978a,

1978b; Ward & Olson, 1980). Nitrate reduction (denitrification) will also inhibit methanogenesis, either by raising the Eh of the environment to inhibitory levels or by substrate competition (Bollag & Czlonkowski, 1973; Macgregor & Keeney, 1973; Balderston & Payne, 1976; Zeikus, 1977). The methanogens are also sensitive to low pH levels with growth in pure culture usually not occurring below pH 6 (Bryant, 1974; Mah & Smith, 1981). This last point has not been extensively studied in the field and, considering the contribution of methane to carbon recycling, it is important to determine how man-made sources of pollution such as acid precipitation (and the sulfate and nitrate derived therefrom) affect methanogens in the natural habitat.

The Methane Fermentation Pathway

Studies carried out upon the rumen fermentation, sewage solids fermentation and bacterial enrichment cultures provide most of the data used to outline the methane fermentation pathway. Very little chemical or microbiological data are available on the nature of these reactions in soil or natural aquatic systems. It does seem likely, however, that processes which take place in natural aquatic and soil systems will be similar to those of other systems (Zeikus, 1977; Mah et al., 1977; Bryant, 1979; Balch et al., 1979).

For the sake of clarity it is most convenient to treat the methane fermentation process in three stages (Mah et al., 1977; Bryant, 1979; Laube and Martin, 1981). In the first two stages complex organic compounds are broken down to such precursors of methanogenesis as hydrogen, carbon dioxide and acetate. These precursors are metabolized

to methane in the third stage.

In stage one various species of non-methanogenic fermentative bacteria hydrolyze polysaccharides, e.g., cellulose, to sugars which are then taken up and fermented via the Embden-Meyerhof-Parnas pathway to pyruvate. The pyruvate undergoes further decomposition at low partial pressure of hydrogen to the end products acetate, CO_2 and H_2 which are immediately utilizable by methanogens (Zeikus, 1977; Mah et al., 1977; Bryant, 1979; Laube & Martin, 1981). In the presence of a high partial pressure of hydrogen the most common end products are ethanol, butyrate, or propionate (Zeikus, 1977; Mah et al., 1977; Bryant, 1979; Laube & Martin, 1981). These bacteria also break down other organic compounds, such as lipids and proteins, and produce primarily the end products listed above. Additional end products of the first fermentation stage include long chain fatty acids, such as stearate and palmitate, and various aromatic acids, such as phenylacetate and indolacetate (Zeikus, 1977; Mah et al., 1977; Bryant, 1979).

In stage two of the fermentation those products of the first stage which are not immediately utilizable by methanogens undergo further decomposition. Compounds such as propionate, the long chain fatty acids and aromatic acids are anaerobically oxidized to acetate or CO_2 plus acetate, depending on the starting compound (Braynt, 1979). In the few cases where it is known, the electron sink product generated in this oxidation is H_2, but production of formate by the reduction of CO_2 is an alternative possibility. In this case the formate generated would most likely undergo further degradation to CO_2 and H_2 by other bacteria or could possibly be directly metabolized by methanogenic

bacteria (Zeikus, 1977; Mah et al., 1977; Bryant, 1979).

The methanogens are active only in the terminal third stage of the fermentation. In this stage these bacteria utilize the final end products of the first two stages. These end products, potentially harmful to the bacteria which produced them, are primarily acetate, CO_2 and H_2 (Zeikus, 1977; Mah et al., 1977; Bryant, 1979; Mah & Smith, 1981). The methanogens are the only anaerobic organisms known which are capable of effectively utilizing electrons in the form of hydrogen (Bryant, 1979) and which are able to break down acetate anaerobically in the absence of light, or other electron acceptors such as sulfate or nitrate. By carrying out this third fermentative step the methanogens fill an important ecological niche. In their absence organic matter could not be decomposed effectively in the anaerobic environment and organic acids which contained almost as much energy as the original undegraded organic matter would accumulate (Zeikus, 1977; Mah et al., 1977; Bryant, 1979; Rudd & Taylor, 1980).

The pathways by which methanogenic bacteria reduce their substrates to methane or incorporate them into cell materials remain largely unknown (Zeikus, 1977; Bryant, 1979; Zeikus, 1980; Kenealy & Zeikus, 1981). The data are consistant with either unified or totally separate pathways so that it is not known if the pathways are connected by common intermediates (Kenealy & Zeikus, 1981). One complication to attempts to determine the pathways is the fact that major differences exist in the intermediary metabolism between diverse methanogenic genera (Weimer and Zeikus, 1979). It is known however, that none of the documented anabolic pathways for growth on one carbon (C-1) substrates (the Calvin cycle,

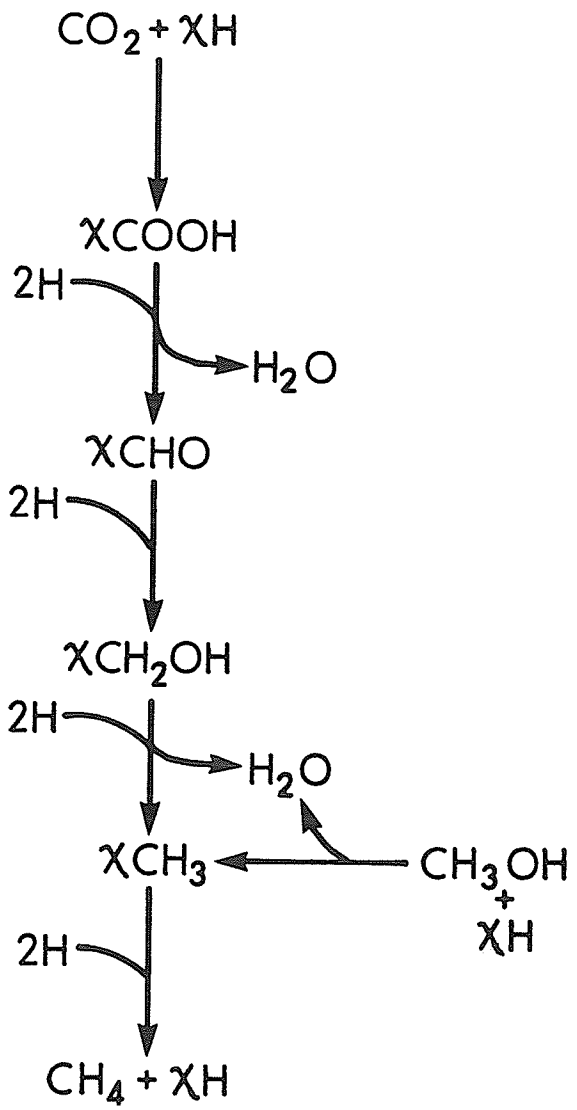
the serine or hexulose phosphate pathways, the tricarboxylic acid cycle) are present in the methanogens (Daniels & Zeikus, 1978; Fuchs and Stupperich, 1978; Zeikus, 1980; Kenealy & Zeikus, 1981).

At present, methane production from C-1 substrates is believed to proceed via the association of the substrate with C-1 carriers which catalyze the sequential reduction to CH_4 (fig. 1; Barker, 1956; Zeikus, 1977; Zeikus, 1980; Kenealy & Zeikus, 1981). With the exception of coenzyme M (CoM), which mediates the terminal step of the reduction, none of the carriers involved have been characterized. CoM is one of the smallest of all known coenzymes and is required by methyl CoM reductase, an enzyme present in all methanogens which catalyzes the final step of the reduction (Taylor & Wolfe, 1974; Balch & Wolfe, 1979; Balch et al., 1979; Zeikus, 1980). Production of CH_4 from the metabolic intermediate $X\text{-}CH_3$ (X is an unknown carrier, the intermediate itself may be simply $CoM\text{-}CH_3$) requires the transfer of the methyl group to CoM via a transferase followed by the reduction of $CH_3\text{-}CoM$ by methyl CoM reductase (Mah et al., 1977; Zeikus, 1980; Shapiro & Wolfe, 1980; Kenealy & Zeikus, 1981). $X\text{-}CH_3$ has also been put forward as a possible common intermediate in the C-1 anabolic and catabolic pathways of the methanogens, but there is no conclusive evidence for or against such a linkage of the pathways (Zeikus, 1980; Kenealy & Zeikus, 1981).

The Problem of Cadmium

Cadmium was first discovered as a separate element in 1817 when it was isolated from samples of zinc carbonate by F. Strohmeyer. In

Figure 1. A proposed mechanism for methane production from one carbon (c-1) substrates. XH is an as yet unknown C-1 carrier(s) which catalyzes the reduction to CH_4 (after Gunsalus et al., 1976)


$CO_2 + \chi H$
$\chi COOH$
2H
H_2O
χCHO
2H
χCH_2OH
2H
H_2O
χCH_3
CH_3OH
+
χH
2H
$CH_4 + \chi H$

nature it is always found in the presence of zinc and cadmium metal is produced as a byproduct of zinc metal production (Chizhikov, 1966; Aylett, 1973; Fulkerson et al., 1973; Nriagu, 1980b). In pure elemental form Cd is a silvery-white metal which is soft, very ductile and highly resistant to corrosion. It is a bivalent element assigned atomic number 48, has an atomic weight of 112.4 and a density of 8.65 (Chizhikov, 1966; Aylett, 1973; Weast & Astle, 1979; Nriagu, 1980b). The concentration of Cd in the lithosphere is normally quite low, ranging from 0.1 to 0.2 ug Cd/g but goes as high as 50 mg Cd/g in some cadmium-rich ores, usually averaging about 3 mg Cd/g in commercially exploitable ores (Chizhikov, 1966; Aylett, 1973; Fleischer et al., 1974; Nriagu, 1980b). In environments contaminated by Cd, concentrations ranging from 0.2 ug Cd/g to as much as 50 mg Cd/g have been recorded (Kneip et al., 1974; Forstner, 1980).

Industrially, cadmium is used primarily for electroplating, as a plastic stabilizer, and in paint pigments, alloys and batteries (Aylett, 1973; Fulkerson et al., 1973; Windholz et al., 1976; Nriagu, 1980b). The use of Cd in all of these products, especially plastics and batteries, is expected to increase dramatically. As recycling Cd is difficult or prohibitively expensive it is expected that the amount of the metal released to the environment from the use and disposal of these products will also increase (Fulkerson et al., 1973; Fleisher et al., 1974; Nriagu, 1980a,b).

Cadmium is one of the most potent physiological toxicants known. In a study conducted by Bienvenue et al. (1963) Cd ranked fourth in overall elemental toxicity behind indium, mercury and uranium. At no

level of intake or in any compound does it serve any known physiological function. It is this ubiquitous toxicity which is the basis of concern about the spread of Cd in the environment (Fulkerson et al., 1973; Fleischer et al., 1974; Nordberg, 1974; Yosumura et al., 1980).

The acute toxicity of cadmium and Cd compounds has been known since medieval times, long before the element itself was discovered (Nriagu, 1980b). It is only in recent times, however, that possible toxic effects of long term exposure to the metal have come to light. In 1968, the Japanese Ministry of Health and Welfare released the results of a seven year study into the cause of Itai-itai disease, found among inhabitants of the Jintsu River basin in Japan. The disease itself is defined medically as a renal tubular dysfunction (kidney failure) combined with osteomalacia and osteoporosis. Osteomalacia is a softening of the bones caused by a deficiency of vitamin D or calcium, and osteoporosis is the abnormal rarefaction of bone tissue. The cause of the disease was found to be chronic Cd poisoning by the ingestion and uptake of Cd from food plants grown in soils irrigated by water polluted by mine effluents and from drinking water taken from polluted wells. Since 1962 over 200 cases of the disease have been recorded with nearly 100 deaths attributed to it by 1965 (Fulkerson et al., 1973; Fleischer et al., 1974; Friberg et al., 1974; Nordberg, 1974; Yosumura, 1980).

Since this study was released, cadmium has also been implicated as a possible factor in hypertension and other cardiovascular diseases, and in various pulmonary disorders including emphysema (Fulkerson et al. 1973; Nordberg, 1974; Fleischer et al., 1974; Yosumura et al., 1980;

Perry & Erlanger, 1981). Animal tests have shown the element to be teratogenic, weakly mutagenic, and possibly carcinogenic. Testicular damage has been shown to occur at doses smaller than those required to cause liver or kidney damage (Parizek, 1957; Fulkerson et al., 1973; Nordberg, 1974; Fleischer et al., 1974; Yosumura et al., 1980).

Higher organisms, and in some instances single celled organisms (Maclean et al., 1972; Prinz & Weser, 1975; Gauthier & Flatau, 1977; Lerch, 1979), may have evolved a defense against small amounts of Cd by means of the metal binding protein metallothionein (Margoshes and Valee, 1957; Nordberg, 1971, 1972; Fulkerson et al., 1973; Cherian and Goyer, 1978; Nordberg & Kojima, 1979). There is controversy over the assignment of a protective role for metallothionein and, indeed, there is some evidence which suggests that the cadmium-thionein complex (Cd bound to metallothionein) is toxic (Nordberg, 1971; Nordberg et al., 1975; Cherian et al., 1976; Cherian & Goyer, 1978; Nordberg & Kojima, 1979).

Of more recent interest is the basis of heavy metal resistance in bacteria which may be related to the more general mechanism of drug resistance (Novick & Roth, 1968; Kondo et al., 1974; Nakahara et al., 1977; Lighthart, 1979; Sterritt & Lester, 1980). For example, the penicillinase plasmid in Staphylococcus aureus which confers resistance to penicillin also contains gene(s) which confer resistance to Cd ions (Novick & Roth, 1968). The resistant cells will allow calcium to enter the cell but not Cd, instead apparently sequestoring it in the cell wall (Chopra, 1971; Kondo et al., 1974). This selection phenomenon is important to the cell because cadmium is known to interfere with

calcium metabolism (Larson & Piscator, 1971; Fulkerson et al., 1973; Kondo et al., 1974).

Relatively little is known about the interaction of Cd or other heavy metals with microorganisms in the natural environment because most investigators deal with the toxic effects of heavy metals upon pure cultures of bacteria in the laboratory (Sterritt & Lester, 1980; Wong et al., 1980). Avakyan (1967) has conducted studies comparing the toxicity of various heavy metals upon a variety of bacteria. He concluded that of the six metals tested (Ag, Hg, Ni, Co, Cd and Pb) Cd was the least toxic to the organisms studied (Avakyan, 1967). Zwarun (1973) found that the threshold level for cadmium toxicity to Escherichia coli was approximately 6 mg/l. Total inhibition occurred at 12 mg Cd/l. Both of these levels are below those recorded in some contaminated environments (Forstner, 1980). Doyle et al. (1975) found that Cd concentrations of 40 mg/l or more could have significant repressive effects upon the growth of common bacteria such as Staphylococcus aureus (non-resistant strains) and Bascillus cereus. More importantly, these workers also found that as the concentration of Cd in the growth medium increased so did the uptake and accumulation of the metal in these bacteria, with possible serious consequences in terms of food-chain magnification (Doyle et al., 1975). Babich and Stotzky (1977a) found that species-dependent inhibition of growth took place at Cd concentrations as low as 0.5 mg/l.

Some mixed culture studies in laboratory microenvironments have been conducted. In a study to investigate the role of sulfide and

carbonate ions in the prevention of cadmium toxicity to anaerobic digestion, Mosey (1971) found that the minimum Cd concentration required to cause inhibition in the controls was approximately 50 mg/l. Bond et al. (1975) in a study on the effects of Cd on forest soil litter decomposition found that 10 mg Cd/l was sufficient to reduce oxygen and CO_2 respiration by 40% in soil/litter micro-environments. Hayes and Theis (1978) in a study of heavy metal effects on anaerobic digestion found their maximum shock dosage of 20 mg Cd/l and chronic dosage of 10 mg Cd/l to be insufficient to cause inhibition. Lester et al. (1979) studied the effects of 50 mg/l shock doses of the metals lead, copper, cadmium and chromium upon four bacterial species grown in mixed culture in chemostats. They concluded that only Cu was more toxic than Cd.

Studies carried out with mixed cultures in pristine and polluted environments have shown that heavy metal pollution can selectively influence bacterial populations. In a series of tests on saprophytic bacterial communities in freshwater systems, Houba and Remacle (1980) found a strong correlation between the degree of contamination of a site and the percentage of resistant strains of bacteria in the community. Mills and Colwell (1977) found that 10 mg Cd/l could cause a 59% reduction of C-14 glucose oxidation by organisms taken from the sediments of a relatively non-polluted environment. Among organisms taken from the sediments of a metal polluted environment the identical dosage (10 mg Cd/l) caused no inhibition of oxidation. They concluded that organisms taken from the polluted site were metal resistant and that Cd input to the site had caused acute disturbances in the local

microbial ecology. In a detailed study carried out on the microbial populations of activated sludge sewage digestors Cenci and Morozzi (1977) found that growth was inhibited by 0.5 mg Cd/1. The inhibition was most pronounced in log phase growth.

The studies conducted in anaerobic digestors are of particular interest to this study because the decompositional processes that take place in such digestors are believed to be very similar to those which occur in the natural environment (Zeikus, 1977; Bryant, 1979; Balch et al., 1979). The very different results obtained by Cenci and Morozzi (1977) and Mosey (1971) or Hayes and Theis (1978) clearly show the need for further research in this area and the impossibility of predicting from laboratory studies the response of a given natural environment.

Acid Precipitation

Water vapour in the atmosphere originates from evaporation and transpiration of water and is essentially pure or distilled water. Once in the atmosphere, water vapour condenses out on solid particles of soot and dust, etc. and reaches a chemical equilibrium with atmospheric gases. One such gas is carbon dioxide, which dissociates incompletely in water to form a weak acid, carbonic acid (H_2O_3). Under normal conditions of CO_2 concentration and pressure in the atmosphere, the pH of precipitation is pH5.6. Acid precipitation is defined as snow and rain with a pH value below 5.6 (Likens et al., 1979).

Acid precipitation is caused by the presence of sulfuric, nitric

and, to a lesser extent, hydrochloric acid in rainwater or snow (Gorham, 1976; Likens et al., 1979). Unlike carbonic acid, these strong acids undergo complete dissociation in a dilute aqueous solution and can lower the pH to below 5.6. Over large areas of the world snow and rain on an annual basis are now from 5 to 30 times more acid than normal, and the precipitation of individual storms can be several hundred to several thousand times more acid than expected (Likens et al., 1979). In 1968, Oden pointed out that rain and snow in Europe was gradually becoming more acidic. A series of maps of volume-weighted mean annual pH of precipitation showed that a region of high acidity (pH 4 to 4.5) had spread from the area of the Netherlands, Luxembourg and Belgium in the late 1950's to include most of Germany, northern France, southern Scandinavia and the eastern British Isles by the late 1960's (Oden, 1963; Brosset, 1973). Similar maps of the eastern seaboard of North America show a region of high acidity (pH 4 to 4.5) spreading from an area centered on the states of Pennsylvania and New York in the mid 1950's to include most of the eastern United States and parts of eastern Canada by the early 1970's (Likens et al., 1979; Babich et al., 1980). More recently, the western United States have also been shown to be receiving acid rain (Lewis & Grant, 1980). A short historical review which outlines the growth of awareness of the acid precipitation problem has been prepared recently by Cowling (1982).

Sulfuric acid is the predominant strong acid present in acidic precipitation and accounts for about 65 to 80% of the total acid content (Cogbill & Likens, 1974; Likens & Bormann, 1974; Gorham,

1976; Ottar, 1978; Likens et al., 1979). The presence of this acid in precipitation is the result of the oxidation of various gaseous sulfur species, primarily SO_2 and H_2S (Brosset, 1973; Eggleton and Cox, 1978; Middleton & Kiang, 1978; Calvert et al., 1978; Likens et al., 1979). Atmospheric H_2S is derived almost entirely from natural sources either biogenic (decaying organic matter) or geologic (volcanic emissions). Sulfur dioxide on the other hand is released to the atmosphere from natural sources as mentioned above, and from anthropogenic activities, primarily the burning of fossil fuels. 95% of their sulfur content is released in the form of SO_2 (Brosset, 1973; Semb, 1978; Dovland & Semb, 1980).

The exact ratio of overall sulfur emissions between natural and anthropogenic sources is difficult to determine. It is currently believed that while the two sources emit roughly equal amounts, the anthropogenic sources are increasing (Robinson & Robbins, 1968; Granat, 1978; Semb, 1978; Dovland & Semb, 1980). Estimates of the total amount of sulfur released to the atmosphere vary widely and range from 40 to 280 x 10^6 tonnes per year. Most evidence indicates that the lower estimates are correct (Robinson & Robbins, 1968; Gorham, 1976; Granat, 1978; Semb, 1978). If so, then the increasing anthropogenic emissions are of even greater concern.

The natural source emissions are not believed to have contributed very much to acid precipitation problems as, in pre-industrial times, these emissions were probably well in balance with natural sources of neutralizing bases such as ammonia in the atmosphere, sea spray in maritime areas and calcareous soils (Gorham, 1976). Anthropogenic

sources have increased since the industrial revolution and occur in small 'hot spot' regions of north-western Europe and the north-eastern United States and Canada. These regions represent only about 1% of the Earth's surface area but account for some 60% of the total sulfur emissions (Husar & Husar, 1978). In these regions the natural source emissions are unimportant to the sulfur budget and natural sources of bases are overwhelmed by the anthropogenic emissions. Furthermore, the residence time of SO_2 and H_2SO_4 in the atmosphere can be as long as 7 days, in which time they can be transported as far as 1000 kilometres from the source so that the areas affected by acid precipitation are not restricted to the hot spot regions of sulfur emissions (Prahm et al., 1976; Wilson, 1978; Husar and Husar, 1978; Gillani et al., 1978). With the increased use of 'superstacks' to prevent local pollution problems, anthropogenic emissions are becoming ever more widely dispersed and, even allowing for the use of flue-gas desulfurization units or 'scrubbers' in all new power plants, the U.S. Environmental Protection Agency (EPA) predicts that the levels of SO_2 emissions will continue to increase (Oden, 1976; Likens et al., 1979). It is unlikely that the magnitude of the sulfur contribution to the acid precipitation problem will begin to decrease in the near future.

Nitric acid is the second major component of acid precipitation and accounts for about 30% of the total acid content (Cogbill & Likens, 1974; Likens & Bormann, 1974; Gorham, 1976; Ottar, 1978; Likens et al., 1979; Babich et al., 1980). It is formed from nitrogen oxides (Nox) by conversion processes similar to those for H_2S and SO_2 (Gorham,

1976; Likens et al., 1979; Dovland & Semb, 1980; Babich et al., 1980). Nox in the atmosphere arise from both natural and anthropogenic emissions; the natural source emissions exceed the anthropogenic by as much as an order of magnitude (Gorham, 1976; Dovland & Semb, 1980). Natural source emissions are primarily biological in nature. The most important is the anaerobic reduction of nitrogen compounds by bacteria. These processes result in the release of an estimated 1.4 to 1.5 x 10^8 tons of Nox annually (Robinson & Robbins, 1975; Gorham, 1976). As with SO_2, anthropogenic emissions of Nox are primarily the result of the burning of fossil fuels, particularly petroleum products in internal combustion engines (Likens et al., 1972; Gorham, 1976; Dovland & Semb, 1980).

Although anthropogenic emissions of Nox are believed to be much smaller than the total natural emissions, they still make a very significant contribution to acid precipitation because they too are concentrated in relatively small 'hotspots' and overwhelm local natural buffers. Nitrous oxides also have residency times in the atmosphere of a week or more and can be transported for hundreds of kilometres from their source (Robinson & Robbins, 1975; Gorham, 1976; Dovland and Semb, 1980).

With the advent of strict SO_2 emission controls the loading of sulfate into the atmosphere may slow down in some of the problem regions. However, unless similar controls are adopted for Nox emissions, the nitric acid component will be sufficient to continue the increase in acidity of precipitation (Lewis & Grant, 1980). There is also evidence that Nox and Nox-related emissions also can increase the

the severity of nearby SO_2 emissions. Hydrocarbons that are released along with Nox emissions seem to enhance the conversion processes of SO_2 which results in an increased formation of H_2SO_4 (Husar and Husar, 1975; Isaksen et al., 1978; Rodhe, 1981).

Hydrochloric acid is the third strong acid found in acidic precipitation but accounts for only about 5% of the total acid content (Gorham, 1976; Babich et al., 1980). Little work has been done to determine the natural and anthropogenic sources of gaseous HCl emissions and their relative contributions to total atmospheric loading. It is known that the primary source of anthropogenic emissions is the burning of fossil fuels, chiefly coal which may contain a high percentage of chlorine (Gorham, 1976; Babich et al., 1980). As with SO_2 and Nox emissions acid precipitation problems arise because such emissions are concentrated in a few relatively small hotspots of activity where any natural neutralizing agents are overwhelmed.

General Effects of Acid Precipitation

When deposited in an ecosystem acid precipitation can exert a toxic effect on the indigenous biota of the system by altering the chemical composition of the system. The ability of an ecosystem to resist such alterations depends in large part on its buffering capacity. Only when this capacity is exhausted will the pH drop. In freshwater or terrestrial environments located in regions of calcareous soils, such as the Canadian and U.S. prairies, there is little cause for immediate concern since such soils have a large buffering capacity and are thus very resistant to acidification. However, in regions of naturally acidic (ie. podzolic) soils underlain by granitic bedrock

as, for example, the Canadian Shield, there is only a very limited neutralizing capacity and thus little resistance in terrestrial or freshwater environments located there to increased acidity caused by acid precipitation (Oden, 1976; Kramer, 1978; Glass et al., 1979; Likens et al., 1979; Petersen, 1980; Wiklander, 1980). Oceanic ecosystems are highly resistant to acidification because of their large sodium content, because of the presence of other alkaline cations such as calcium and magnesium, and because of the bicarbonate-carbonate system (Gorham, 1976; Babich et al., 1980).

When the pH of podzolic soils is lowered one of the results is an accelerated leaching of minerals such as calcium and magnesium, both of which are important nutrients for the indigenous biota. This increased leaching may lead to a decrease in the fertility and productivity of the soil (Gorham, 1976; Oden, 1976; Abrahamsen, 1980; Hutchinson, 1980; Wiklander, 1980; Gorham & McFee, 1980). The mobilization of metal ions such as aluminum, cadmium, mercury, lead and manganese from rock into the soil is also increased under acidic conditions. Since all of these metals are toxic to plants and animals, even in small amounts, their presence places additional stresses on soil ecosystems undergoing acidification. Further, metal ions eventually enter aquatic ecosystems and cause severe damage there as well (Gorham, 1976; Schofield, 1976; Tyler, 1978; Hutchinson, 1980; Wiklander, 1980; Babich et al., 1980; Cowling and Linthurst, 1981).

In freshwater ecosystems undergoing acidification, reduction in pH causes a variety of other chemical changes. For example, in lake waters acidified to pH 5.5 or less, bicarbonate is transformed to

carbonic acid which is then converted to carbon dioxide. With the loss of anionic bicarbonate from the waters, sulfate often becomes the dominant anion (Johnson et al., 1972; Wright et al., 1976; Henriksen, 1980; Babich et al., 1980; Likens et al., 1980). In lake systems acidified to pH 5 or less, collodial aluminum oxides become soluble and potentially toxic aluminum ions are released.

Acid Precipitation Effects on Terrestrial Biota

Few studies have been conducted to determine the effects of acid precipitation on terrestrial animals. Most problems and injuries to such organisms appear to arise as a result of major inputs of acidic precipitation into porrly buffered waters in which sensitive life stages of the organisms live (Cowling & Lithurst, 1981). For example, it has been demonstrated that acid precipitation may adversely affect the reproductive success of the terrestrial salamander, Ambystoma maculatum, which breeds in temporary ponds formed by the accumulation of melted snow and spring rains. Laboratory and field studies have shown that the greatest hatching success of this organism occurs at pH levels near neutrality and that egg mortalities under such conditions are under 1%. At pH levels below 6 egg mortalities rose to over 60%. The developmental anomalies and embryonic stages at which death occurred were similar at the same pH in both the field and lab studies, strongly suggesting that pH was the critical factor (Pough, 1976; Pough & Wilson, 1977).

The directly toxic effects of acid precipitation on terrestrial plants have been studied and demonstrated much more extensively. The most common response reported is the formation of lesions on the upper

epidermis of leaves following exposure to acidic precipitation (Jacobson, 1980). For example, simulated acid rain at pH levels of 3 or less induced lesion formation on the foliage of kidney bean, sunflower and soybean plants (Evans et al., 1977; Evans & Curry, 1979). Lesion formation was also induced on specimens of bracken fern (Evans and Curry, 1979) and several species of poplar (Evans et al., 1978). The exposure of kidney bean and birch seedlings to simulated acid rain at pH levels of 3 or less caused necrosis of the leaves, poor root development and, reduced growth (Wood & Bormann, 1974; Ferenbaugh, 1976).

Acid precipitation may also affect plant reproduction. For example, the germination of spruce seeds was reduced by 80% in soils which had been acidified to a pH of 3.8 by simulated acid rain (Babich et al., 1980). The bracken fern produces motile spermatozoids during the process of sexual reproduction. Laboratory studies have shown that a two minute exposure to a solution at pH 5.1 can reduce motility of the spermatozoids to 30% of that in control solution at pH 6.1 (Evans, 1979).

Long term effects have not been investigated as thoroughly as the directly toxic effects and the results of such investigations are often inconclusive. A decrease in growth rates in pine forests of southern New Jersey on the past 25 years has been tentatively linked with acid precipitation. The data are not unequivocal, however, and acid precipitation has not been definitely shown to be the cause of the reduced growth (Johnson et al., 1981). Other studies carried out in Scandinavia have shown that acid precipitation may actually have a beneficial effect on forest growth by supplying N and S and thus acting

as a fertilizer (Abrahamsen, 1980; Tveite and Abrahamsen, 1980). Further research on the complex interactions between acid precipitation and forest ecosystems is clearly required.

It has been demonstrated that acid precipitation can adversely affect the microbial biota of soils. For example, soil respiration, used as an indicator of the overall soil microbial biochemical activity, was reduced after soil acidification by a simulated acid rain of pH 2 (Baath et al., 1979). Other important bacterial processes, such as leaf-litter and cellulose decomposition, glucose mineralization, nitrogen fixation and nitrification, were all adversely affected in tests with simulated acid precipitation (Leivestad et al., 1976; Tamm, 1976; Denison et al., 1977; Shriner, 1977; Lohm, 1980; Francis et al., 1980; Strayer & Alexander, 1981; Strayer et al., 1981). Counts of micro-organisms have shown that, in general, soil populations decrease with increased soil acidity (Francis et al., 1980). If soil microbial processes and populations are indeed inhibited by acid precipitation then similar effects on the micro-organisms of freshwater ecosystems can reasonably be expected.

Lake Acidification

The acidification of lakes in regions susceptible to acidic precipitation is a well documented phenomenon. For example, Schofield (1976) found that by 1975 the average pH of lakes in the Adirondack Mountains of New York state had dropped from a range of 6 - 8 in the 1930's to a range of 4.1 - 4.3. The percentage of lakes with a pH of less than 5 had increased from only 4% in the 30's to 51% by 1975 (Schofield, 1976). Lakes in the immediate area of the metal smelters

at Sudbury, Ontario, have registered pH values as low as 3.2 (Gorham, 1976). Emissions from these same smelters are believed to have acidified lakes in the La Cloche Mountains region of Ontario where pH levels as low as 4.8 have been recorded (Beamish, 1976; Beamish & Van Loon, 1977; Likens et al., 1979). In Scandinavia, several thousand lakes have been acidified to pH 5 or less, apparently by acid precipitation (Gorham, 1976; Wright et al., 1976; Wright and Snekvik, 1978; Likens et al., 1979; Babich et al., 1980).

The directly toxic effects of low pH on fish are varied. At extremely low pH levels of 3 or less, mucous coagulates on the gill surfaces and causes death by anoxia (Robinson et al., 1976). Between pH 4 - 5, the most likely cause of death is a disturbance of normal ionic and acid-base balances (Schofield, 1976). A reduction in the concentration of inorganic ions of blood plasma due to a net loss of salt is caused by interference with ion transport mechanisms through fish gills. The ultimate result is a decrease of plasma osmolality. This reduction in plasma ions and osmolality can affect intracellular ion concentrations and cellular volume, and can disturb normal physiological functions that relate to microcirculation within body tissues. In extreme cases of direct acid stress the organism involved will die (Leivestad & Muniz, 1976; Wright and Snekvik, 1978; Muniz & Leivestad, 1980). It takes a profound acid stress to cause direct fish deaths but, if taken in conjunction with other related stresses, such as starvation due to the loss of prey populations from acidification (Økland & Økland, 1980) or metal poisoning from mobilized metals such as aluminum (Schofield, 1976), then even a relatively slight direct acid stress can have cumulative and fatal consequences.

Typically, the effects of acidification on fish populations are subtle and progressive. Sudden, massive fish kills caused by increased environmental acidity occur very rarely (Leivestad & Muniz, 1976; Wright et al., 1976). Instead, fish populations decline gradually because of reproductive failure. Causes of this failure include the inability of females to release eggs, and egg and fry mortality. The young fry are much more sensitive to acidity than older fish and are especially vulnerable to the early spring 'pulses' of low pH runoff waters from melting snowpacks (Jensen & Snekvik, 1974; Beamish, 1976; Schofield, 1976; Harvey, 1980; Muniz & Leivestad, 1980; Rosseland et al., 1980).

Sensitive macrophytes in lakes undergoing acidification will often be supplanted by more acid tolerant organisms and this replacement can cause further complications. For example, studies conducted on some Scandinavian lakes have shown that when the pH fell below 6 the growth of Sphagnum moss was enhanced on the lake bottoms so that eventually they were entirely covered with a dense mat-like growth (Grahn et al., 1974; Grahn, 1977). All other macrophytes in the lakes were replaced by the moss and, because of the density of the covering, nutrients were trapped in the sediments and could not be recycled (Grahn et al., 1974; Wright et al., 1976; Grahn, 1977; Hultberg, 1977; Hendrey & Bertucci, 1980; Babich et al., 1980).

The effects of lake acidification on the planktonic and microbial populations of freshwater lakes have received much study. For the most part these effects appear to be deleterious, but there are reports of some beneficial effects. For example, in a whole lake acidification

experiment conducted at the Experimental Lakes Area (ELA) in northwestern Ontario, it was found that, despite a species composition change from chrysophytes to chlorophytes, the total phytoplankton biomass and productivity increased as the study lake (L. 223) became more acidic. The increases were attributed to the deepening of the euphotic zone and higher average water temperatures which apparently resulted from a change in the color of the dissolved organic matter in the lake as the pH dropped (Schindler, 1980; Findlay & Saesura, 1980; Schindler et al., 1980a). In general, however, acidification has been found to cause a reduction in the total numbers and species diversity of phytoplankton communities in affected freshwater systems (Almer et al., 1974; Wright et al., 1976; Crisman et al., 1980; Raddum et al., 1980). One explanation for the apparent discrepancy between the ELA study and others is simply that the pH in the ELA study lake may not as yet have reached the critical value for extreme effects, such as loss of population, to occur.

It has also been consistantly demonstrated that zooplankton populations undergo a similar reduction in numbers and diversity as the acidity of their environment increases. Species begin to disappear as the pH falls below 6 and the rate of disappearance accelerates rapidly as the pH drops below 5.5 (Almer et al., 1974; Hendrey and Wright, 1975; Wright et al., 1976; Crisman et al., 1980; Raddum et al., 1980; Malley et al., 1982). For example, in the ELA whole lake study mentioned earlier, three species of zooplankton had been lost from the lake by the time the pH had been reduced to 5.6. An increase in the abundance of herbivorous zooplankton was apparently in response

to the increased food supply offered by the increased phytoplankton population (Malley et al., 1982). The duration of this increase in herbivore numbers is as yet unknown but, in light of the results from other studies, their numbers can be expected to fall with further reductions in lake pH.

Most bacteria are not tolerant of acidic conditions and so in freshwater systems undergoing acidification changes can be expected in the bacterial population as the pH drops below optimum levels. The accumulation of leaves and other organic debris on the bottoms of acidified lakes indicate that a reduction in bacterial decomposition occurs under acidic conditions (Grahn et al., 1974; Hendrey et al., 1976; Almer et al., 1978; Friberg et al., 1980). Studies conducted using litter bags under various pH regimes tend to support such an hypothesis (Hendrey et al., 1976; Traaen, 1980). These studies are not conclusive evidence of bacterial inhibition however, as certain species of zoobenthos also play a major role in the decomposition of leaves and other organic debris by breaking down large detrital particles into smaller ones. Thus, the accumulation of coarse debris on the bottoms of acidified lakes may be caused in large part by the disappearance of these organisms. Other experiments which deal more specifically with acidification effects on bacterial decomposition or organic matter have yielded mixed results. In a series of lab experiments Bick and Drews (1973) found that microbial decomposition of peptone and ammonia was reduced as the pH decreased and that below pH 5 decomposition of ammonia ceased altogether. These investigators also found that the total cell counts of bacteria and the total cell

counts and number of species of ciliated protozoa declined as the pH was lowered (Bick & Drews, 1973). Laake (1976) found that when the pH of overlying waters was lowered, a reduction in the rate of oxygen utilization occurred at the surface of test sediments which indicated that heterotrophic microbial activity was inhibited by the increased acidity. Further, the utilization of radioactive glucose in the water just above the sediments decreased by up to 98% when the acidity of the water was increased from pH 6 to pH 5 (Laake, 1975). Hambrick et al., (1980) found that hydrocarbon mineralization rates were very dependant upon pH and redox potential conditions. Given equal pH levels, aerobic sediments had higher mineralization rates than did anaerobic sediments. Under conditions of equal redox potential, sediments at near neutral and slightly alkaline pH values (6.5 and 8.0 respectively) were found to have much higher mineralization rates than sediments of pH 5. Of particular interest to this paper is the fact that the lowest mineralization rates recorded in their study occurred in anaerobic sediments that had been acidified, a demonstration that anaerobic decomposer bacteria are indeed affected by low pH (Hambrick et al., 1980). In field experiments Fleischer and Granèli (1979) found that glucose turnover times in the sediments of an acidified lake were up to eight times longer than in the sediments of a eutrophied lake. In a series of respirometer studies Traaen (1974, 1980) determined that, when no change in the composition of a microbial community occurred, low pH inhibited the decomposition of glucose, glutamic acid, and homogenized leaf litter. When the structure of the microbial community did change from one that was

dominated primarily by bacteria to one dominated by fungi, it was found that the decomposition of glucose and glutamic acid proceeded fairly rapidly, even at pH levels as low as 4, and that oxygen consumption was little reduced. On the homogenized leaf litter substrate no conspicuous change in the microbial community structure occurred and oxygen consumption at pH 5.2 was only half that at pH 7 and substrate decomposition proceeded very slowly. It was suggested that the overall microbial ability to decompose complex substrates, such as cellulose and lignin, is more easily and severely affected by low pH than is the ability to decompose more readily degradable substrates, such as glucose. The end result of such selective inhibition is a build up of coarse debris on the bottoms of acidified lakes (Traaen, 1974, 1980). Andersson et al., (1978, 1980) compared various indicators of microbial metabolism, such as carbon dioxide production and oxygen consumption, in sediment samples from neutral and acidified lakes and found few differences between them. They speculated that only the very top sediment layers (2 - 4 cm) in acidified lakes were affected by the acidity and that in the deeper sediments metabolic activity went on as usual. The result of such selective inhibition would be that only the decomposition of debris on the sediment surface would be reduced by low pH (Andersson et al., 1978, 1980). Gahnstrom et al. (1980) determined in a series of laboratory experiments that glucose turnover rates in sediment samples from acidic and neutral oligotrophic lakes were low and not significantly different from one another. Likewise, the oxygen consumption in profundal sediments was found to be of the same magnitude in both

acidified and neutral lakes. However, when the uptake of oxygen in the littoral sediments of the acid lakes was compared to that in the profundal sediments, consumption was found to be much lower in the littoral sediments. This depressed oxygen consumption indicated a reduced microbial activity in the littoral sediments, possibly caused by the inflow of acid runoff waters during snowmelt and rainfall. It was concluded that acidification does not appear to affect profundal sediments very seriously, possibly because of the large buffering capacity of such sediments or because of increased sedimentation of organic matter. That acidification did have some effect on the microbial activity of the profundal sediments was shown by the fact that after liming of one of the acid lakes to increase the pH, the oxygen consumption and glucose turnover rate increased in the profundal sediments of the lake. The increase in activity which lasted for well over a year following the lime treatment indicated that accumulation of organic matter had increased during the acidification period. The liming may have produced a more favorable abiotic environment so that easily degradable organic compounds which had accumulated in the sediments were then more available to the microbiota (Gahnstrom et al., 1930).

Clearly the effects of acidification on the biota of affected freshwater ecosystems are not as straightforward as once thought. Much study remains to be carried out before the processes involved in lake acidification are well understood.

Management and Control Strategies

Control and amelioration strategies for lakes in the process of

being acidified are rather limited. To date the best results have been obtained by adding lime to affected lakes (Scheider et al., 1975; Broberg, 1978; Hultberg & Andersson, 1982). Liming increases the pH but otherwise alters lake water chemistry to a minimally (Grahn & Hultberg, 1975). The treatment does appear to reverse the effects of acidification (Scheider et al., 1975; Broberg, 1978; Dickson, 1978; Dillon et al., 1979; Gahnstrom et al.,; Hultberg & Andersson, 1982), but the reversal is temporary unless fresh lime is added, since the influx of fresh acidic waters uses up the buffering capacity of the lime fairly quickly unless the lake waters have a long residence time (Broberg, 1978). The amounts of lime required per lake are on the order of tonnes, so that the difficulties of transporting the required amounts, and the large number of lakes affected by acid rain, effectively preclude the widespread use of this treatment as a control strategy. Recent work by Kelly et al. (1981) and Dillon (personal communication) has shown that productive lakes appear to have larger buffering capacities than do relatively unproductive lakes in the same region. If this is indeed the case, then increasing the productivity of lakes in susceptible regions, possibly by the addition of phophorous (Schindler et al., 1971, 1973, 1973; Dillon et al., 1979; Kelly et al., 1981), would render such lakes less vulnerable to acidification. The difficulties involved, and the effectiveness of such a treatment remain to be studied. For example, one possible difficulty lies in the fact that the buffering ability of a productive lake is apparently highly dependent on the charge of the nitrogen source of the lake; if it is NH_4 then the lake will undergo acidification

(Schindler, personal communication). Nevertheless, as increasing the productivity of a lake by (for example) phosphorous addition is relatively simple and requires only a few kilograms of material per year, this method may prove to be a widely usable alternative to liming as a control strategy for combating lake acidification.

Combined Low pH and Heavy Metal Effects

One well documented consequence of lake acidification is an increase in water column concentrations of heavy metals (Schofield, 1976; Wright & Gessing, 1976; Wright et al., 1976; Dillon et al., 1978; Schindler et al., 1980a, 1980b; Schindler & Turner, 1982). These increases in the metal levels are attributed to deposition along with acidic precipitation (Wright et al., 1976; Beamish & Van Loon, 1977; Hutchinson & Whitby, 1977; Franzin & McFarlane, 1980), increased leaching from drainage basin soils (Malmer, 1976; Beamish & Van Loon, 1977; Harriman & Morrison, 1980; Keller et al., 1980), leaching from lake sediments (Beamish & Van Loon, 1977; Schindler et al., 1980b), and combinations of any of the above. Several studies have been conducted to examine the possible synergistic and/or antagonistic interactions between these related pollutants (Babich & Stotzky, 1978; Gadd & Griffiths, 1978; Brungs et al., 1978; Rai et al., 1981b). The need for such studies is underscored by a recent report from Sweden which states that in a hospital which relied on waters acidified by acid precipitation for its needs, the water concentrations of cadmium, copper, lead and mercury had reached toxic levels. At least three kidney patients on dialysis equipment died as a result of receiving fatal doses of aluminum from the water used in the machines (anony-

mous, 1982).

In terrestrial environments most studies of the combined effects of low pH and heavy metals have dealt with plants and the results yielded have often been contradictory. For example, John (1972) found that roots from radish plants grown in pH 4.1 soil which had been supplemented with an equal amount of Cd. Other studies have confirmed the uptake of Cd from the soil to be pH dependent, the more acidic the soil the greater the uptake (and thus increased toxicity) of Cd by plants (John et al., 1972; Smith & Huckabee, 1973; Andersson & Nilsson, 1974; Williams & David, 1976; Shen-Miller et al., 1976). However, other studies have found that uptake of cadmium by plants was independent of the soil pH. For example, Davies and Roberts (1975) examined radishes harvested from several gardens in Wales which had been contaminated with Cd. They found no correlation between the soil pH and Cd uptake by the plants (Davies & Roberts, 1975). Other investigators have either obtained similar results (Lagerwerff, 1971; Lagerwerff & Biersdorff, 1972; Jones et al., 1975), or found the uptake was also dependent on the concentration of Cd in the soil. Miller et al., (1976) found that in soils which had been supplemented with 1 or 10 mg Cd/kg soil, the uptake of Cd by soybeans was dependent on the soil pH with a decrease in the pH resulting in an increase in the uptake of Cd. However, with supplements of 100 mg Cd/kg soil there was no correlation between the soil pH and uptake and accumulation of Cd by the soybeans (Miller et al., 1976). There exists some speculation as to the reason(s) for these contradictory results, but no definitive causes have been identified.

Somewhat more extensive studies have been conducted on the combined effects of metals and high acidity on the biota of aquatic environments. With a few exceptions most such studies have indicated that these pollutants tend to act in combination, that is, metals exert a greater toxic effect at acid pH levels and acid toxicity is potentiated by the presence of heavy metals.

Schofield (1976), Cronan and Schofield (1979), and Baker and Schofield (1982), have found the combination of high aluminum levels and low pH to be extremely toxic to fish, causing rupture of gill filaments. Gothberg and Nagell (1977) found that Atlantic salmon (Salmo salar) fingerlings could survive at a pH of 5 for over 21 days, but when 0.2 mg Al/l was present in the environment the fingerlings would die within 6 days at the same pH. Other investigators working with chromium (Van er Putte et al., 1981), copper (Chaloumakos et al., 1979), lead (Merlini & Pozzi, 1977; Hodson et al., 1978), zinc (Schofield, 1965), and other metals (Muniz & Levistad, 1980) have all found that metal uptake by, and toxicity to, fish increases as the pH drops.

Studies on microorganisms have found similar results, with a few exceptions (see Gachter, 1976; Babich & Stotzky, 1977a,b; Say & Whitton, 1977; Babich & Stotzky, 1979). Rai et al. (1978a) working with the metals zinc, mercury, and methyl mercury, found that their toxicity to Chlorella vulgaris increased as the acidity increased. At the same concentration of zinc or mercury, growth of the alga at pH 4 was reduced to less than 10% of that at pH 7. At the same concentration of methyl mercury growth at pH 4 was only 25% of that

at pH 7 (Rai et al., 1981a). Harding and Whitton (1977) working with both Zn tolerant and sensitive populations of the alga Stigeoclonium tenue found that a decrease in the pH increased the toxicity of Zn to both populations, with the Zn sensitive population also being more sensitive to pH changes. Ko and Hora (1972) working with the fungus Neurospora tetrasperma found that at pH 4.5, 0.65 mg Al/l completely inhibited spore germination, whereas at pH 7 the same concentration of aluminum would cause less than a 5% reduction of spore germination. Avakyan (1971) found that at the same concentration of copper the growth of the food yeast Candida utilis at pH 4.5 was reduced to less than 50% of the growth at pH 7. Zwarun and Thomas (1973) found that 1 mg Al/l at pH 4.5 caused a reduction of 95% in the growth of the bacterium Pseudomonas stutzeri when compared to a pH 7 control with the same amount of aluminum. Titus and Pfister (1982) found that the uptake and accumulation of Cd by a Pseudomonas sp. increased at reduced pH levels. They attributed this increase to the increased mobility of the metal under acidic conditions (Titus & Pfeister, 1982).

Acid mine drainage streams polluted by metals provide some idea of the effects that the input of high concentrations of metals and acid can have on an ecosystem. Acid mining wastes are very toxic (Hale, 1977; Hoehn & Sizemore, 1977) and can have devastating effects on a pristine environment. In one study Hoehn and Sizemore (1977) found that as a result of acid mine drainage a 6 mile stretch of a creek in Virginia is devoid of algal, benthic macroinvertibrate, and fish life. Although such polluted streams will still support microbial

life (Erlich, 1963; Whitton & Say, 1975) the community is dominated by those organisms which are highly tolerant of metals and acidity and is not likely to bear much resemblence to the microbial community present prior to the onset of acid mine drainage input.

Much work remains to be done on the problem of combined metal and acid pollution. Sites sensitive to such pollution and tolerant and sensitive species must be identified. Control and amelioration mechanisms require further study. The problem of food chain magnification of heavy metal content under acidic conditions must be studied. Especially required are more studies which examine the effects of metals and pH both separately and in combination to determine if their combined effects are additive, synergistic, or even antagonistic. The acid precipitation problem is tremendous in scope and a more thorough understanding of all its aspects is required if viable solutions are to be found.

MATERIALS AND METHODS

MATERIALS AND METHODS

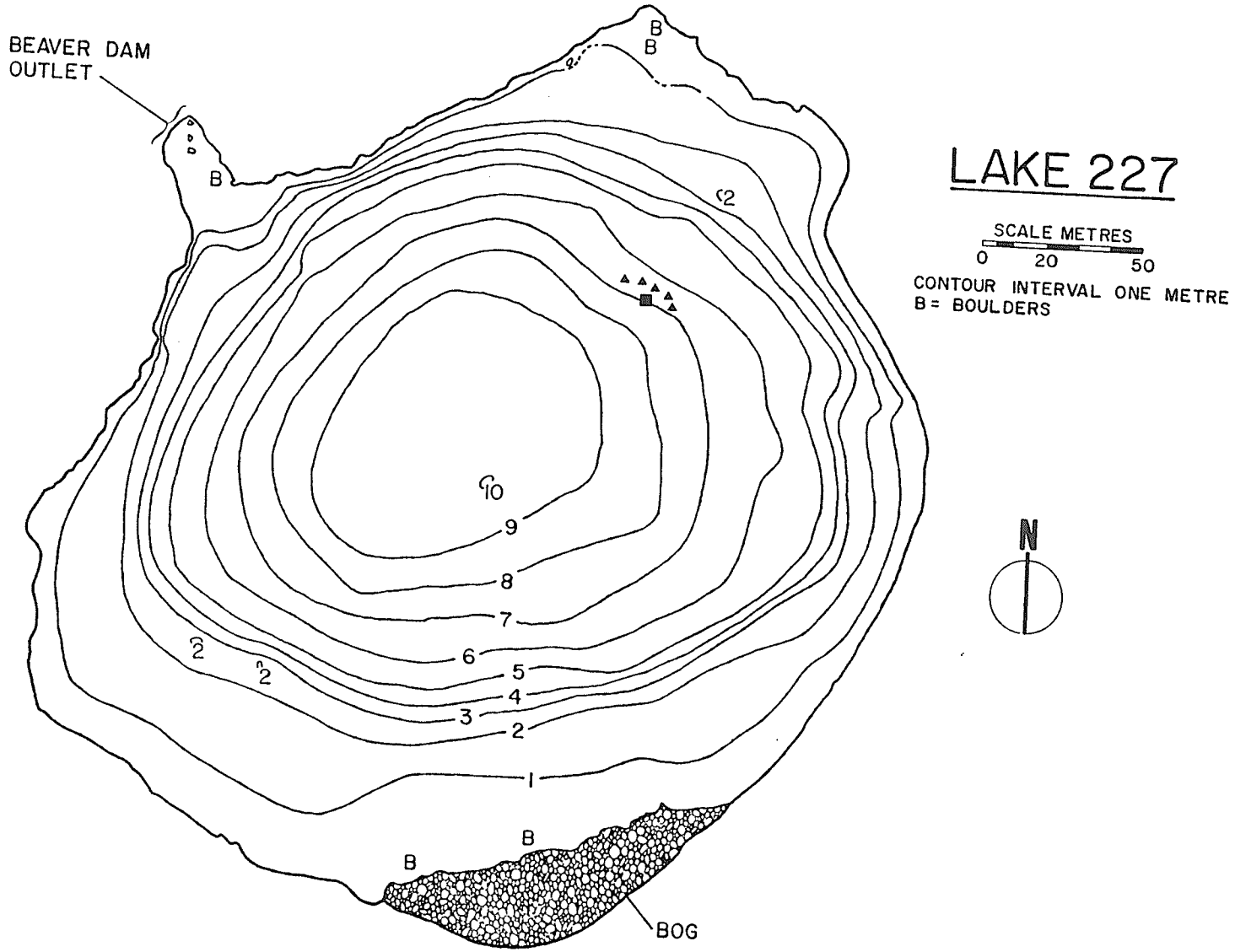
Lake Description

All of the field studies and a major portion of the laboratory work were conducted at the Experimental Lakes Area (ELA) located approximately 50 kilometres east southeast of Kenora, Ontario, at $93^{o}30'$ - $94^{o}00'$ W, $49^{o}30'$ - $49^{o}45'$N (Johnson & Vallentyne, 1971). Since the summer of 1969 whole lake experiments have been carried out at this station under the direction of the staff of the Freshwater Institute (FWI) in Winnipeg. The background environmental data on ELA and progress in a number of research programs being conducted there have been well documented (Stevenson, 1971; Schindler, 1973; Watson, 1980) and will not be discussed here.

Lake 227 (figure 2), the study lake, is located approximately 3 km northeast of the ELA base camp (Cleugh & Hauser, 1971). It is a small lake, 5.0 hectares in surface area, roughly circular in shape and with a maximum depth of 10 metres. Further physical and some chemical characteristics of the lake and its sediments may be found in Schindler (1971), Brunskill and Schindler (1971), Brunskill et al. (1971) and Schindler et al. (1971).

Since the summer of 1969 this originally oligotrophic lake has been loaded with nitrogen and phosphorous on an experimental basis and the budgets for these nutrients closely monitored. From 1969 to 1974 inclusive, the lake was enriched weekly during the ice-free seasons with nitrogen as nitrate and phosphorous as phosphate (7.0 g N m^{-2} yr^{-1} and 0.50 g P m^{-2} yr^{-1}, a raio of 14:1 (Findlay, 1981).

Figure 2. A bathymetric chart of Lake 227. The approximate locations of the field experiment tubes are marked by the triangles (▲) and the adjacent lake site (lake site I) by the square (■) (after Brunskill and Schindler, 1971).

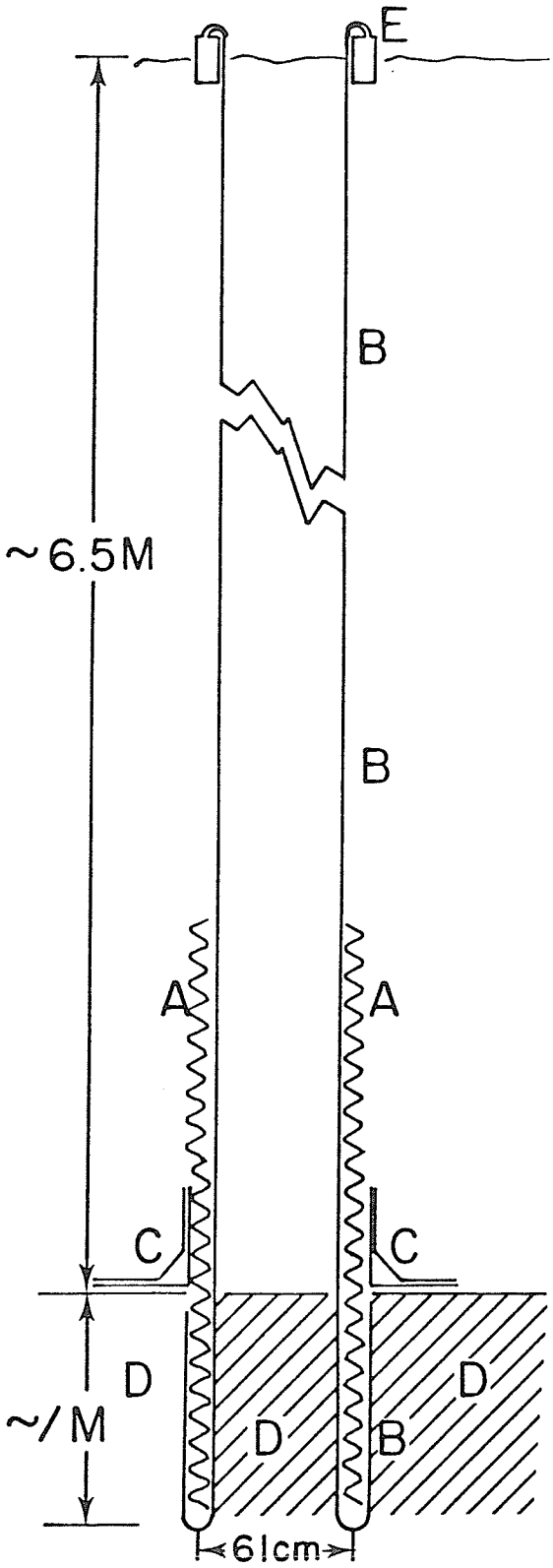

BEAVER DAM
OUTLET
B
B
B
2
10
9
8
7
6
5
4
3
2
1
2
2
B
B
B
BOG
LAKE 227
SCALE METRES
0
20
50
CONTOUR INTERVAL ONE METRE
B = BOULDERS
N

From 1975 on the loading rate was reduced to 2.25 g N m^{-2} yr^{-1} and 0.46 g P m^{-2} yr^{-1}, a ratio of 5:1 (Findlay, 1981). The immediate effect of these manipulations could best be described as artificial eutrophication. To date, Lake 227 remains highly eutrophic (Schindler, 1977; Schindler et al., 1971, 1973, 1978). It was because of the rich background data available on this lake and because the investigations of Rudd et al. (1974) had shown the 227 sediments to be highly methanogenic that it was chosen for study.

Field Systems, Design, Studies & Sampling

The field studies were carried out in five tubes which, in late July 1979, were sunk into sediments of Lake 227 overlain by 6.5 metres of water (fig 2). The tubes were constructed of woven polyethylene (Curry Industries, Winnipeg) approximately 61 cm (2 ft) in diameter by 10 metres long. The bottom end of the tubing was fastened to a length of galvanized steel culvert (West-Man Culvert and Metal Co., Winnipeg) about 2.4 metres (8 ft) long by 61 cm wide, in such a manner that the bottom of the tubing was kept open (fig. 3). The bottom end of each tube was sunk into the sediments at the 6.5 m site with the culvert and attached tubing entering approximately 1 metre into the sediments. The culverts played a dual role in these systems: they anchored the polyethylene tubing firmly into the sediments and, because the polyethylene was permeable to methane, they were an impermeable barrier preventing the lateral loss of methane from within the tubing above the sediments. The top of the polyethylene tube was fastened to a floating framework which kept it open and above the water surface.

Figure 3. Cross-sectional diagram of a field experiment tube. Legend: A - steel culvert; B - woven polyethylene tube; C - supports to hold the culvert upright on the sediments; D - lake sediments; E - floatation collar.


E
B
~6.5M
B
A
A
C
C
D
~/M
D
D
B
D
61cm

Of the five tubes set up, numbers 1 and 3 were used as controls, number 2 to study the effects of decreased pH, number 4 to study the effects of added cadmium, and number 5 to study the effects of both low pH and added Cd.

Test solutions were added to the tubes using a Masterflex model 7570 sampling pump and a special 'additions rig' which permitted the additions to be made at constant depths of 1, 2.5, 4, 5.5 and 6.5 m over the course of the study period. The use of five introduction points allowed the test additions to be distributed fairly evenly throughout the water column of a tube without having to stir or otherwise disturb it. The additions rig was made of 5 lengths of polyethylene tubing (6 cm interior diameter, Canlab) banded together and weighted to hang vertically in the tube water column. When making additions the test solution was divided up into 5 equal aliquots which were introduced into each line and then pumped to depth. To keep air from entering the addition line, and thus to minimize atmospheric invasion of the lower water column, a second line was used to pump water up from the appropriate depth and this water followed the test solution through the addition line.

Water samples for analysis were collected using a Masterflex model 7570 peristalitic sampling pump and a 10 metre long by 9 mm interior diameter polyethylene sampling line (Cole Parmer # 6408-09) which had been marked off at half metre intervals. The line was fitted with a sampling head which kept the intake of the line 10 cm above the sediments when the bottom of the head was resting on the sediments.

When taking the maximum depth sample the head was gently lowered onto the sediments to minimize disturbance, the sample was taken and the head then slowly raised. This method allowed the maximum depth samples to be taken at a constant depth above the sediments regardless of the level of the lake water.

Using the sampling system described above, 25 ml water samples for the determination of water column methane concentration were collected in 50 ml plastic syringes (Plastipak - Becton, Dickinson & Co., Missisauga, Ontario). The water samples were immediately stripped of their methane content in the field using the syringe stripping technique of Rudd et al. (1974). The stripped gas was then immediately stored in 10 ml serum vials according to the method of Rudd et al. (1974) until analyzed. Duplicate samples were taken to check the reproducibility of the stripping technique.

Water samples for pH, nitrate and sulfate analysis were collected from the sampling line in 125 ml glass sample bottles (Pyrex - Fisher Scientific) equipped with ground glass stoppers. At least twice the volume of the bottle was displaced as recommended by Stainton et al. (1977) to minimize atmospheric invasion into the samples. The samples were returned to the lab as quickly as possible and either analyzed immediately or stored at $4^{\circ}C$.

Water samples for cadmium and zinc determinations were collected from the sampling line in acid washed polyethylene scintillation vials (New England Nuclear) allowing at least twice the volume of the vial to be displaced to minimize atmospheric invasion. Upon return to the lab the samples were preserved by the addition of one drop of ultra

pure redistilled nitric acid to each vial. This treatment gave the samples an almost unlimited storage life.

Dissolved oxygen content and the temperature of the tube water columns were determined using a Yellow Springs Instrument (YSI) model 54 oxygen meter equipped with a YSI model 5419 pressure compensated probe on a 15 metre line which had been marked off at 1 metre intervals. Before going into the field the probe and meter calibrations were checked and standardized against known dissolved oxygen and temperature standards.

Winter Sampling

The equipment used in the winter was essentially the same as that used during the ice-free months. All sampling lines, pumps and sample bottles were transported and used in insulated, heated boxes in order to prevent ice formation and clogging. Once taken, all samples were stored in heated boxes for transportation back to the ELA labs to prevent freezing. Water samples for methane, pH and dissolved oxygen analysis were collected in 300 ml BOD bottles (Wheaton) according to the method of Stainton et al. (1977) using the same sampling pump, head and line as used in the summer. Preparation and analysis of the samples began within an hour of their return to the laboratory. Two 25 ml subsamples were removed from the BOD bottles using two 50 ml plastic syringes each fitted with an 18 gauge, 38 mm needle (Yale - Becton Dickinson & Co.). The bottle was then fitted with a YSI model 5420a BOD probe attached to a YSI model 54 oxygen meter and the remaining 250 ml of water was analyzed for the dissolved

oxygen. Removal of the 2 subsamples and fitting of the BOD probe to the bottle would normally take under 30 seconds. The 50 ml of water removed for the subsamples was slightly less than the amount of water normally displaced by the probe, so that atmospheric invasion into the bottle during subsample removal and the dissolved oxygen determination was minimal. Concurrently with the oxygen measurement one subsample would be stripped of its methane content with the stripped gas being stored for later analysis. Both stripping and storage were done according to the method of Rudd et al. (1974). The remaining subsample would be subjected to pH analysis immediately following storage of the gas sample. Typically, the entire operation took approximately 3 minutes per bottle.

Heavy metal samples were collected in the same manner as in the summer and preserved by acidification within 4 hours of arrival at the laboratory.

Temperature profiles in the winter were taken with a YSI model 43 thermistor.

Nitrate and sulfate levels were not monitored during the winter months because there was no spectrophotometer available at ELA at that time and the samples would have deteriorated significantly by the time they could have been returned to the FWI labs.

Analytical Methods, ELA

All methane analyses done at ELA were carried out on a Pye Unicam model 104 gas chromatograph fitted with a flame ionization detector and a Poropak Q column. The sample size injected was 0.2 cc taken

directly from the storage vials using a 0.5 cc glass syringe (Claspak Becton, Dickinson & Co.) fitted with a 26 gauge, 12.5 mm needle.

The pH of the water samples was determined within two hours of returning the samples to the lab using the method of Galloway et al. (1979) on a Radiometer/Copenhagen PHM 63 digital pH meter, or in the winter on a Radiometer/Copenhagen PHM 29b pH meter. If the interval between return and analysis was anticipated to be more than 2 hours, the samples were stored at 4^{o}C. until analyzed. Prior to analysis the samples were allowed to return to room temperature.

Determination of nitrate and/or sulfate content of the samples took place within 24 hours of sampling, the samples being stored at 4^{o}C. until analysis. Both determinations were done spectrophotometrically on a Coleman Junior II Spectrophotometer model 6/20. Nitrate content was determined using the Bausch and Lomb Spectrokit reagent system for nitrate which is adapted from the U.S. Environmental Protection Agency publication Methods for Chemical Analysis of Water and Wastewater, page 175. The sulfate content was determined with the Bausch and Lomb Spectrokit reagent system for sulfate which is adapted from the American Public Health Association's Standard Methods for the Examination of Water and Wastewater, 13th. ed., page 334.

Heavy metal water samples were transported back to the FWI labs for analysis. Analysis was carried out on a Varian Techtron model AA5 atomic absorption spectrograph according to the method of Culver (1975).

Sediments and Waters used

Two types of laboratory systems were used in the course of these studies. The sediment samples used for these systems were taken from the 10 metre sampling site of Lake 227 (fig. 2) with an Eckmann dredge. Typically only the top 10-12 cm of the lake sediment bed were sampled, these sediments being a deep blue-black in color and very fluid in nature (Brunskill et al., 1971).

Depending on the size of the sample required, the sediments were stored in either a 1 litre glass jar (Bellco) fitted with a ground glass stopper, or a 20 litre thick-walled polyethylene carboy (Nalgene) with a screw cap. The storage vessels were filled to overflowing with the sediments before capping so that there was no air space above the sediments. The samples were then transported to the lab as quickly as possible and stored at 4^{o}C. until used. Sediments were sampled throughout the year as old supplies were depleted and fresh sediments required.

The second laboratory system used required large amounts of anaerobic lake water (see description of 'microbasin' lab systems, pgs 60-64) which was also obtained from Lake 227. The water was taken from a depth of 9 metres in the lake with a 4 litre Van Dorn sampler and was kept in 20 litre polyethylene carboys (Reliance Products Ltd., Winnipeg) for transport and storage. Once back at the lab the water samples were stored at 4^{o}C. in the dark until used. Prior to use the oxygen content was determined with a dissolved oxygen meter and, if necessary, the water was bubbled with ultra-pure nitrogen to remove any water present. Water sampled more than one week prior to an experi-

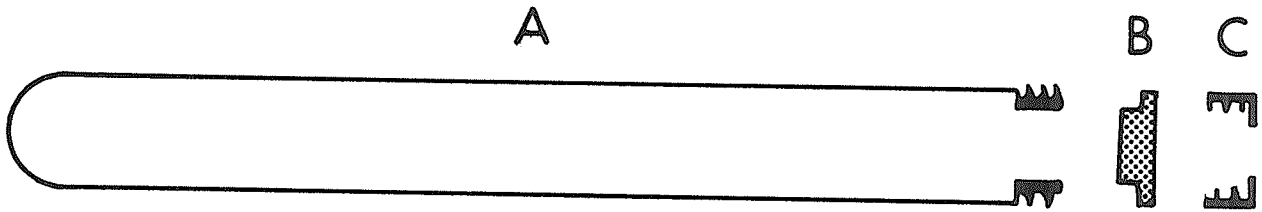
ment was not used.

Laboratory Systems, Design and Use

The first lab system used was the modified Hungate anaerobic culturing technique (Hungate, 1969) described by Zeikus and Winfrey (1976) and Winfrey et al. (1977). All experiments were carried out in quadruplicate in 15 X 130 mm anaerobic test tubes (fig. 4) which contained 9 ml of sediments and 1 ml of various test solutions. The 227 sediments, being extremely fluid, were added to each tube with a 10 cc glass syringe fitted with an 18 guage, 38 mm needle, through the septum in the tube cap. Prior to and during the addition the tube was gassed with ultra-pure nitrogen (Liquid Carbonic Canada) flowing at approximately 500 ml/min. Injection of the sediments was completed in 30 seconds. Additions of test solutions were made anaerobically using 1 ml volumes of the solution injected through the septum by a 1 cc glass syringe fitted with a 22 gauge, 16 mm needle. Appropriate dilutions of the test solutions were made up in 10 ml volumes from 1 molar stock solutions and then stored under a nitrogen atmosphere in spare anaerobic tubes. After additions of the test solution the tubes were stirred by vortex for 3 minutes under a flow of nitrogen (500 ml/min) to ensure complete mixing of the addition into the sediments and also to purge the tube of any remaining methane. All of the tubes were incubated in the dark in an environmental chamber (Coldstream) set to $12^{o}C$. Each experimental series was repeated at least once.

The second laboratory system used was based on the microbasins systems of Wright (1978) as modified to form continuous culture

Figure 4. Cross-sectional diagram of an anaerobic test tube. Legend: A - 15 x 130 mm glass screw cap test tube; B - rubber septum; C - screw-on cap with centre punched out.

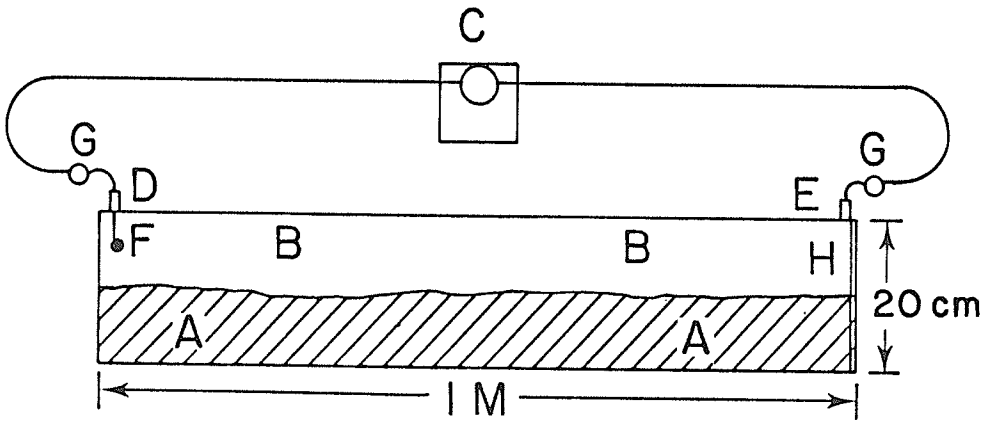

A
B
C

apparatus. Each microbasin is a plexiglass box 1 metre long by 10 cm wide and 20 cm high, and is equipped with numerous sampling ports and one removable end (fig. 5).

In use, a basin was filled 35-40% with sediments through the removable end and then lakewater taken from the hypolimnion of Lake 227 (as described earlier) was added to almost fill the basin. Once the basin was filled the end was replaced and sealed with CGE Silicone seal (Canadian General Electric). When the seal had set, the basin was shaken by hand for approximately 5 minutes in order to distribute the sediments evenly across the basin bottom. A settling period of at least 24 hours followed, after which the basin was connected to a Cole Parmer Ultra Masterflex drive system peristaltic pump with a Master Servodyne speed controller and the water above the sediments was circulated through the basin at a rate of approximately 10 ml/min or about 14.4 litres per day. At this rate the water would circulate completely every 20 to 24 hours. Current flow studies showed complete mixing of the water and any test additives within 24 hours of the addition.

It was necessary to flush the methane from the microbasin water before the addition of a test solution because the methane present in the system would tend to mask the effect of the test solution. This flush was done by pumping the water through a nitrogen bubbling flask at a rate of about 3 litres per hour. The flow rate of the ultra-pure nitrogen was about 2 litres per minute. This high speed pumping of the water was maintained for at least one full circulation of the microbasin water through the bubbling flask, 6 to 8 hours. Once the

Figure 5. Cross-sectional diagram of a microbasin (after Wright, 1978). Legend: A - sediments; B - anaerobic lake water; C - peristaltic pump; D - inflow line; E - outflow line; F - inflow line outlet port which ran parallel to the sediment surface; G - three way valves; H - the removable end of the basin.

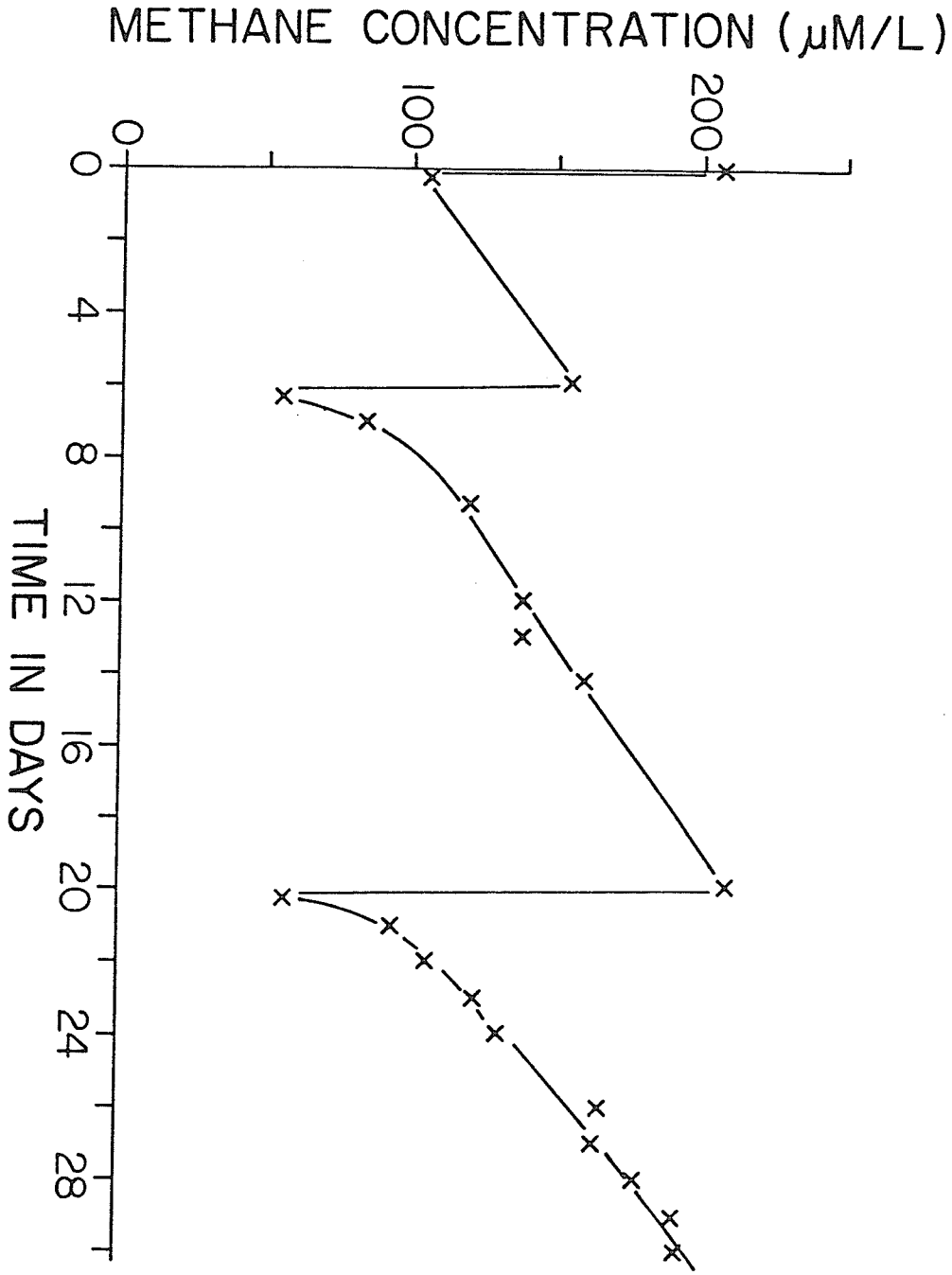

C
G
G
D
E
F
B
B
H
20 cm
A
A
1 M

flush sequence was finished the normal flow lines were reconnected, the test solution added to the inflow line, and an initial methane sample taken from the outflow line. High speed pumping of the basin water was maintained for 1 hour after the addition to help mix the test solution and the water. At the end of this mixing period normal speed circulation (14.4 l/day) was resumed and another methane sample taken.

To determine if flushing would have any effect upon methane production in a basin, several control basins were subjected to repeated flushing and recovery cycles (fig. 6). In the flush phase the methane content fell to typically less than 25% of previous levels. A rapid recovery phase lasting approximately 24-36 hours then followed during which the methane concentration in the basin free water roughly doubled. This phase may have been caused in part by the equilibration of methane remaining in the sediments during the flush with the low methane content water after the end of the flush. Thereafter there was a steady increase in the methane concentration in the free water due to normal methanogenic metabolism. It should be noted that there was no apparent loss of methanogenic activity after the repeated flush sequence and that this held true even in cases where the basin underwent the flush and recovery sequence 5 times.

The methane flush was also used to check the sediments in the test basins for activity. Prior to the addition of any test solution all test microbasins were subjected to one complete flush and recovery sequence to ensure that their methanogenic population was active. Only

Figure 6. Typical nitrogen flush and recovery profile for a control microbasin. Clearly visible are the sudden drop in methane concentration during the flush (e.g. at approximately day 20 the methane concentration fell from over 200 to almost 50 uM/l during the flush), the rapid recovery phase (e.g. from day 20 - 21 when the methane concentration climbed from 50 to just under 100 uM/l), and the steady recovery phase which followed the initial rapid recovery phase (e.g. from day 21 to day 30).


METHANE CONCENTRATION (μM/L)
0
100
200
TIME IN DAYS
0
4
8
12
16
20
24
28

after a basin had shown a complete recovery phase was it again flushed and the test solution added.

All of the basins were incubated in the dark at 12°C.; those used at ELA were kept in a waterbath and those at FWI in an environmental chamber.

Laboratory Sampling and Analyses

The anaerobic test tubes were sampled at 24 hour intervals until methane production leveled off. Immediately prior to sampling the tubes were vortexed for 30 seconds to drive evolved methane from the sediments. A 1 cc plastic syringe fitted with a 22 guage, 13 mm needle, was used to remove a 0.4 cc gas sample from the headspace of the tube. The gas was immediately injected into a Hewlett Packard model 5750 gas chromatograph equipped with a flame ionization detector and a Poropak Q column. Tests using methane standards and both glass and plastic syringes showed no loss of methane from the plastic syringe.

Water samples were removed from the microbasins with a 50 ml plastic syringe through a 3-way valve on the outflow line of the basin (fig. 5). The methane content of the water was determined on a 25 ml sample using the syringe stripped and storage method of Rudd et al. (1974). Methane samples taken at FWI were analyzed on the Hewlett Packard 5750 (described above), while those taken at ELA were analyzed on the Pye Unicam (described above).

Twenty-five ml water samples were removed from the basins for heavy metal analysis and 30 ml samples for pH determination. Both these analyses were carried out in the same manner as at ELA.

Standard acid test solutions were made using concentrated reagent grade sulfuric acid (Fisher) diluted as required with deoxygenated, distilled water. Stock cadmium solutions were made up using reagent grade cadmium chloride (Fisher) in deoxygenated distilled water. All other stock solutions were made up in a similar manner, all other chemicals used being Fisher reagent grade or the equivalent. All stock solutions were stored under a nitrogen atmosphere.

RESULTS

RESULTS

Test Tube Studies

In preliminary testing the modified Hungate method was found to be a fairly useful rapid screening technique (fig. 7). In tubes which received acetate (5 mM), a precursor in the methanogenic pathway, methane production was more rapid and the final concentrations reached greater than in the control tubes. In tubes which received nitrate (5 mM), an inhibitor of methanogenesis, little CH_4 was detected and quantities remained fairly constant throughout the test period. In tubes to which glucose (5 mM) was added the methane levels rose slowly for the first 72 hours as the glucose was broken down to CH_4 precursors. After 72 hours the CH_4 content increased rapidly to the highest levels recorded in this test series. In tubes which received cadmium (44.5 uM, final concentration 5 mg/l) methane production was low, similar to that found in the tubes which received nitrate.

A series of tests was conducted to determine if a threshold value for the apparent inhibitory effect of cadmium on methanogenesis could be found (fig. 8). The amounts of Cd added were 8.9 uM to the first set, 44.6 uM to the second set and 89 uM to the third set of tubes (1,5 and 10 mg Cd/l respectively). At all three Cd concentrations tested methane production closely resembled that in tubes to which nitrate was added. None of the tests resembled the controls in terms of rapidity of CH_4 production or the final concentrations reached.

Several tests were conducted to determine what effect acetate would have upon the inhibitory effect of cadmium (fig. 9). In tubes

Figure 7. Methane production preliminary test data. Methane production in anaerobic test tubes following the addition of various test solutions. Legend: x - control tubes; □ - 5 mM acetate added; △ - 5 mM glucose added; ▽ - 5 mM nitrate added; ○ - 5 ppm cadmium added.

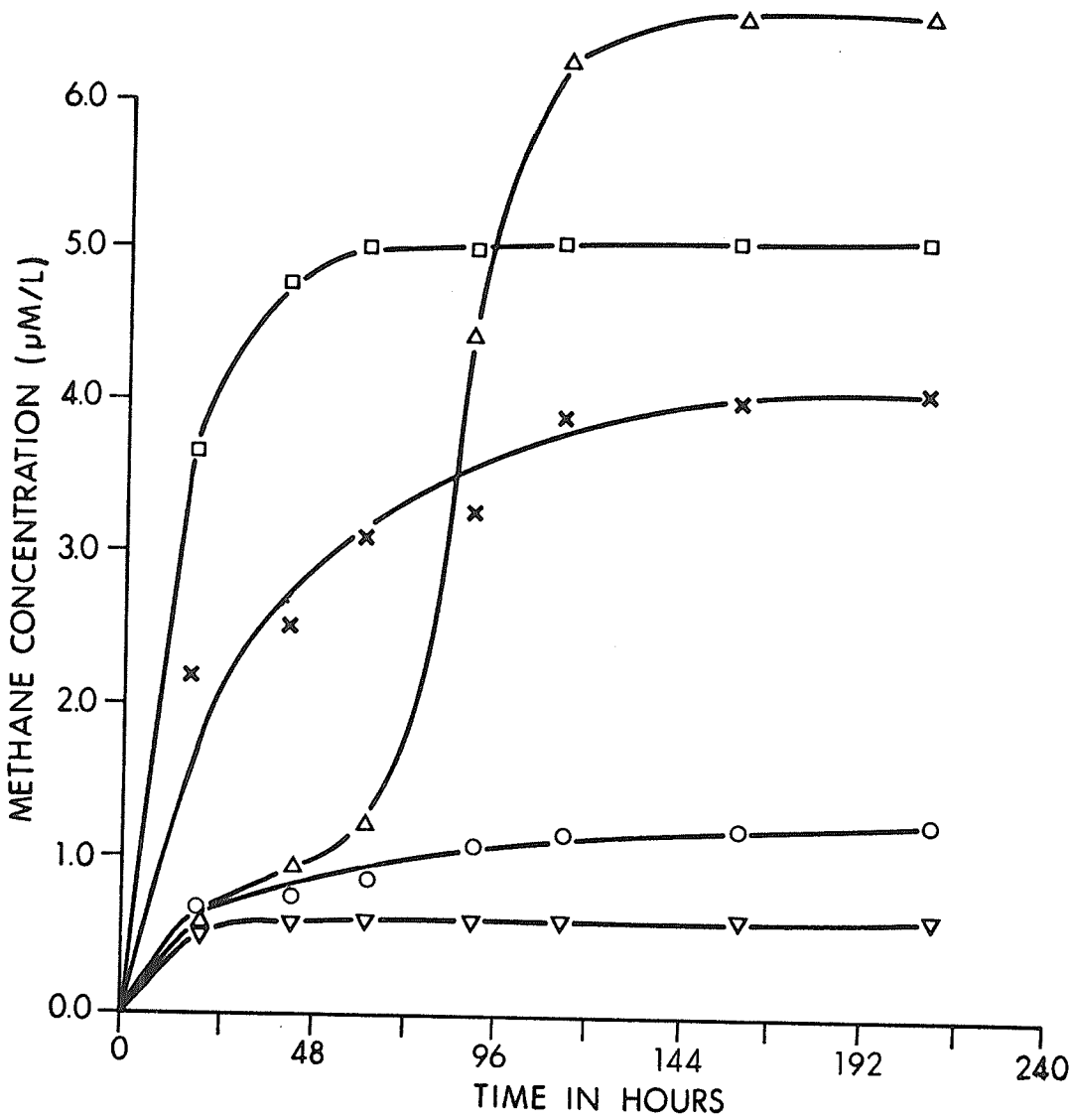

METHANE CONCENTRATION (μM/L)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
48
96
144
192
240
TIME IN HOURS

Figure 8. Anaerobic test tubes, cadmium test series data. Legend: x - control tubes; ○ - nitrate addition tubes; □ - ppm cadmium addition tubes; △ - 5 ppm Cd addition tubes; • - 10 ppm Cd addition tubes.

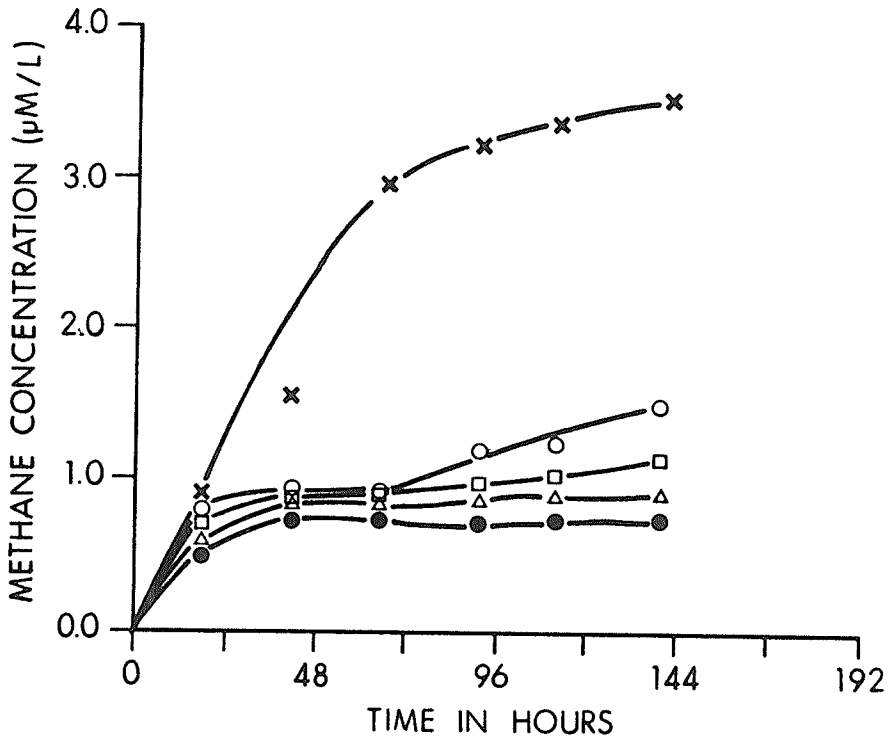
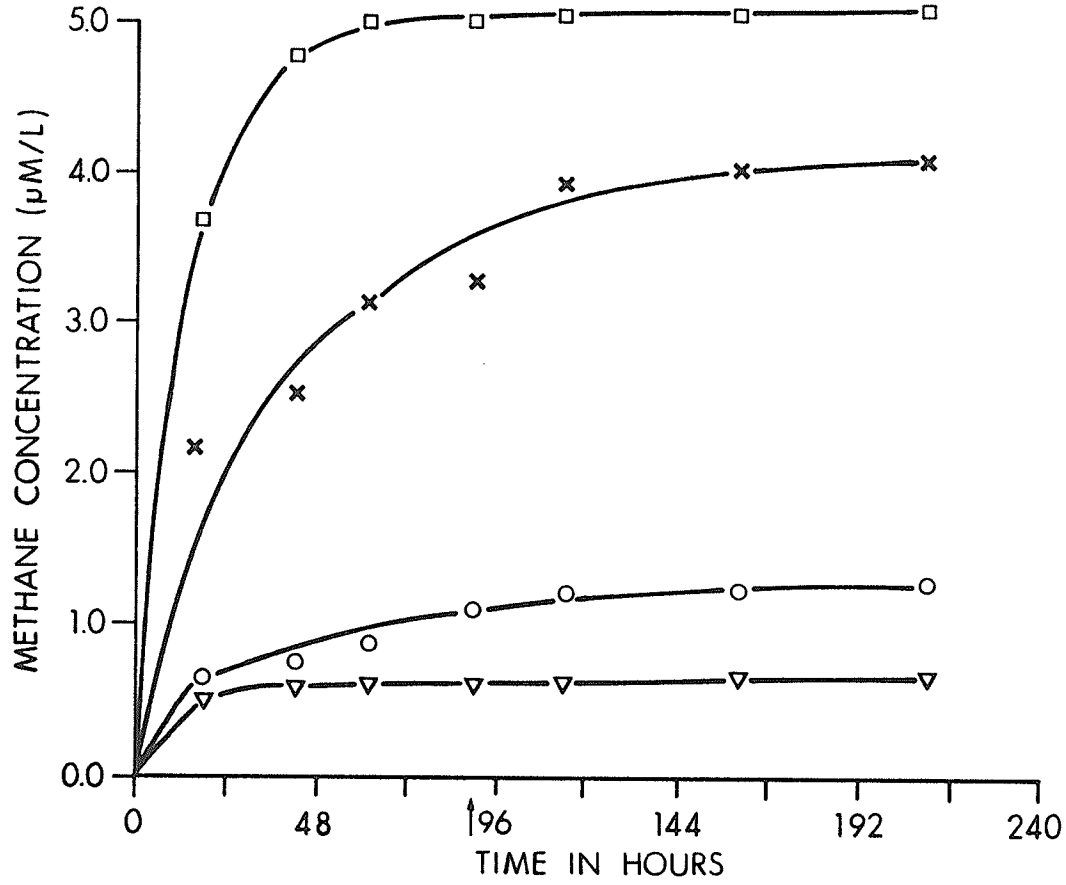

METHANE CONCENTRATION (μM/L)
4.0
3.0
2.0
1.0
0.0
0
48
96
144
192
TIME IN HOURS

Figure 9. Anaerobic test tubes, methane precursor + cadmium addition test series data. Legend: x - control tubes; ○- nitrate addition tubes; □- 5 ppm Cd + 5 mM acetate, simultaneous addition; ▽- 5 ppm Cd + 5 mM acetate, delayed addition, ↑ marks the time of the delayed acetate addition.


5.0
4.0
3.0
2.0
1.0
0.0
METHANE CONCENTRATION (μM/L)
0
48
96
144
192
240
TIME IN HOURS

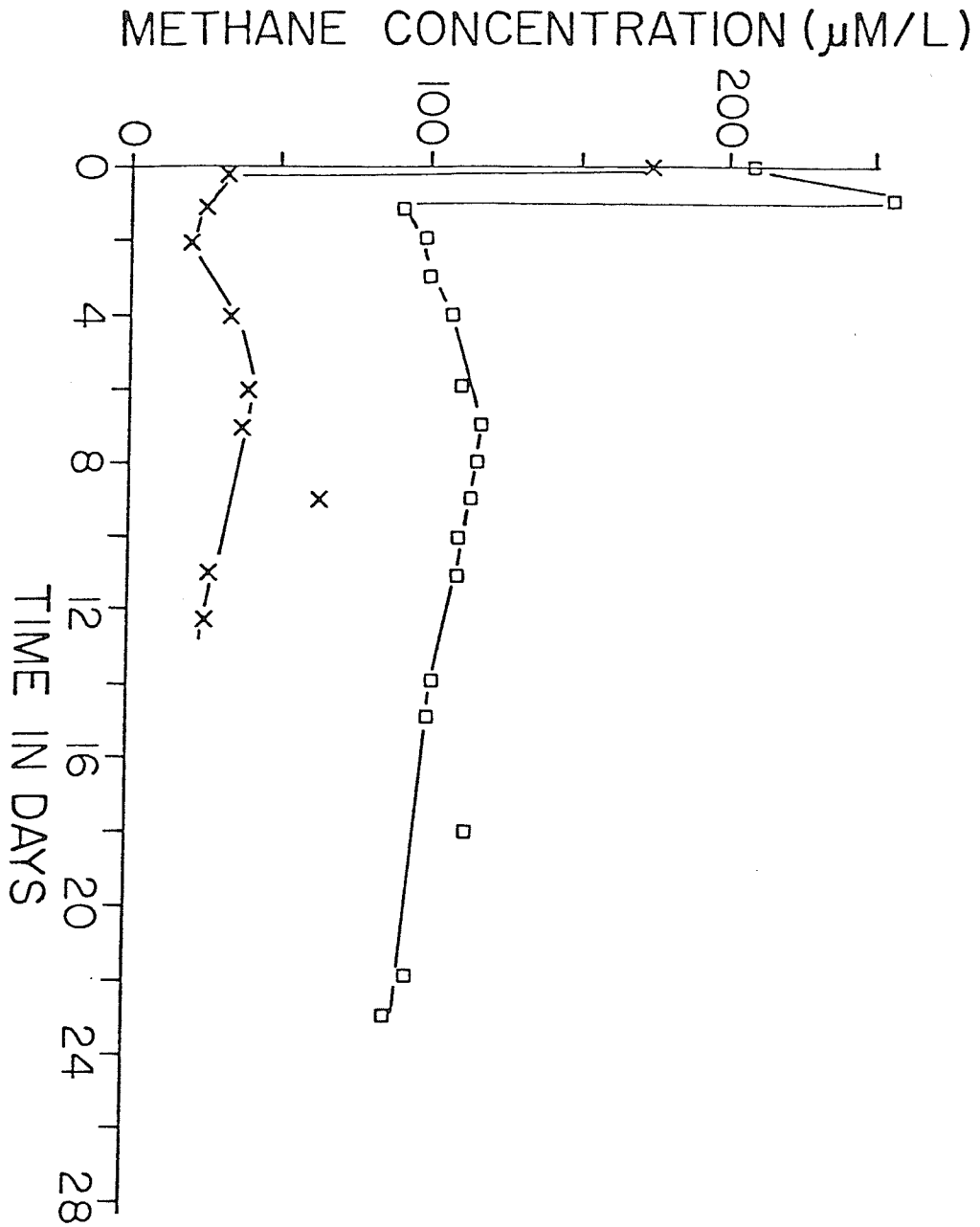
to which acetate (5 mM) and Cd (44.5 uM) were added simultaneously the Cd had no apparent effect upon methane production from the acetate. In tubes where 5 mM of acetate were added approximately 90 hours after the Cd addition (44.5 uM) there was no change in the CH_4 concentrations after the acetate addition and the CH_4 production closely resembled that found in the tubes to which nitrate was added.

Microbasin Acidification

The pH of the free water in the microbasins even after repeated flush and recovery cycles (fig. 6), was normally in the range 6.5 to 6.8. Figure 10 shows results typical of some acidification studies conducted in the basins. In both cases, at pH 3.5 and 5.5, a slight recovery took place until approximately day 7 after the acid addition. Past this point the methane content of the waters fell, as if the CH_4 were being removed and not replaced. It should be noted than in neither case shown in figure 10 does the CH_4 concentration graph resemble the recovery graph shown in figure 6.

Major qualitative changes took place in the acidified microbasins. The basin free water was normally so murky that the 10 cm width of a basin could not be seen through. Within 48 hours of the acid additions the waters cleared to such an extent that objects on the far side of the basins were clearly visible. In the microbasins where the free water pH was adjusted to 3.5, the sediments, normally a dark blue-black in color, became a light brown.

Figure 10. Typical post-flush profiles for microbasins which had undergone acidification. Legend: x – microbasin in which the water had been acidified to pH 3.5; □– microbasin in which the water had been acidified to pH 5.5


METHANE CONCENTRATION (μM/L)
0
100
200
0
4
8
12
16
20
24
28
TIME IN DAYS

Microbasin Cadmium Addition

Cadmium concentrations used in the microbasin experiments were three orders of magnitude lower than those tested in the anaerobic tubes (fig. 11). In basins to which 2.67×10^{-2} uM Cd (final concentration 3 mg Cd/l) the methane concentration did not increase. In the basins to which 8.9×10^{-3} uM Cd (final concentration 1 ug Cd per litre) was added the CH_4 content showed a slight, steady increase, (fig. 11) but not as rapid as in the control basins (see also fig. 6).

No qualitative changes in the waters or sediments of these basins were noted.

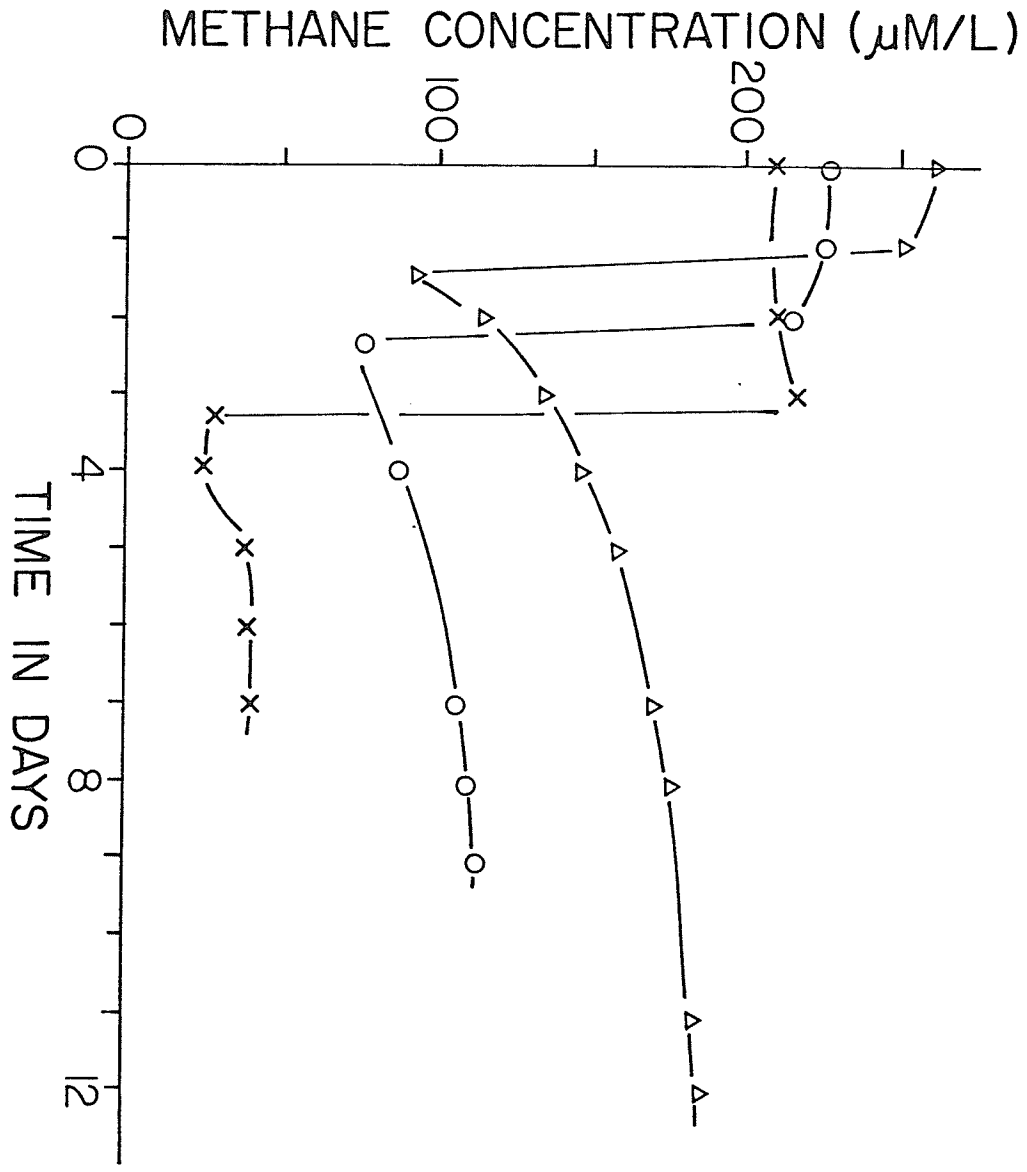
Analyses of dissolved cadmium showed that the metal normally disappeared from the microbasin free water into the sediments within 120 hours of addition.

Field Studies

The methane concentrations in the tubes and adjacent lake site (located approximately 1.5 metres northeast of tube 3, fig. 2) were monitored over the period 1 August, 1979 to 22 October, 1930 (figs. 12 to 15), approximately 64 weeks.

Over the study period the meta- and hypolimnetic pH values in the control tubes and adjacent lake site were normally in the range 6.3 to 6.5 (appendix A), the concentrations of Cd below the practical limit of detection (appendix A), and the concentrations of sulfate well below reported inhibitory values (appendix B). Prior to the additions made to the experimental tubes these conditions held true for them also.

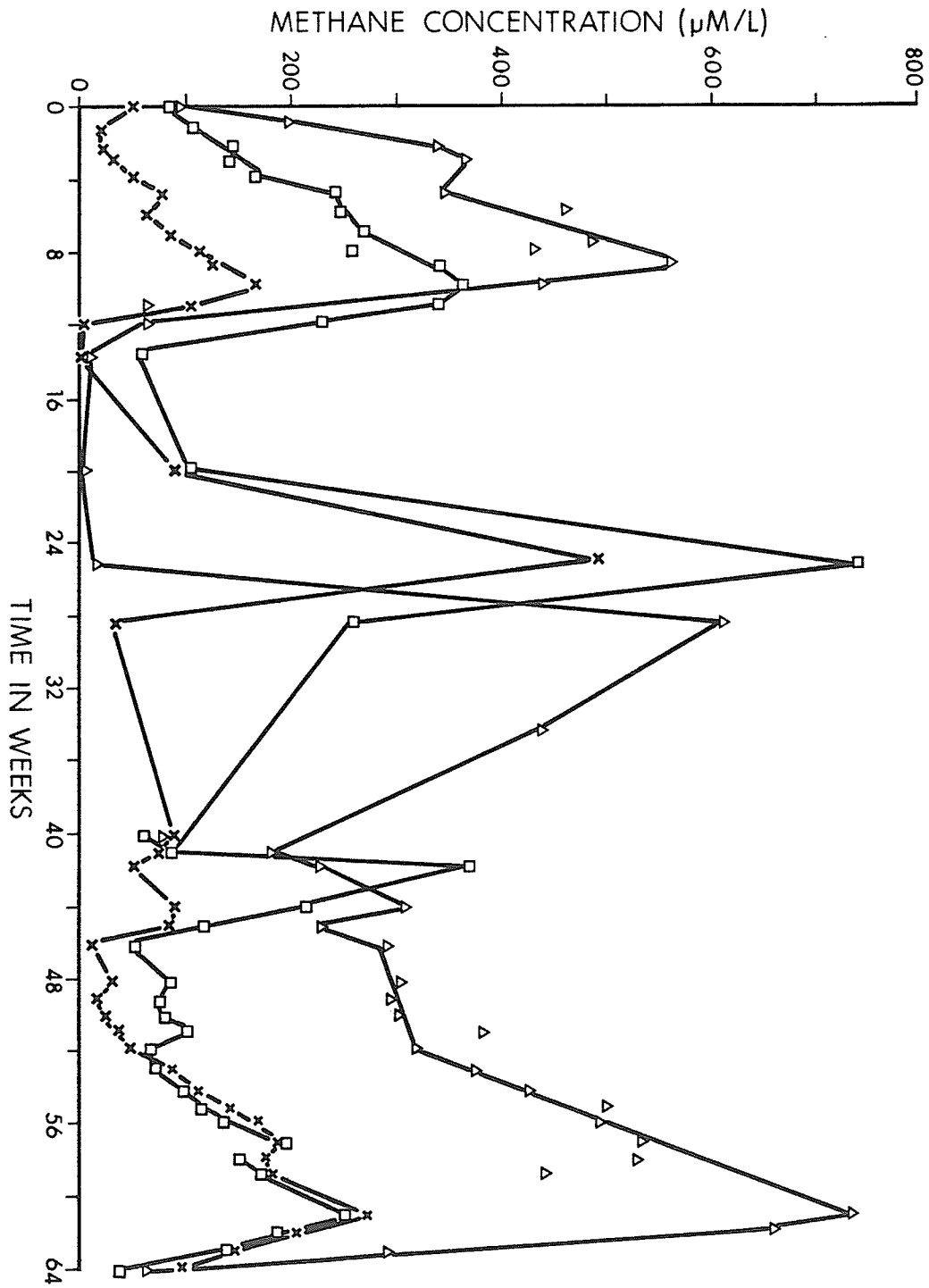
Figure 11. Typical post-flush profiles for microbasins which had been spiked with cadmium. Legend: △ - control basin; ○ - basin spiked with 1 ug Cd/l in the free water; x - basin spiked with 3 ug Cd/l in the free water.


METHANE CONCENTRATION (μM/L)
0
100
200
TIME IN DAYS
0
4
8
12

Nitrate levels in all the tubes and the lake were normally near the detection limit of the method and remained well below values reported to be inhibitory to methanogenesis for the entire study period (appendix B). The galvanized steel of the culverts released significant amounts of zinc into the tube water columns and the lake (appendix A); however, no correlation between the amount of Zn released and other factors, such as tube water column pH, was found.

The methane concentration patterns at 6.5 m in the control tubes and adjacent lake site paralleled one another fairly well, except for early winter 1979 to early spring 1980 approximately weeks 26 to 42 (fig. 12). Although there were large differences in the CH_4 levels between the control tubes and the lake, the general pattern of concentration increase and decrease was the same. Beginning with a steady increase in CH_4 from the start of sampling, there was a sudden, rapid decrease at the onset of fall overturn and the resulting oxgenation of the water columns. The peak methane concentrations in both the control tubes occurred some 7 to 10 days after the peak in the lake. In the early winter there was little or no CH_4 being produced; however, once all of the oxygen near the sediments had been utilized the CH_4 content rose very rapidly. Once all the available organic matter in the sediments had been utilized the methane concentrations again peaked and fell as the water column CH_4 was oxidized. The mid-winter increase and decrease occurred earlier in the control tubes than in the lake. In the spring to very early summer, weeks 42 - 46, there was some variation in the CH_4 levels as the lake underwent stratification. From early to mid summer the CH_4 contents did not change,

Figure 12. Methane concentrations at a depth of 6.5 m in the field control tubes and the adjacent lake site over the period 1 Aug., 1979 (week 0) to 22 Oct., 1930 (week 64). Legend: x - field tube #1; □- field tube #3: △- lake site I data.

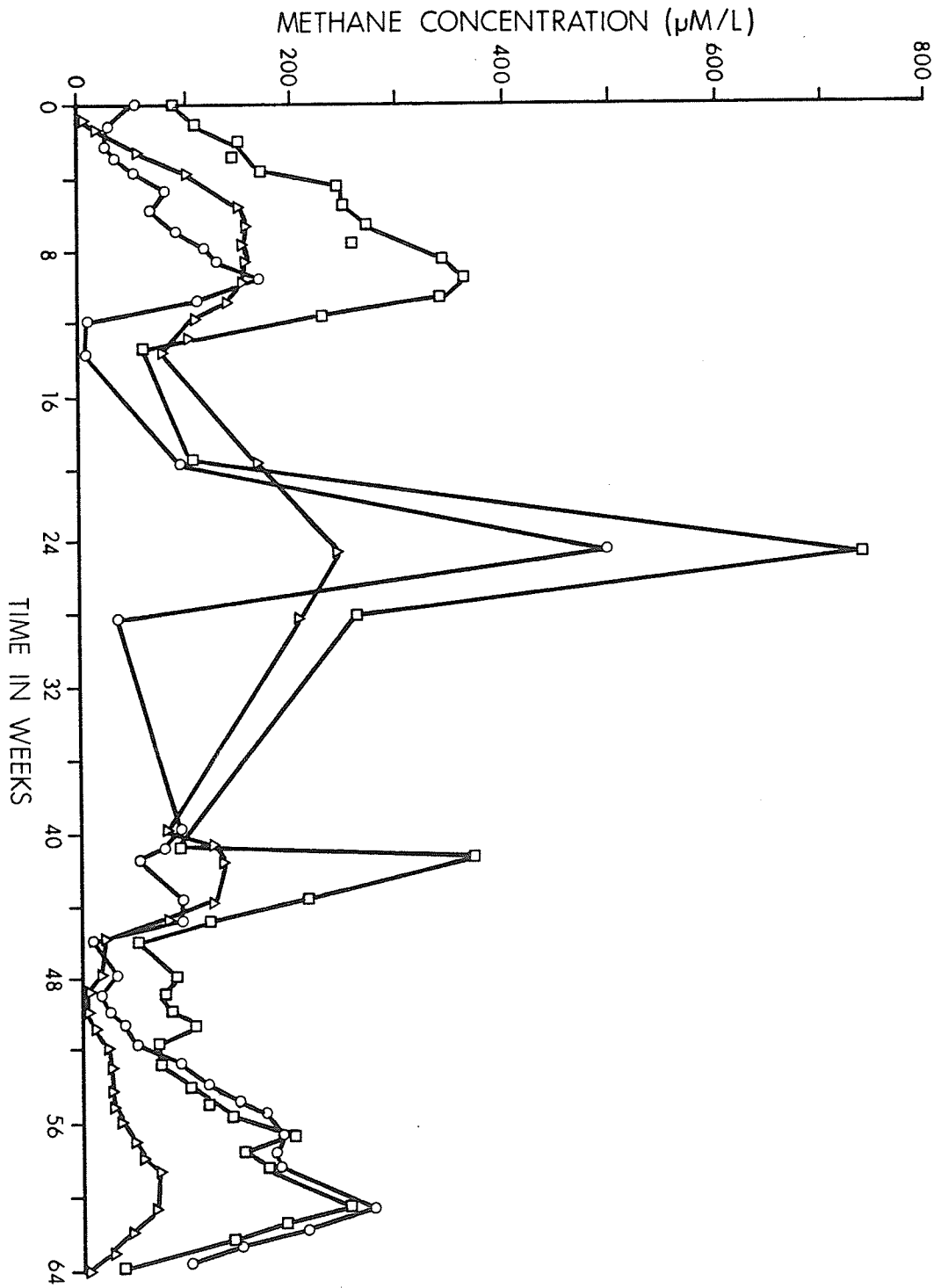

METHANE CONCENTRATION (μM/L)
0
200
400
600
800
TIME IN WEEKS
0
8
16
24
32
40
48
56
64

indicating little activity on the part of the methanogenic populations. In late summer, about week 52, the methane concentrations began to rise. Finally, in the autumn, overturn took place and the CH_4 levels fell. It should be noted that the control tubes showed remarkable agreement in the last 12 weeks of the study.

In the late summer and fall of 1979 the hypolimnetic pH of the tube 2 water column fell to near 3 at a depth of 6.5 m and to near 5.6 at 5 m (appendix A). Over the winter the acid additions were stopped and the water column pH returned to near normal. In the 1980 ice-free season the hypolimnion was kept at approximately pH 4.8 by additions of acid (appendix A). The extremely low pH at 6.5 metres in the 1979 sampling season was caused by an error in the initial acid addition to that depth. Immediately after the acid additions there was a dramatic increase in the sulfate content of the tube water column (appendix B). The hypolimnetic sulfate levels remained near values reported to be inhibitory to methanogenesis (20 mg SO_4/l; Winfrey & Zeikus, 1977) until almost the time of fall overturn. The zinc levels in the tube increased after the initial acid additions in 1979 (appendix A) but, since similar increases were noted in the non-acidified tubes and the adjacent lake site, it appears unlikely that the increased acidity of the tube water column was responsible for the increased Zn content.

The tube 2 methane concentration pattern at 6.5 m depth showed a steady increase in CH_4 content until approximately week 6 when the CH_4 concentration reached a plateau where it stayed until fall overturn,

Figure 13. Methane concentrations at a depth of 6.5 m in the field control tubes and the 'acid only' field tube over the period 1 Aug., 1979 to 22 Oct., 1980. Legend: ○- #1 field tube, control; △- #2 field tube which received additions of acid only; □- #3 field tube, control.


METHANE CONCENTRATION (μM/L)
0
200
400
600
800
TIME IN WEEKS
0
8
16
24
32
40
48
56
64

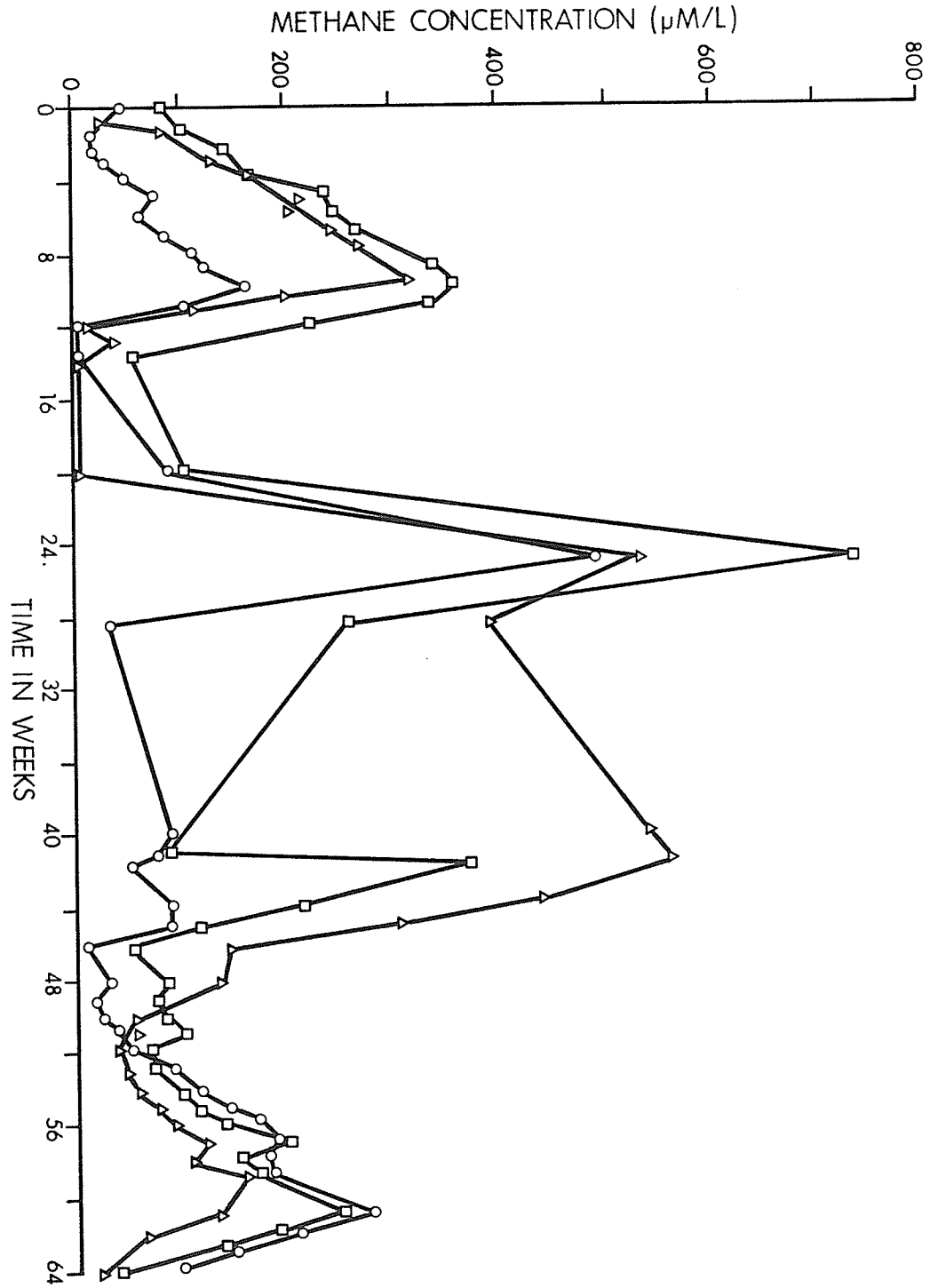
indicating a reduction in methanogenic activity in the tube (fig. 12). During fall overturn the CH_4 content fell more slowly than in any of the other tubes, possibly due to inhibition of the methane oxidizers. In the winter there was a slow, steady increase and decrease in the CH_4 content of the tube with a peak much lower than in the controls. In spring and early summer the CH_4 concentration showed some variability and then reached a low steady state level. After week 52 the CH_4 content rose slowly and peaked earlier and at a lower concentration than in the controls. The fall decrease in CH_4 was again slower than in the control tubes. The tube 2 methane concentration pattern bore the least resemblance to the control tube patterns and showed decreased methanogenic activity.

The cadmium levels in the hypolimnion of the tube 4 water column were initially very variable as a timetable for the Cd additions was being worked out (appendix A). Once the timetable was set the Cd concentration at 6.5 m depth remained above 5 ug/l for the remainder of the 1979 field season to near midwinter. Cadmium additions to the tube halted in late fall 1979 and the Cd levels returned to near normal over the winter except at 6.5 m where the Cd concentration remained above 2.5 ug/l (appendix A). In the 1980 ice-free season Cd was again added to the tube and the hypolimnetic concentration of the metal rose steadily, if somewhat erratically, peaking at slightly over 14 ug Cd/l near the end of sampling (appendix A). The zinc levels in the tube also rose dramatically over the 1980 season, exceeding those found in any of the other tubes (appendix A).

The methane concentration pattern of tube 4 paralleled the control tube patterns closely from the start of sampling, through fall and into winter (fig. 14). At approximately week 40 the CH_4 concentration reached an all time high in tube 4 and then fell rapidly through the spring and early summer (weeks 40 - 50). At week 52 the tube 4 methane level again began to parallel the control tube levels and, until the peak concentration was reached, bore a close resemblance to the tube 5 concentration pattern. Tube 4 peaked some two weeks before the controls and at a much lower concentration of CH_4 . With the exception of the anomalies mentioned above, the tube 4 methane concentration pattern paralleled the control patterns closely with few indications of effects caused by the cadmium additions.

The hypolimnetic pH in tube 5, which received both acid and Cd additions, fell slightly in 1979, remaining in the range 5.8 - 6.0 and returning to normal over the winter months. With the resumption of the acid additions in 1980 the hypolimnetic pH was reduced to between 5.5 to 5.0. The hypolimnetic cadmium levels in this tube showed the same initial variability as those in tube 4 and remained high throughout fall and much of winter (appendix A). By spring 1980 the concentration of Cd at all depth had fallen to near 1 ug/l. With the resumption of Cd additions the levels rose and peaked at about 14 ug Cd/l near the end of sampling. As in tube 2 the sulfate levels rose after the acid additions to reported inhibitory values, but fell below those values more rapidly than in tube 2 (appendix B). Zinc levels in tube 5 increased over the course of sampling, but they remained low in comparison with most of the other tubes and the adjacent lake site

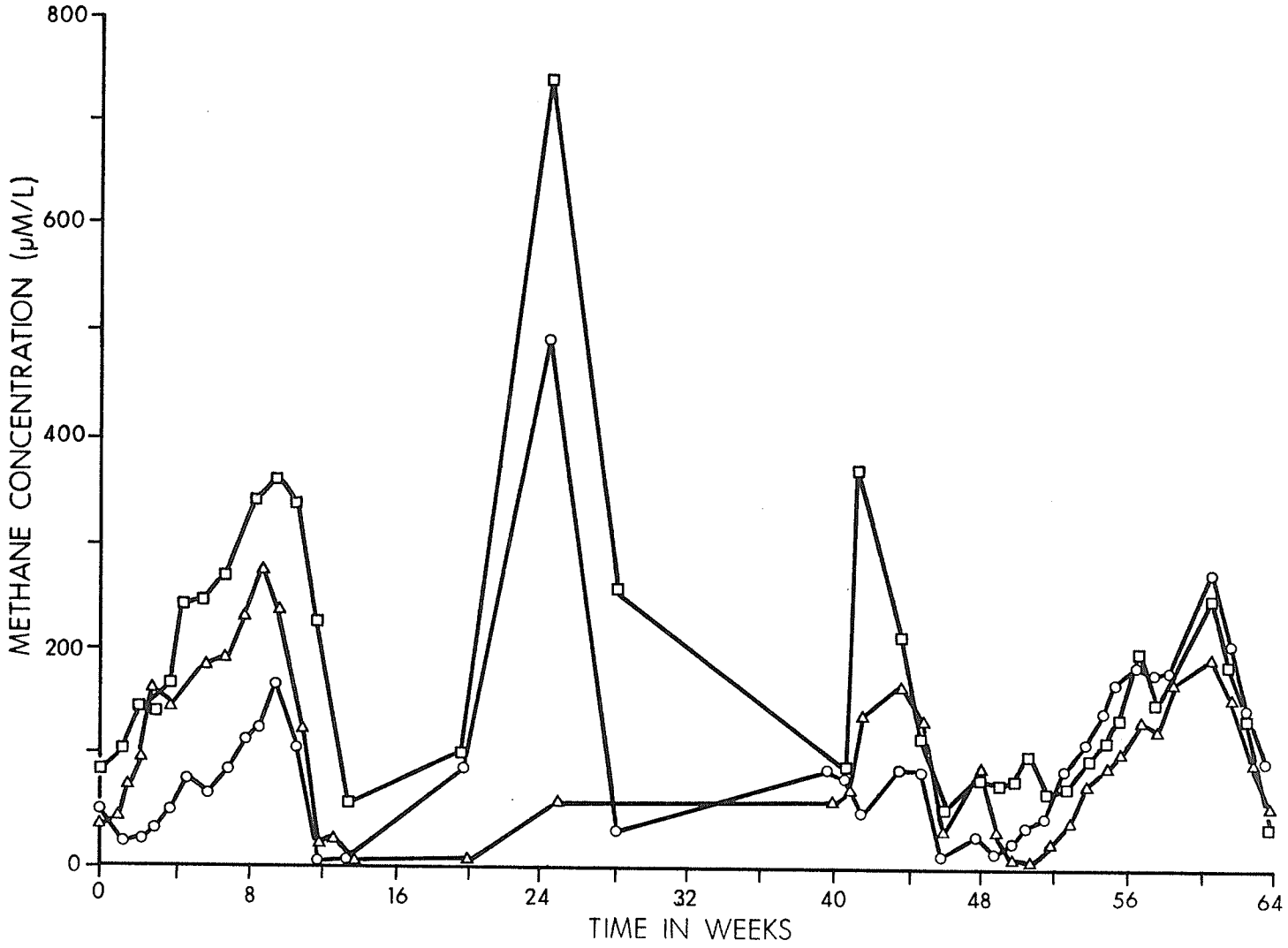
Figure 14. Methane concentrations at a depth of 6.5 m in the field control tubes and the 'cadmium only' addition field tube. Legend: ○ - #1 field tube, control; □ - #3 field tube, control; △ - #4 field tube which received additions of Cd only.


METHANE CONCENTRATION (μM/L)
0
200
400
600
800
TIME IN WEEKS
0
8
16
24
32
40
48
56
64

(appendix A).

The increase in methane content at 6.5 m in tube 5 during the summer of 1979 paralleled the increases in the control tubes fairly closely, but the decrease in autumn began at least one week earlier than in the controls (fig. 15). Recovery of methane content in the winter was slower than in the controls, following the lake values closely until about week 25. After this point it was impossible to sample from tube 5 as shifting ice had closed it off. Sampling was resumed from this tube once the ice was off the lake. In spring and early summer the CH_4 content showed variability and then a low steady state concentration. In summer the methane concentration pattern paralleled the control patterns closely although the CH_4 levels were consistently lower in tube 5 than in the controls. In fall the CH_4 concentration peaked at the same time as in the controls, though at a lower level. The tube 5 methane concentration pattern paralleled those of the control tubes fairly closely with only the exceptions mentioned above giving any indication of the possible effects of the acid and cadmium additions.

Figure 15. Methane concentrations at a depth of 6.5 m in the field control tubes and the 'cadmium + low pH' field tube over the period 1 Aug., 1979 to 22 Oct., 1980. Legend: ○- #1 field tube, control; □- #3 field tube, control; △- #5 field tube which received additions of both acid and Cd.


800
600
400
200
0
METHANE CONCENTRATION (μM/L)
0
8
16
24
32
40
48
56
64
TIME IN WEEKS

DISCUSSION

DISCUSSION

Test Tube Studies

The results of the anaerobic test tube experiments are fairly straightforward and show that cadmium does inhibit methanogenesis under these laboratory conditions. When methane production in the control tubes is compared to that in the tubes to which nitrate or cadmium was added (figs. 7 & 8) it is evident that, at all Cd concentrations tested, inhibition of methanogenesis equivalent to that caused by nitrate occurred. Conversely, in those tubes to which acetate or glucose, precursors of methanogenesis, were added, the CH_4 production was enhanced to well above the control values. The limitations of the technique did not allow for lower concentrations of Cd to be tested and thus no apparent threshold level for cadmium sensitivity was found in these initial experiments.

The inhibitory effect of cadmium can be prevented by the addition of the methane precursor acetate (fig. 9). The production of CH_4 in those tubes to which acetate and Cd were added simultaneously was just as great as in those tubes to which acetate alone was added, with no signs of inhibition. However, in those tubes to which acetate was added some 90 hours after the Cd addition the production of CH_4 was inhibited and no signs of increased methanogenic activity were noted following the acetate addition. These data raise the interesting possibility that it is organisms functioning earlier in the anaerobic decomposition pathway which are inhibited by Cd, rather than the methanogens themselves. If the methanogens had been affected then

methane production in the tubes receiving acetate and Cd simultaneously would have remained low, showing an inhibition pattern similar to those in figures 7 and 8. As there was a large amount of methane produced, greater than that in the control tubes, the acetate must have been metabolized anaerobically by an active methanogenic population. The absence of methanogenic activity in the tubes to which acetate was added some time after the Cd may be due to the inactivation of the methanogens by a simple starvation stress, or by a combination of Cd toxicity and starvation stress. Further work, using methanogenic isolates, would be necessary to determine the validity of this hypothesis.

Microbasin Studies

The results of the microbasin experiments are also straightforward. Inhibition of methane production occurred in all of the test basins to which acid and/or Cd had been added (figs 10 and 11). The inhibition was apparently immediate and total at both pH 3.5 and 5.5 and when the Cd concentration in the basin free water was 3 ug Cd/l (2.67×10^{-2} micromoles Cd added). Inhibition was total because, while there was CH_4 present in the basin waters following the nitrogen flush and test additions, the CH_4 concentration did not increase, indeed it fell with time, showing that no new methane was being produced and that the methanogens were inhibited. When the Cd concentration in the water was only 1 ug Cd/l (8.9×10^{-3} uM Cd added) inhibition was immediate but apparently incomplete. Some recovery of methane production took place after the addition of cadmium which indicated that a threshold level for Cd inhibition of the methanogenic population in the micro-

basins had been reached. The fact that CH_4 production was not as rapid in this basin as in the controls shows that some of the bacteria in the pathway had been affected. Whether these bacteria were in the first, second or third stage of the methanogenic pathway is unknown.

Field Studies

Due to a number of factors in the field which were either not present or were controlled in the lab environment the results of the field studies, with some exceptions, are not as straightforward as those of the lab experiments. For example, the tube material used in the field experiments may have interfered to some extent with the circulation of water within the tubes. Evidence for such interference is the presence of the late and early methane peaks noted in the control tubes as compared to the lake (fig. 12). If there had been no interference in the circulation of the tube waters then these peaks should have occurred simultaneously in both the tubes and the lake.

Upon consideration of the tube methane concentration graphs in figures 12 to 15, with the exception of the number 2 tube (fig. 13), there was little evidence of methane production inhibition. In the case of the tube 2 graph, the plateaus and slow increases in methane content indicated that partial inhibition of methanogenesis occurred. When compared to the control tubes, with the exceptions of some early and low peaks mentioned earlier, tubes 4 and 5 (figs 14 & 15) showed no signs of any inhibition caused by the experimental additions. These results are rather surprising when one considers the fact that the pH values reached in the field were much lower than those shown

to cause complete inhibition of methanogenesis in the microbasins. Likewise, the cadmium levels recorded were well above values shown to cause complete inhibition within the microbasins. There are several possible reasons for the apparent discrepancies between these two sets of data.

One possibility is increased sensitivity on the part of the bacteria in the sediments used in the lab experiments. These sediments had undergone extensive manipulation prior to the test additions, including removal from the lake, exposure to oxygen during removal and subsequent loading into the lab systems, physical disturbance by transportation and shaking during the lab set up. It is unlikely that the physical structure of the sediments was the same as it was in the lake so that the bacteria may have been more sensitive to the test solutions.

Zinc is known to interfere with cadmium toxicity (Webb, 1972; Fulkerson et al., 1973; Mitra et al., 1975; Macara, 1978) and large amounts of Zn were being released from the culverts into the tube water columns (appendix A). It is probable that the Zn in the water columns of tubes 4 and 5 exerted an antagonistic effect upon the Cd added.

Babich and Stotzky (1977b) found that decreasing the pH reduced the toxicity of Cd to soil bacteria and fungi. In the case of tube 5 it is possible that the reduced pH had a similar protective influence.

The buffering capacity of the sediments <u>in situ</u> may have been greater than that of the sediments in the laboratory. As mentioned

earlier, the sediments used in the laboratory underwent extensive manipulation prior to the test additions. Set up and the initial flush and recovery sequence in the microbasins took at least two weeks, during which time the microbial population would be actively metabolizing the nutrients in the sediments and releasing byproducts. As well, in the field there would have been a constant influx of fresh buffers into the field tubes from the surrounding lake and watershed. Once the buffers in a microbasin were used up however, they were not replaced. The combination of these factors could easily have combined to reduce the buffering capacity of the microbasin sediments so that they would be more easily acidified than those in the field. Thus, although the water column pH in the field tubes was below values shown to be inhibitory in the lab, the higher buffering capacity of the field sediments kept the reduced pH from affecting the methanogens.

Whatever the reasons for the discrepancies it is evident that extrapolation of the results obtained with the lab systems to the natural environment cannot be done. Despite this caution, the combination of lab and field systems appears to be a useful one for exploring the reactions of organisms, such as methanogens, which are difficult to culture by more conventional techniques. In particular, the microbasins proved to be very useful systems for screening potentially toxic substances.

In conclusion, methanogens are adversely affected by cadmium and low pH in laboratory situations, but it appears that in the field a very profound acid and/or Cd stress is required to inhibit

their activity. Further study on this subject would be required to determine at just what levels of pH and/or Cd the inhibition of methanogenesis in the field begins. Other research projects which are suggested by data in this paper are studies to determine the effects of low pH and/or metals on the other anaerobic terminal decomposers, the sulfate reducers and the denitrifying bacteria. It would be of particular interest to learn what effect, if any, the input of sulfuric and nitric acids in acid precipitation has on the metabolism and ecology of these organisms. Another suggested study would be conducted with pure and mixed cultures of methanogenic and pre-methanogenic decomposer bacteria to determine just where in the methane fermentation Cd has its effect. Much work remains to be conducted on acid precipitation and its related phenomena. This project was designed to answer some very preliminary questions about the interaction of acid precipitation and heavy metals with methanogenic bacteria. Hopefully it has also served to raise some new ones.

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LITERATURE CITED

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APPENDICES

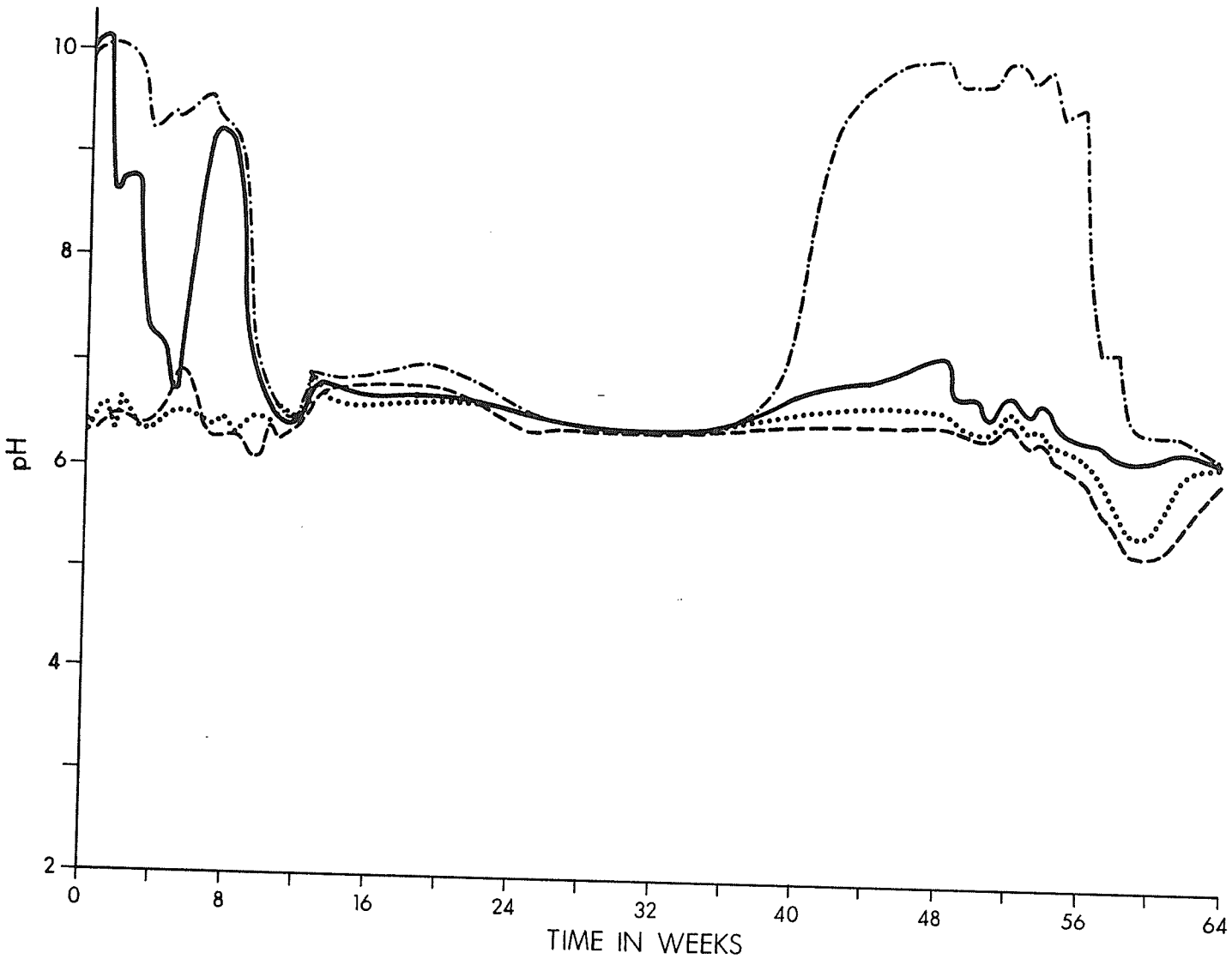
APPENDIX A

pH and Heavy Metal Data

The pH data for the non-acidified tubes and adjacent lake site over the period 1 August, 1979 to 22 October, 1980, were very similar to one another (figs. 16, 18, 19 & 21). With the exception of the tube 1 data (fig. 16) at approximately week 56, the hypolimnetic pH in the tubes and the lake stayed consistantly between 6.2 - 6.8 through the year. The 3 metre data showed great variability in all four data sets during the early part of the study until about week 10 when the pH fell to approximately 6.5 in response to fall overturn. The pH remained at this value throughout the winter and in the following ice free season (weeks 40-64) varying only slightly in comparison with the previous year, rarely going above 7. The only exception was in the lake data, as conditions progressed towards fall overturn the pH at 3 metres rose sharply. The surface pH levels all showed the drop caused by fall overturn, the relatively steady state at between pH 6.5 to 7 in the winter, and the rapid increase and high levels in spring and summer. The only major difference between the data sets occurred when the drop in surface pH during fall 1980 overturn occurred some 4 to 6 weeks earlier in the control tubes than in the lake and tube 4.

The acidified tube pH data (figs. 17 & 20) did not resemble the data discussed above and clearly showed the effects of the acid additions. In tube 2 (fig. 17) the pH at 6.5 m fell dramatically to below 3 after the first acid addition and then rose gradually over fall and winter to approximately 6.5 by early May 1980 (week 40).

Figure 16. The pH in field tube #1 (control) over the period 1 Aug., 1979 to 22 Oct., 1980, at depths of 6.5 m (- - -), 5 m (••••), 3 m (——), and 0 m (-•-•-).


10
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6
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pH
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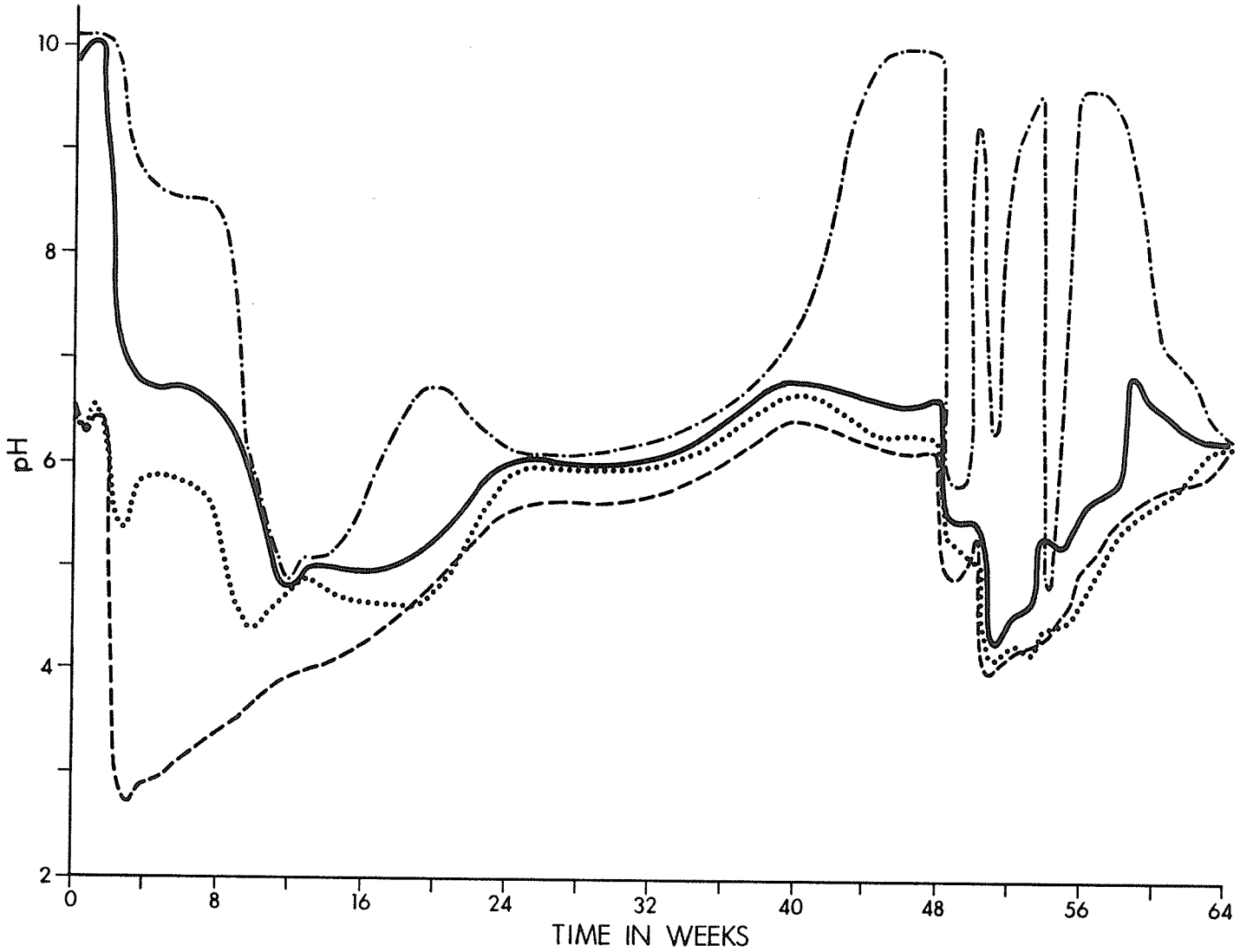
32
40
48
56
64
TIME IN WEEKS

Figure 17. The pH in field tube #2 (acidified only) over the period 1 Aug., 1979, to 22 Oct., 1930, at depths of 6.5 m (- - -), 5 m (••••), 3 m (——), and 0 m (-•-•-).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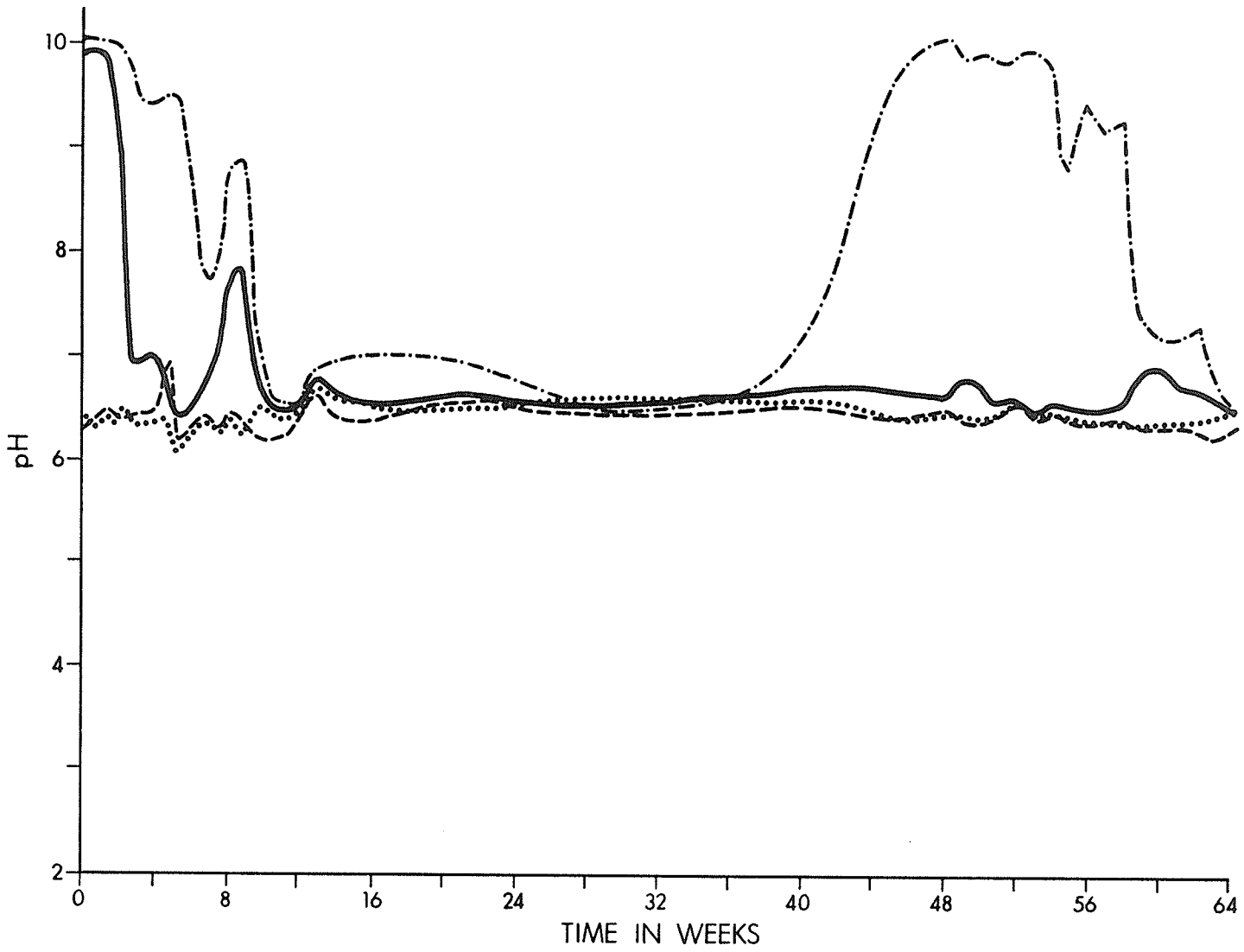
TIME IN WEEKS

Figure 18. The pH in field tube #3 (control) over the period 1 Aug., 1979, to 22 Oct., 1980, at depths of 6.5 m (- - -), 5 m (••••), 3 m (——), and 0 m (-•-•-).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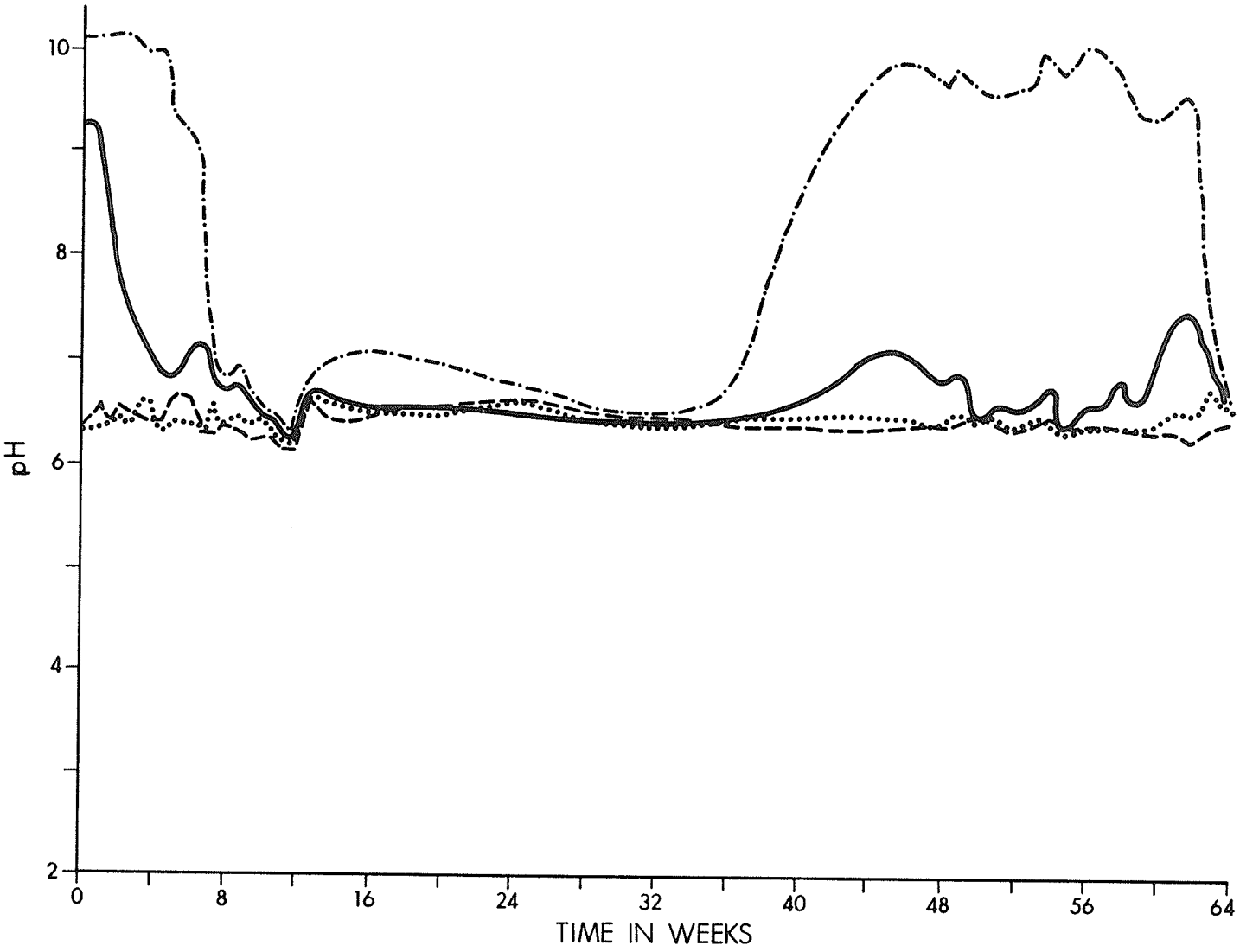
TIME IN WEEKS

Figure 19. The pH in field tube #4 (cadmium addition only) over the period 1 Aug., 1979 to 22 Oct., 1980, at depths of 6.5 m (- - -), 5 m (••••), 3 m (———), and 0 m (-•-•-).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pH
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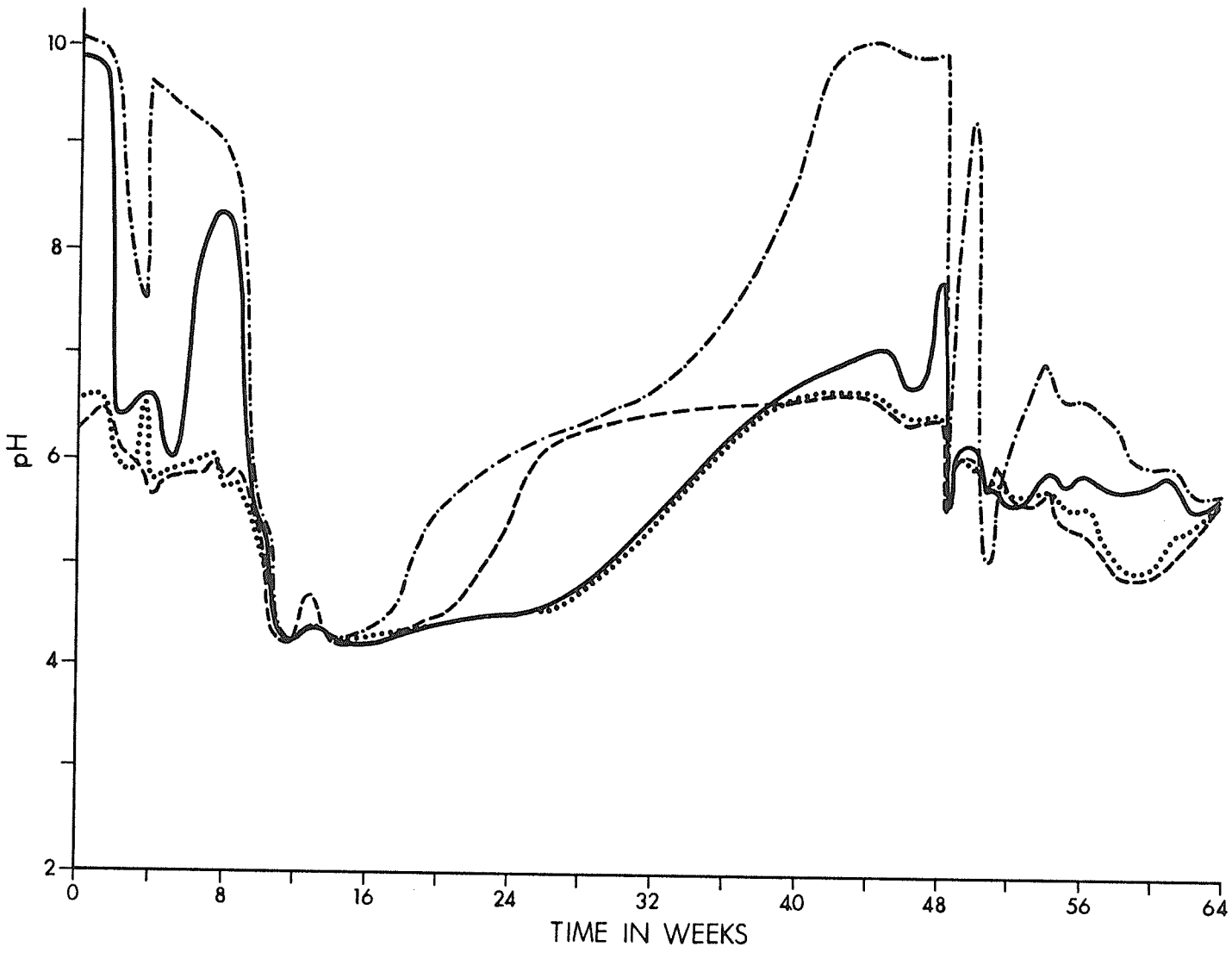
64
TIME IN WEEKS

Figure 20. The pH in field tube #5 (acid and Cd additions) over the period 1 Aug., 1979 to 22 Oct., 1980, at depths of 6.5 m (- - -), 5 m (••••), 3 m (——), and 0 m (-•-•-).


10
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pH
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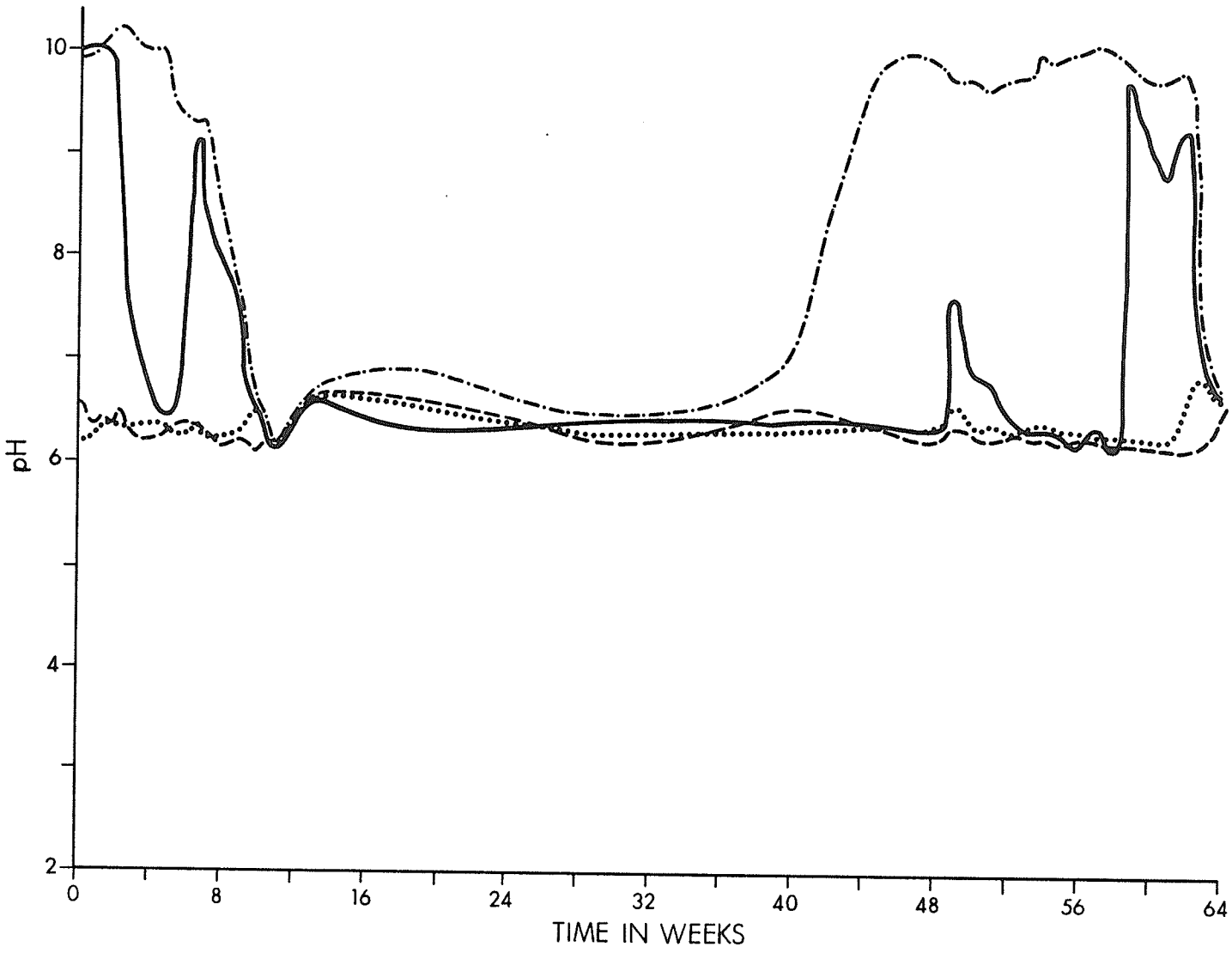
64
TIME IN WEEKS

Figure 21. The pH at the adjacent lake site (lake site I) over the period 1 Aug., 1979 to 22 Oct., 1980, at depths of 6.5 m (- - -), 5 m (••••), 3 m (——), and 0 m (-•-•-).


10
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pH
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TIME IN WEEKS

The effects of the acid additions in the 1980 ice-free season, starting at about week 48, were not quite as dramatic as in the previous season but were still sufficient to keep the pH at 6.5 m below 5.5 until the onset of fall overturn at week 60. At a depth of 5 metres the pH did not drop as much as at 6.5 m during the 1979 season and showed some signs of recovery at about week 4. During fall overturn the pH again fell as the waters mixed, reaching a low of approximately 4.5. In the winter and following summer the pH at 5 m paralleled that at 6.5 m closely and remained below approximately 5.5 until the onset of fall overturn. At a depth of 3 metres the pH fell over 3 units after the initital acid addition and then leveled off until the onset of fall overturn when it again fell to below pH 5 by week 12. Over the winter the pH increased until by spring it was about 6.5. After the acid additions in summer 1980 the pH fell again and paralleled the pH levels at 6.5 and 5 m until the start of overturn at week 60 when it increased sharply to over 7 and then fell slowly to approximately 6.2. As at 3 and 5 metres, the surface pH also fell rapidly after the initial acid addition, leveled off briefly, fell during overturn and recovered in the winter. In early spring the pH rose as rapidly as in the non-acidified tubes and the lake, but then fell rapidly with the acid additions. Recovery was always rapid at the surface with pre-acidification pH values being reached within 2 to 3 weeks of the acid addition. The reduction in surface pH caused by fall overturn occurred at the same time as in the control tubes. In the spring and early summer of 1980, weeks 40 - 48, the pH levels at all depths tested in tube 2 were close to those in the non-acidified tubes and the lake,

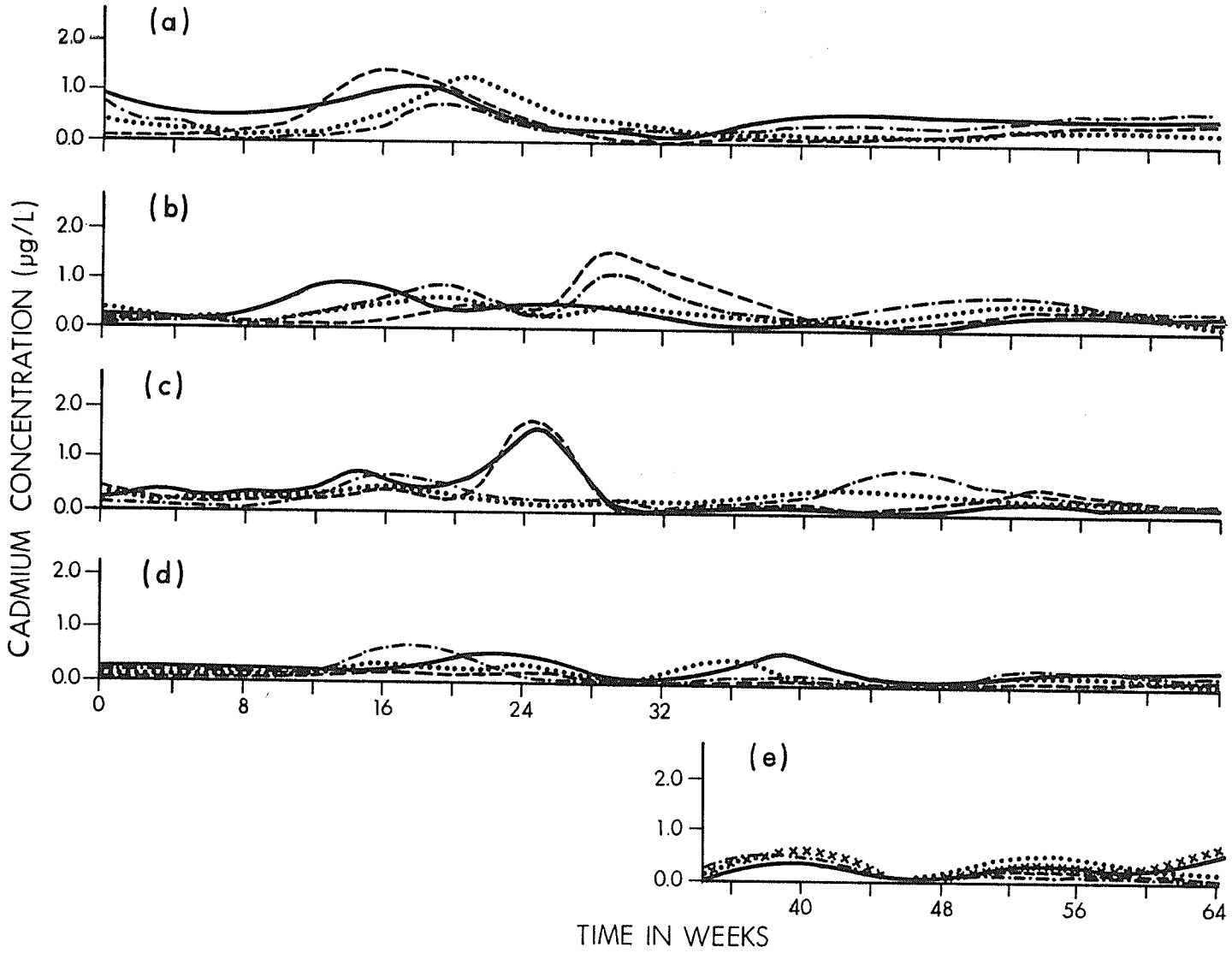
which showed that recovery in the winter had been complete. The large variation in pH between the depths in the early part of the experiment showed the difficulty in acidifying a tube water column to a uniform pH. The large variation in surface pH during the summer 1980 season illustrates the effect that a large, well established algal population can have on the buffering capacity of epilimnetic waters.

From the data for tube 5 (fig. 20), it is evident that the acid addition program affected this tube differently than tube 2. The drop in pH after the initial acid addition was not as dramatic as in tube 2. The pH at 5 and 6.5 m was reduced to just under 6 and stayed at this level until overturn when mixing of the waters drove the pH down to approximately 4.2. Over the winter the pH returned to pre-acidification levels, with the pH at 6.5 m recovering more rapidly than at 5 m, possibly due to the close proximity of the lower waters to buffers in the sediments. During the summer 1980 acidification program the pH again fell more slowly at 5 and 6.5 m in tube 5 and stayed more nearly at a constant level than in tube number 2, remaining between roughly pH 5.5 and 6 after the first 1980 acid addition and not showing recovery until the onset of fall overturn. The pH at 3 metres in tube 5 fell drastically with the initial acid addition and reached a level almost as low as at 5 and 6.5 m. At the onset of fall 1979 overturn there was a rapid recovery followed by an equally rapid drop as the tube water mixed fully, reaching a low of approximately pH 4.2. Over the winter there was a slow recovery which followed the 5 m values closely until, by summer 1980, immediately prior to the first acid addition of the new season, the pH was rising rapidly to the 1979 pre-

acidification level. After the acid addition, the pH at 3 m fell to about 6 and stayed near this value for the rest of the experiment. The surface pH in tube 5 fell rapidly after the initial acid addition and then rose equally rapidly to near the pre-acidification value. With the onset of fall overturn the pH fell until the entire water column was at (approximately) pH 4.2 at about week 11. By early spring (about week 40) the surface pH had recovered fully to pre-acidification levels. With the start of the summer 1980 acidification program the surface pH in tube 5 initially showed similar variability to that seen in tube 2. However, after the second acid addition (about week 48) the recovery in pH was neither as rapid or complete as it was after the first 1980 addition (about week 46) or in tube 2. After the second acid addition the surface pH did not rise above 7 again and after week 52 fell slowly to near 6 by the end of sampling. As was suggested earlier, the great recovery power of the surface pH in tube 2 may have been due to the buffering capacity of a well established algal community. If this were the case, then, as tube 5 was receiving both acid and Cd additions, it is possible that the additional stress imposed by the cadmium disrupted the algal community. Evidence that the algal population was severely effected in tube 5 is the fact that water clarity in this tube was much greater after the second acid addition than in any of the other tubes or the lake. Typically the water sampling line head was not visible past approximately 50 cm depth in the other tubes or the lake. In tube 5 after the second acid addition the head was visible to a depth of approximately 2 metres

Figures 22a to 22e show the results of the cadmium analyses for

Figure 22. Cadmium levels at selected depths in the tubes which did not receive Cd supplements and at lake site I (adjacent) over the period 1 Aug., 1979 to 22 Oct , 1980, and at lake site II (10 m site) over the period 28 March, 1980 to 22 Oct., 1980. Legend: figure 22a, tube #1 (control); figure 22b, tube #2 (acid only addition; figure 22c, tube #3 (control), figure 22d, lake site I; figure 22e, lake site II; sample depths, 0 m (-•--•-). 3 m (——), 5 m (••••), 6.5 m (- - -), and 10 m (XXX, lake site II only).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CADMIUM CONCENTRATION (µg/L)
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64
TIME IN WEEKS

the three tubes not spiked with Cd and the adjacent lake site (figs. 22a -d) over the period 1 August, 1979 to 22 Oct., 1980, and data obtained at the 10 m lake sampling site for the period 28 March, 1980 to 22 Oct., 1980 (fig. 22e). In no case did the concentration of Cd rise above 2 ug/l and for the most part the concentrations remained well below 1 ug/l. When the heavy metal samples were first returned for analysis this value (1 ug/l) was cited as the practical limit of detection and that readings below this should be accepted with caution (A. Lutz, pers. comm.). Since this was the case it is difficult to make more than broad generalizations about the data. There appeared to be a pattern of low levels in the summer of 1979, followed by a slow increase starting at about weeks 10 - 12, possibly a consequence of fall overturn. Over winter the Cd levels decreased slowly in the lake and tube 1, but continued to increase at certain depths in tubes 2 and 3, before finally dropping to near zero by spring. With ice-out in spring (about week 38) there was again an increase and slow decrease in the Cd levels until the summer when the concentrations remained fairly stable. The tube patterns followed the lake patterns fairly closely with the exceptions of the increases in tubes 2 and 3 mentioned earlier. Due to the extremely low Cd concentrations recorded, other than noting that for the most part Cd levels stayed below the practical limit of detectability and that the culverts did not seem to contribute significant amounts of Cd to the tubes, few generalizations should be made.

The effects of the cadmium additions on the Cd levels in tubes 4 and 5 (figs. 23 & 24 respectively) were readily visible. In general,

Figure 23. Cadmium levels at selected depths in field tube #4 (Cd supplements only) over the period 1 Aug., 1979 to 22 Oct., 1980. Legend: 0 m (-•-•-), 3 m (———), 5 m (••••), 6.5 m (- - -).

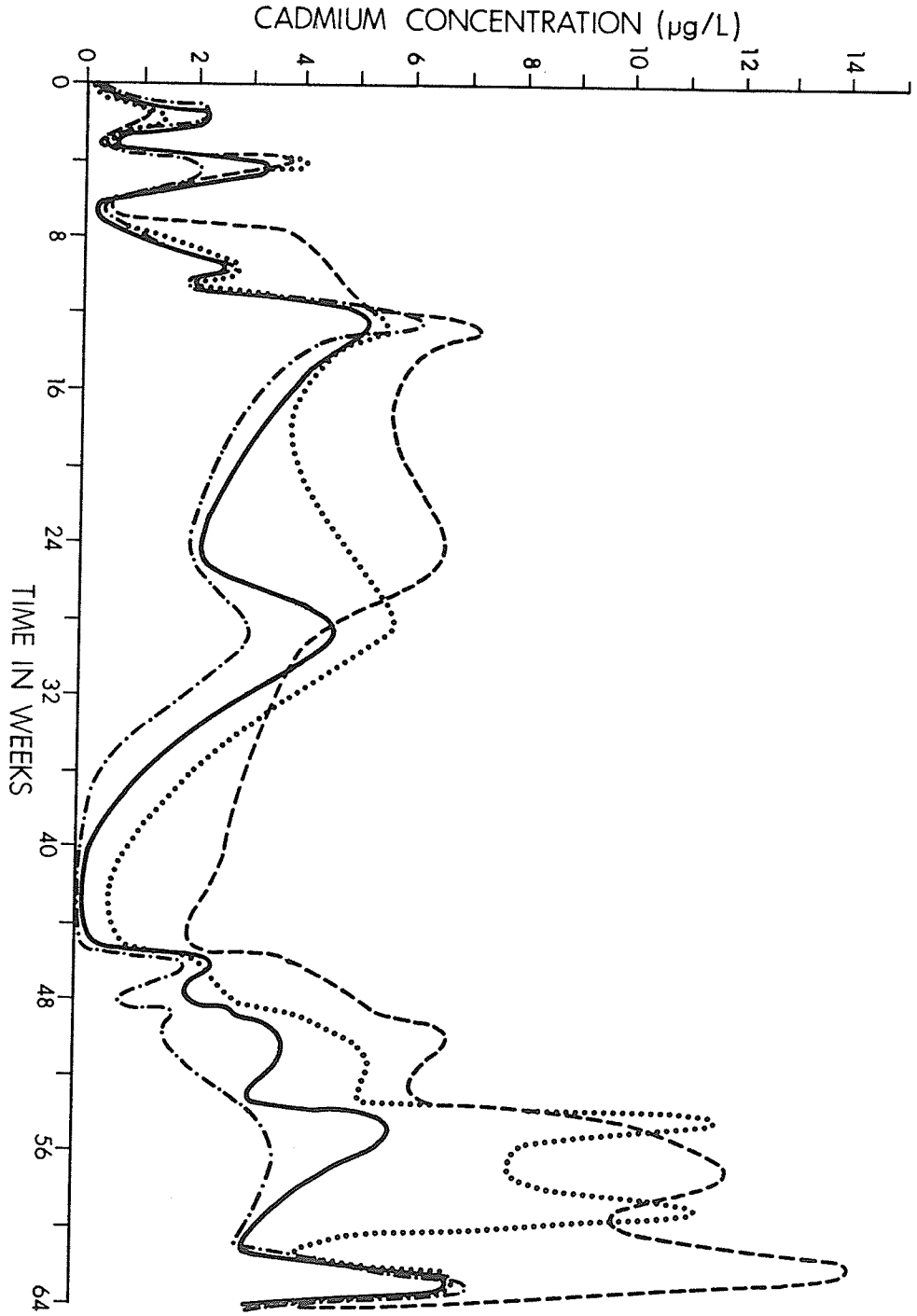
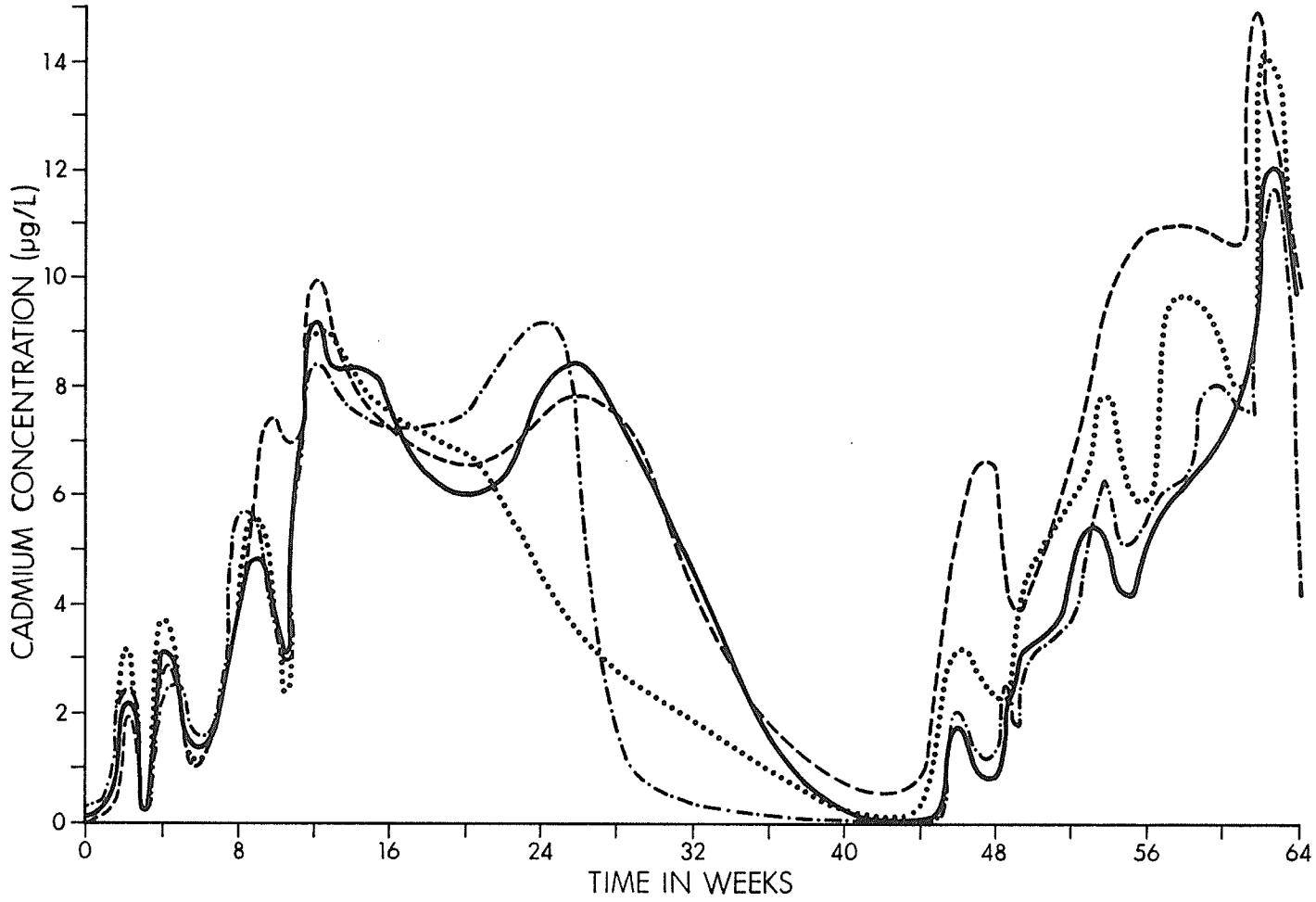

CADMIUM CONCENTRATION (µg/L)
0
2
4
6
8
10
12
14
TIME IN WEEKS
0
8
16
24
32
40
48
56
64

Figure 24. Cadmium levels at selected depths in field tube # 5 (Cd and acid supplements) over the period 1 Aug., 1979 to 22 Oct., 1930. Legend: 0 m (-•-•-), 3 m (——), 5 m (••••), 6.5 m (- - -).


CADMIUM CONCENTRATION (µg/L)
0
2
4
6
8
10
12
14
0
8
16
24
32
40
48
56
64
TIME IN WEEKS

in both sets of data, there was large variation in the Cd content in early part of the experiment, until the rate at which the added Cd would disappear from the water column was determined. After week 6 (approximately 15 Sept., 1979) a weekly addition schedule had been settled upon and was carried out through the remainder of the season. After week 12 all additions were halted for the winter. The combination of this program and fall overturn raised the Cd concentrations in the water columns of tubes 4 and 5 to the peaks recorded at week 12. With the exception of tube 4 at a depth of 6.5 m, where the concentration was approximately 2 ug/l, all of the Cd concentrations recorded prior to the resumption of weekly additons on 18 June, 1980 (about week 45), were below 1 ug/l, indicating that almost all the Cd had left the water column. With the resumption of the cadmium additions the Cd levels in the tubes rose through the summer months of 1980 and peaked about 2 weeks prior to the end of the sampling season when the additions were halted on 3 October, 1980. The great variability of the Cd concentration patterns in the tubes illustrates the difficulty in the maintenance of a given low concentration of Cd in any experimental system which come near to the natural environment because the metal is constantly being taken up and sequestored by the sediments of such a system. That the Cd values were almost always highest near the sediments is not surprising since any detrital particle to which the metal was bound would eventually reach the sediments, thus raising the local cadmium concentration.

Table 2 shows selected zinc data for the tubes and lake sites over the period 1 August, 1979 to 23 Oct., 1980. The zinc analysis samples were very variable and prone to contamination (A. Lutz, pers. comm).

Table 2. Selected Zinc data for the field experiments.

Site	Date	sample depth			
		surface	3 metres	5 metres	6.5 metres
tube 1	Aug. 1/79	0.8	0.3	0.2	∠0.1
	Jan. 23/80	3.0	13.0	11.0	12.0
	Feb. 15/80	0.6	0.1	0.2	0.3
	May 8/80	10.0	20.0	60.0	20.0
	June 11/80	9.0	20.0	20.0	40.0
	Oct. 23/80	26.0	26.0	33.0	40.0
tube 2	Aug. 1/79	-	-	0.3	0.9
	Sep. 25/79	7.0	8.0	11.0	59.0
	Nov. 4/79	38.0	30.0	34.0	60.0
	Dec. 18/79	19.0	22.0	32.0	48.0
	Jan. 23/80	19.0	26.0	45.0	53.0
	Feb. 15/80	1.3	0.6	20.0	65.0
	May 8/80	10.0	80.0	80.0	540
	June 11/80	10.0	50.0	210	340
	Oct. 23/80	26.0	26.0	26.0	33.0
tube 3	Aug. 1/79	-	-	∠0.1	0.3
	Nov. 4/79	30.0	10.0	0.5	53.0
	Jan. 23/80	57.0	34.0	79.0	150
	Feb. 15/80	0.4	0.5	1.7	6.2
	May 8/80	70.0	250	380	370
	June 11/80	20.0	120	400	650
	Oct. 23/80	33.0	26.0	26.0	53.0
tube 4	Jan. 28/80	14.0	10.0	26.0	87.0
	Feb. 15/80	10.0	10.0	20.0	300
	May 8/80	∠10.0	20.0	400	3000
	June 11/80	∠6.0	80.0	410	1330
	Oct. 23/80	40.0	40.0	47.0	60.0
tube 5	Aug. 1/79	-	-	0.3	0.3
	Nov. 4/79	12.0	19.0	14.0	11.5
	Dec. 18/79	32.0	12.0	26.0	12.0
	Jan. 23/80	19.0	15.0	10.0	42.0
	May 8/80	∠10.0	30.0	50.0	50.0
	June 11/80	∠6.0	30.0	70.0	100
	Oct. 23/80	20.0	33.0	33.0	40.0
6.5 m lake site	Aug. 1/79	∠0.1	∠0.1	∠0.1	∠0.1
	Nov. 4/79	3.2	2.3	4.7	12.0
	Jan. 11/80	9.0	10.0	11.0	19.0
	Feb. 15/80	6.2	5.0	11.0	2.6
	Mar. 28/80	20.0	∠10.0	20.0	150
	May 8/80	∠10.0	∠10.0	20.0	50.0
	June 11/80	∠6.0	10.0	20.0	60.0
	Oct. 23/80	10.0	9.0	46.0	20.0
10 m lake site		surface	3 m	5 m	7 m	10 m
	Mar. 28/80	∠10.0	∠10.0	∠10.0	∠10.0	10.0
	May 8/80	10.0	10.0	10.0	20.0	30.0
	June 11/80	10.0	10.0	20.0	20.0	20.0
	Oct. 23/80	40.0	20.0	10.0	9.0	13.0

Nevertheless, the increases in zinc levels after the culverting installations were clearly visible. There were no apparent differences in zinc levels between the tubes being acidified and those which were not. Indeed, the highest levels recorded were in tube 4, which was not acidified. There was also little difference between the lake sites and the tubes, indicating a rapid spread of the contaminating zinc throughout the lake.

APPENDIX B

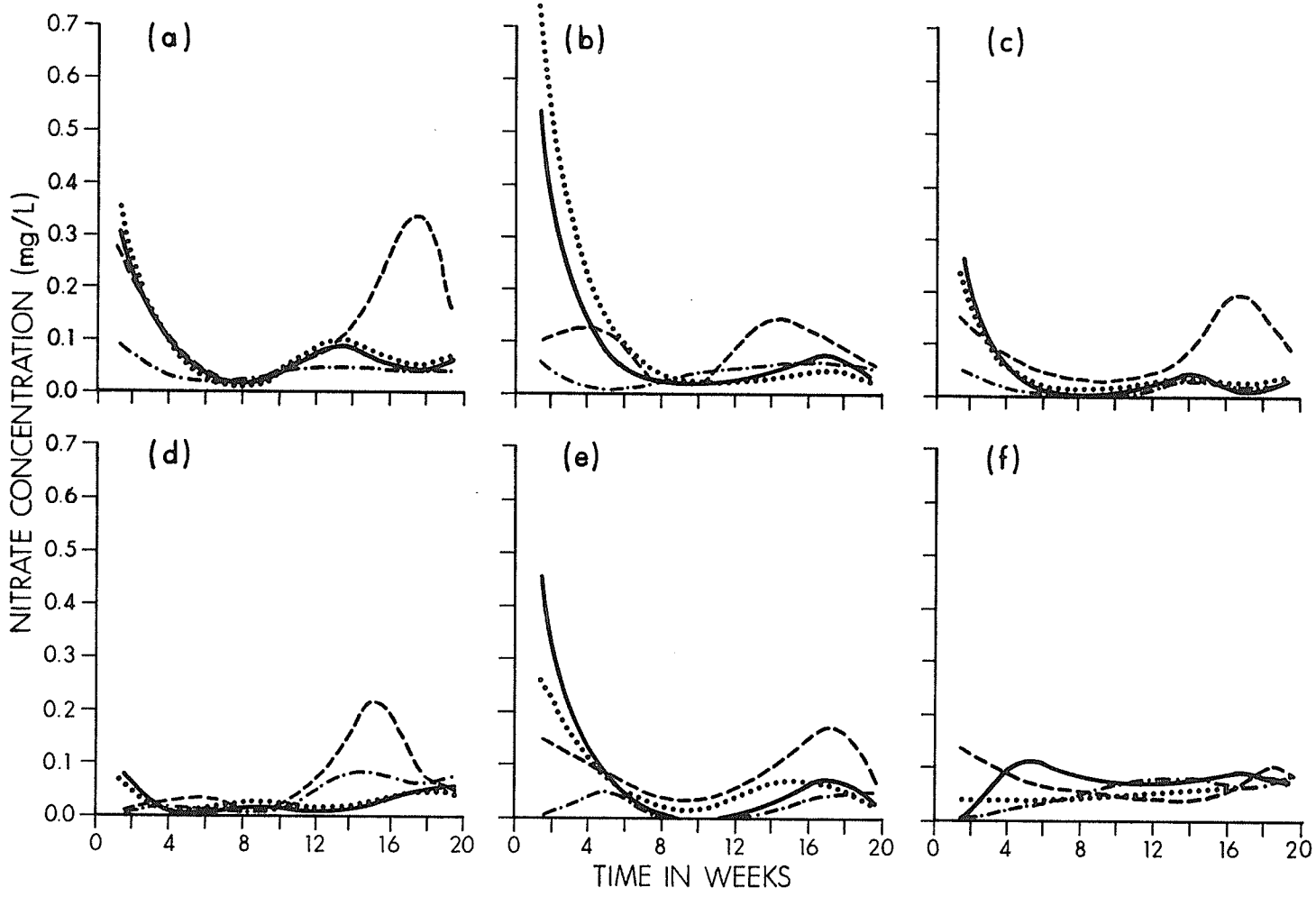
Nitrate and Sulfate Data

In the 1980 sampling season nitrate and sulfate concentrations were monitored over the period 1 June, 1980 to 22 October, 1980, about 20 weeks.

There were few differences between the sets of tube nitrate data (figures 25 a to 3). Except for tube 4 (fig. 25 d), all started with relatively high concentrations of nitrate in the hypolimnetic waters in the early summer, possibly as a result of enrichment from the sediments during spring turnover. The nitrate levels throughout the water columns then dropped to almost zero for the remainder of the season. With the onset of fall overturn at about week 14 there was a large increase in the nitrate concentrations at 6.5 metres in all five of the tubes, to levels as high as in early summer. Nitrate concentrations then declined to near zero by the end of sampling. The lake data (fig. 25 f) differed in that the nitrate levels remained fairly constant throughout the season, there was no early summer high, nor was there a peak in nitrate content at 6.5 m during fall overturn. The differences between these two data sets (lake and tubes) were probably caused by the interference of the culverts and tubing with normal water circulation. The fall overturn peaks at 6.5 m in the tubes were almost certainly caused by the inference of the culverting with horizontal mixing of lake and tube waters.

In no case did the nitrate levels recorded in these experiments exceed the value of 50 mg/l, reported to be sufficient to inhibit

Figure 25. Nitrate levels at various depths in the field tubes and the adjacent lake site over the period 1 June, 1980 to 22 Oct., 1980. Legend: figure 25a, tube #1 (control); figure 25b, tube #2 (acid addition only); 25c, tube #3 (control); fig. 25d, tube # 4 (Cd addition only); fig. 25 e, tube #5 (Cd + acid addition); fig. 25f, lake site I; 0 m (-•-•-); 3 m (——); 5 m (••••); 6.5 m (- - -).

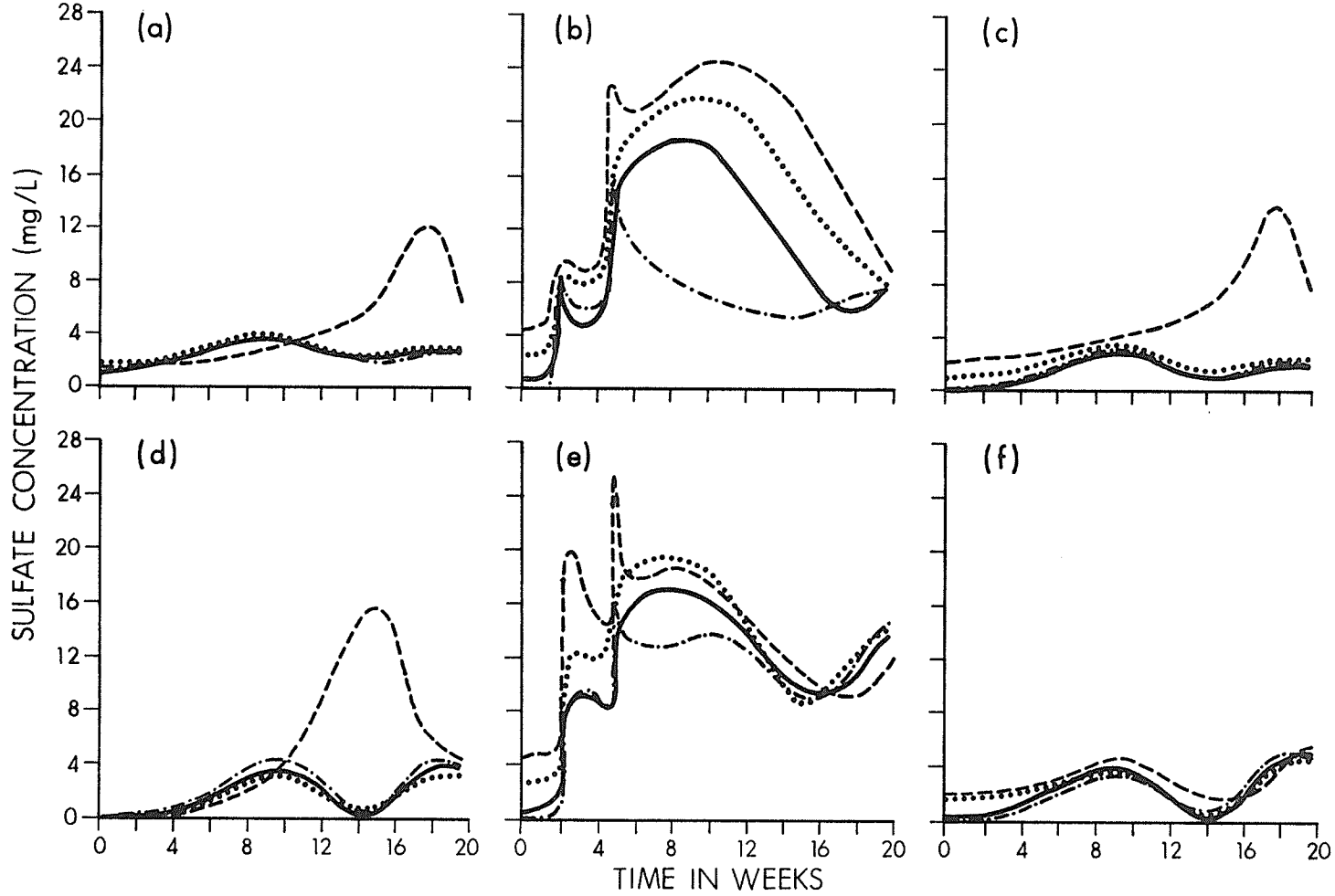

(a)
(b)
(c)
(d)
(e)
(f)
NITRATE CONCENTRATION (mg/L)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
4
8
12
16
20
TIME IN WEEKS

methanogenesis (Bollag & Czlonkowski, 1973). It was therefore unlikely that inhibition of methanogenesis by nitrate took place in the field over the course of the studies.

There was little difference in the sulfate data sets between the lake and the three non-acidified tubes (figs. 26 a, c, d & f). All four sets showed extremely low sulfate levels in the early summer which built to a small peak at all depths by late summer, approximately week 8. Thereafter, above 6.5 m depth in the tubes, the sulfate levels fell and then rose again towards the end of the sampling season. At a depth of 6.5 m there were increases in sulfate content after about week 12 sulfate concentration peaked at about week 16 and then fell near the end of sampling. As with nitrate there was no large peak in sulfate content in the lake during fall overturn and the tube peaks were most likely a consequence of the culverts interfering with horizontal mixing of the tube and lake water columns.

In the case of the acidified tubes (figs. 26 b & e, tubes 2 & 5 respectively) the effects of the acid additions upon the sulfate levels were readily visible. In both data sets there was a sudden, dramatic increase in the sulfate concentrations at all depths in the tubes after the acid additions. The sulfate levels in these tubes remained very high when compared to the other tubes and the lake for the entire summer and autumn. In tube 2 (fig. 26 b) the sulfate concentration remained very high in the hypolimnetic water until near the onset of fall overturn when it decreased. The sulfate levels in tube 5 remained fairly high throughout the entire water column, the high sulfate content of the epilimnetic waters perhaps being a consequence of the relatively

Figure 26. Sulfate levels at various depths in the field tubes and the adjacent lake site over the period 1 June, 1980 to 22 Oct., 1980. Legend: figure 26a, tube #1 (control); figure 26b, tube #2 (acid addition only); figure 26c, tube #3 (control); figure 26d, tube #4 (Cd addition only); figure 26e, tube #5 (Cd + acid addition); figure 26f, lake site I; 0 m (-•-•-), 3 m (——),; 5 m (••••); 6.5 m (- - -).


(a)
(b)
(c)
(d)
(e)
(f)
SULFATE CONCENTRATION (mg/L)
TIME IN WEEKS
0
4
8
12
16
20
24
28

oligotrophic waters within this tube.

The sulfate levels in the non-acidified tubes and the lake remained below the value of 20 mg/l which was shown to inhibit methanogenesis (Winfrey & Zeïkus, 1977) In the acidified tubes the sulfate levels exceeded this value. In tube 5 levels exceeded the inhibitory limit for only a short while following the acid addition, which may account for the lack of inhibition within this tube. In tube 2 the sulfate content remained above the level for several weeks. The high hypolimnetic sulfate content of tube 2 may be the explanation for the partial inhibition of methanogenesis which occurred in this tube.

APPENDIX C

Temperature and Dissolved Oxygen Data

All six of the isotherm data sets (figs. 27 - 32) are virtually identical to one another. Each data set shows the development of homothermic conditions in autumn 1979 at approximately weeks 4 to 8 and in spring of 1980 at approximately weeks 32 to 36. The winter 1979-80 and summer 1980 isotherms show a high degree of similarity among the data sets with few major variations. In the fall of 1980, homothermic conditions again occurred simultaneously. Therefore, the tube material did not have a significant effect on thermal stratification within the tube water columns as compared to the lake.

In the 1980 sampling season (14 May, 1980 to 27 Oct., 1980) all of the dissolved oxygen isopleths from the tubes showed the same general patterns (figs. 33, 34, 35a). Starting in the spring, high concentrations of oxygen were present at depths of 1 to 2 metres and the deoxygenated waters were restricted to a fairly thin layer at depths of 5 metres or more. As the surface waters warmed during late spring and summer the dissolved oxygen concentrations fell, a reflection of the reduced solubility of oxygen in warmer waters. At the same time the bottom layer of deoxygenated water grew deeper as the available oxygen was metabolized, reaching depths of some 4 metres. Over the summer there were sudden incursions into the surface waters of water which had greater or lesser concentrations of dissolved oxygen than the surface waters. These incursions occurred simultaneously in all five tubes and reflect possible sudden increases in surface water temperature,

Figure 27. Isotherms for field tube #1 (control) for the period 16 Sept., 1979 to 26 Oct, 1980. Values are given in degrees centigrade.

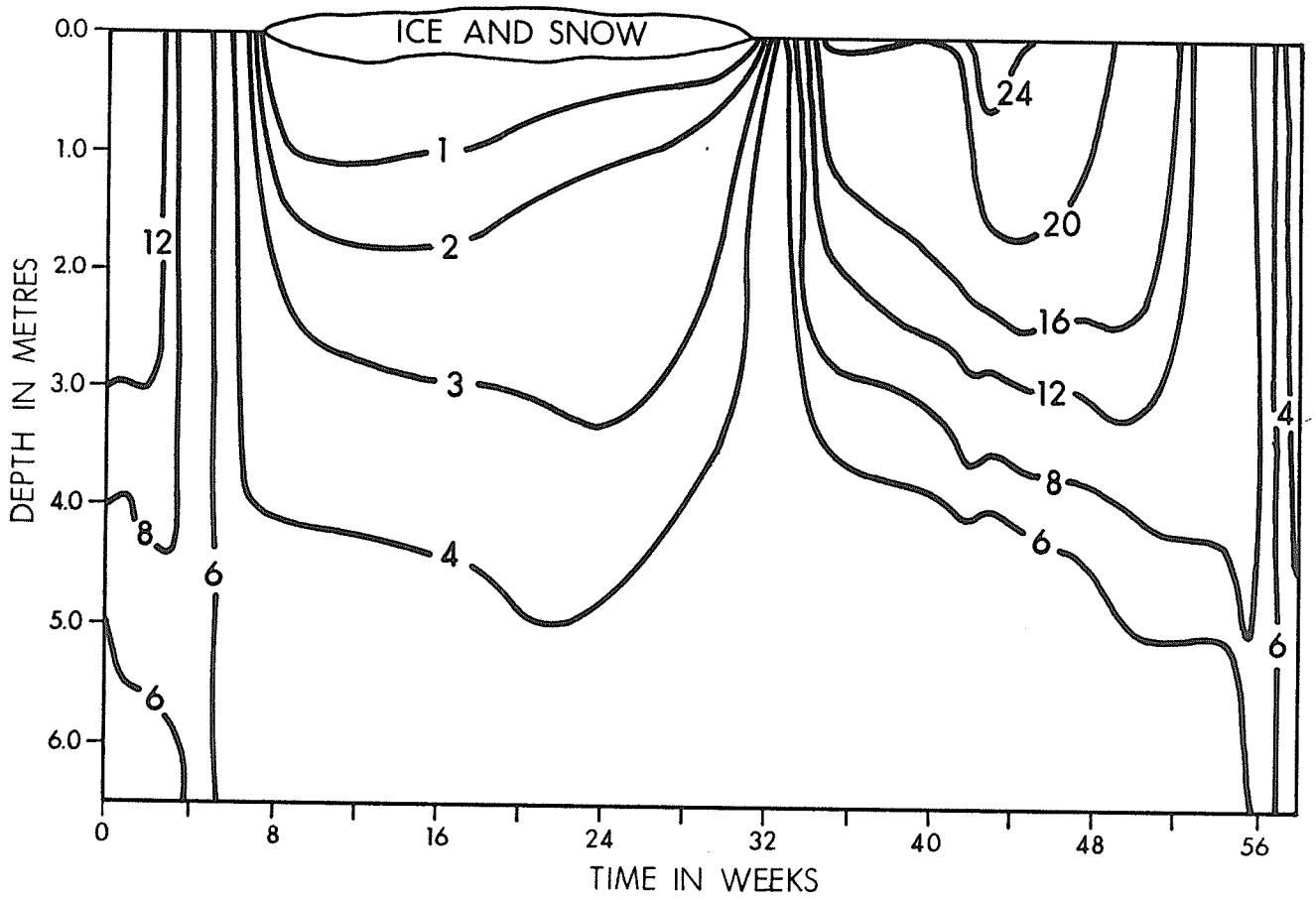

ICE AND SNOW
DEPTH IN METRES
TIME IN WEEKS
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0
8
16
24
32
40
48
56
1
2
3
4
6
8
12
16
20
24

Figure 28. Isotherms for field tube #2 (acid addition only) for the period 16 Sept., 1979 to 26 Oct., 1930. Values are given in degrees centigrade.

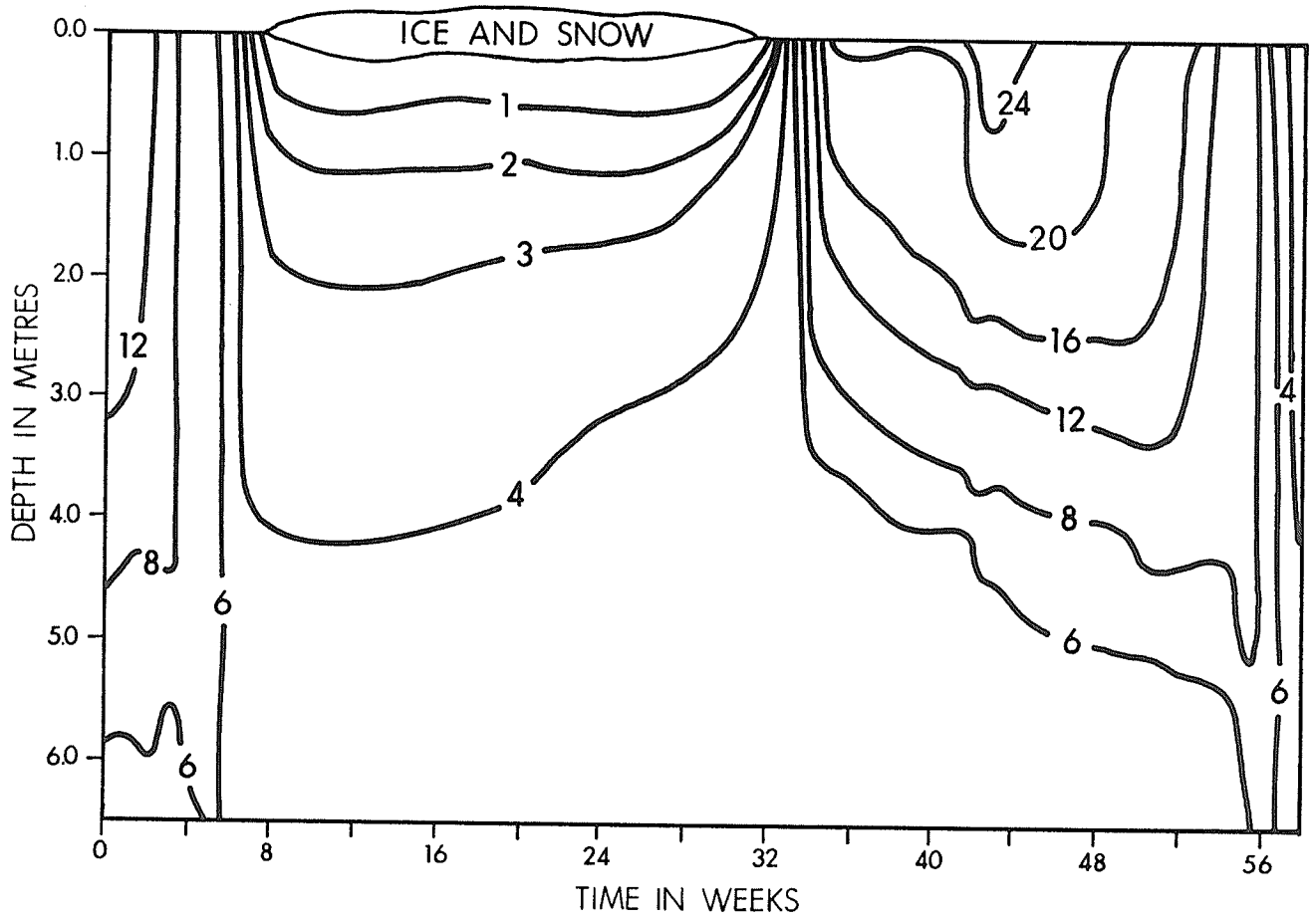

ICE AND SNOW
0.0
1.0
2.0
3.0
4.0
5.0
6.0
DEPTH IN METRES
1
2
3
4
6
8
12
24
20
16
12
8
6
4
6
6
0
8
16
24
32
40
48
56
TIME IN WEEKS

Figure 29. Isotherms for field tube #3 (control) for the period 16 Sept., 1979 to 26 Oct., 1980. Values are given in degrees centigrade.

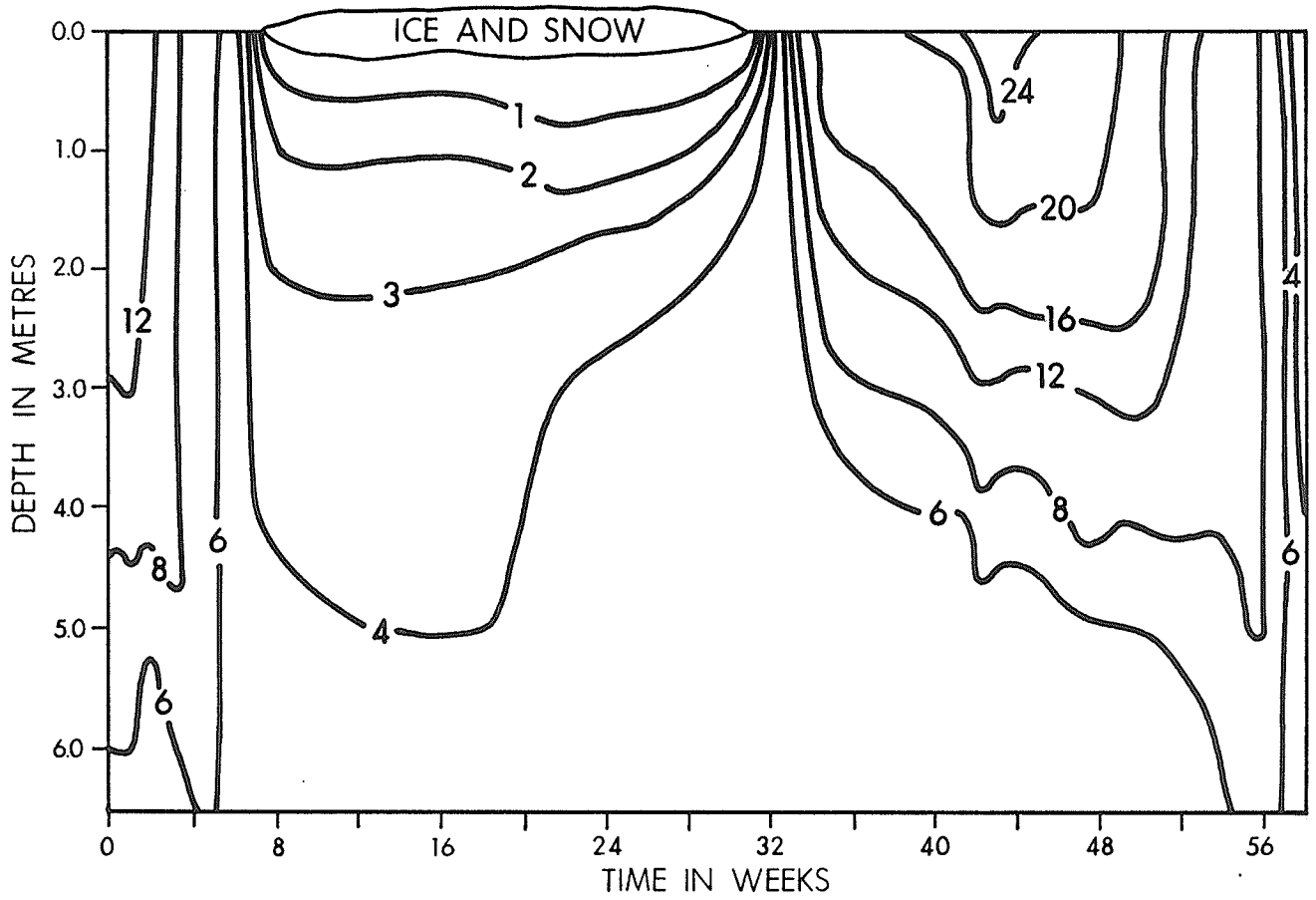

ICE AND SNOW
1
2
3
4
6
8
12
24
20
16
12
8
6
4
6
0.0
1.0
2.0
3.0
4.0
5.0
6.0
DEPTH IN METRES
0
8
16
24
32
40
48
56
TIME IN WEEKS

Figure 30. Isotherms for field tube #4 (Cd addition only) for the period 16 Sept., 1979 to 26 Oct., 1980. Values are given in degrees centigrade.

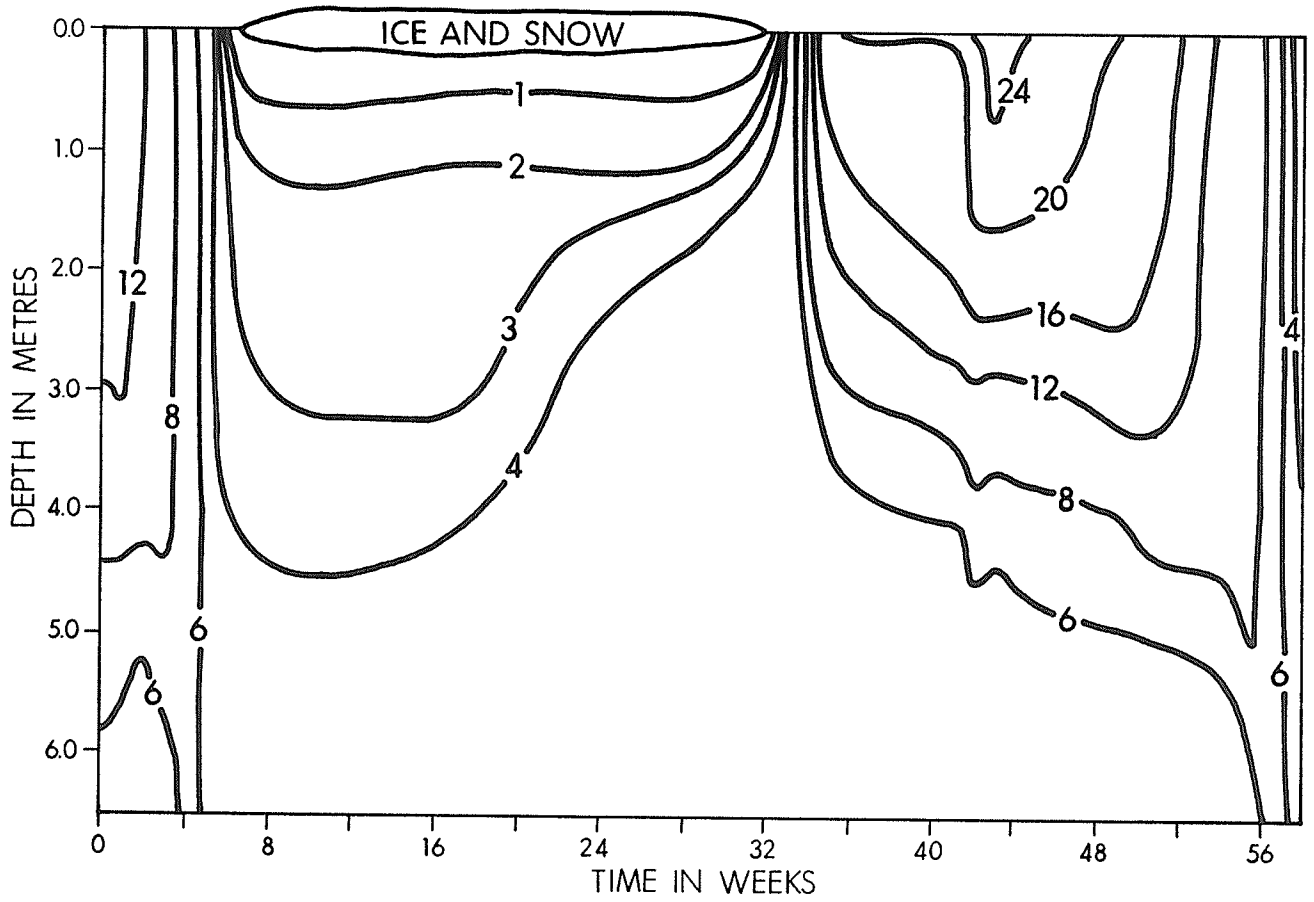

ICE AND SNOW
1
2
3
4
6
8
12
16
20
24
4
6
6
8
12
16
20
24
DEPTH IN METRES
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0
8
16
24
32
40
48
56
TIME IN WEEKS

Figure 31. Isotherms for field tube # 5 (Cd + acid additions) for the period 16 Sept., 1979 to 26 Oct., 1980. Values are given in degrees centigrade.

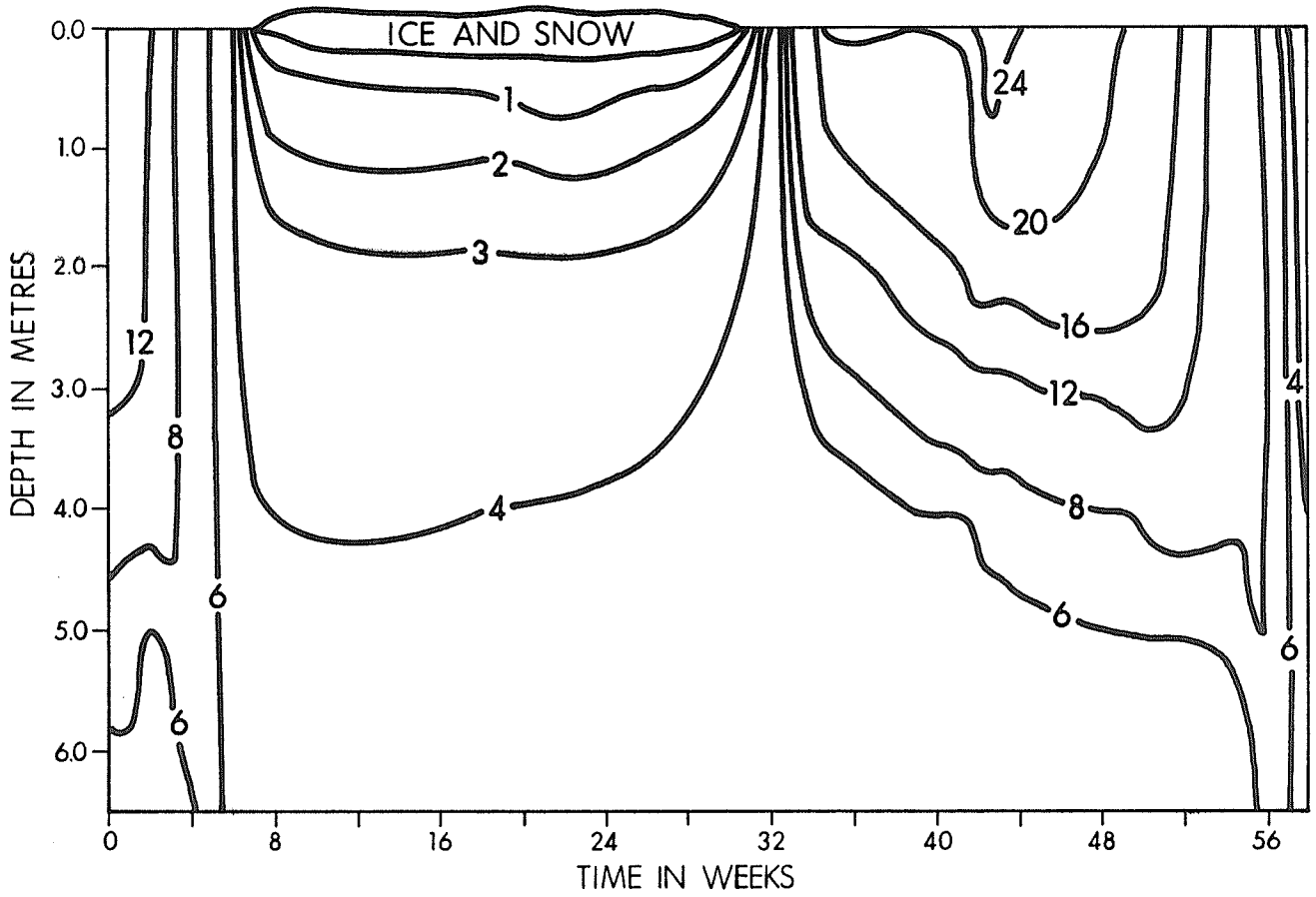
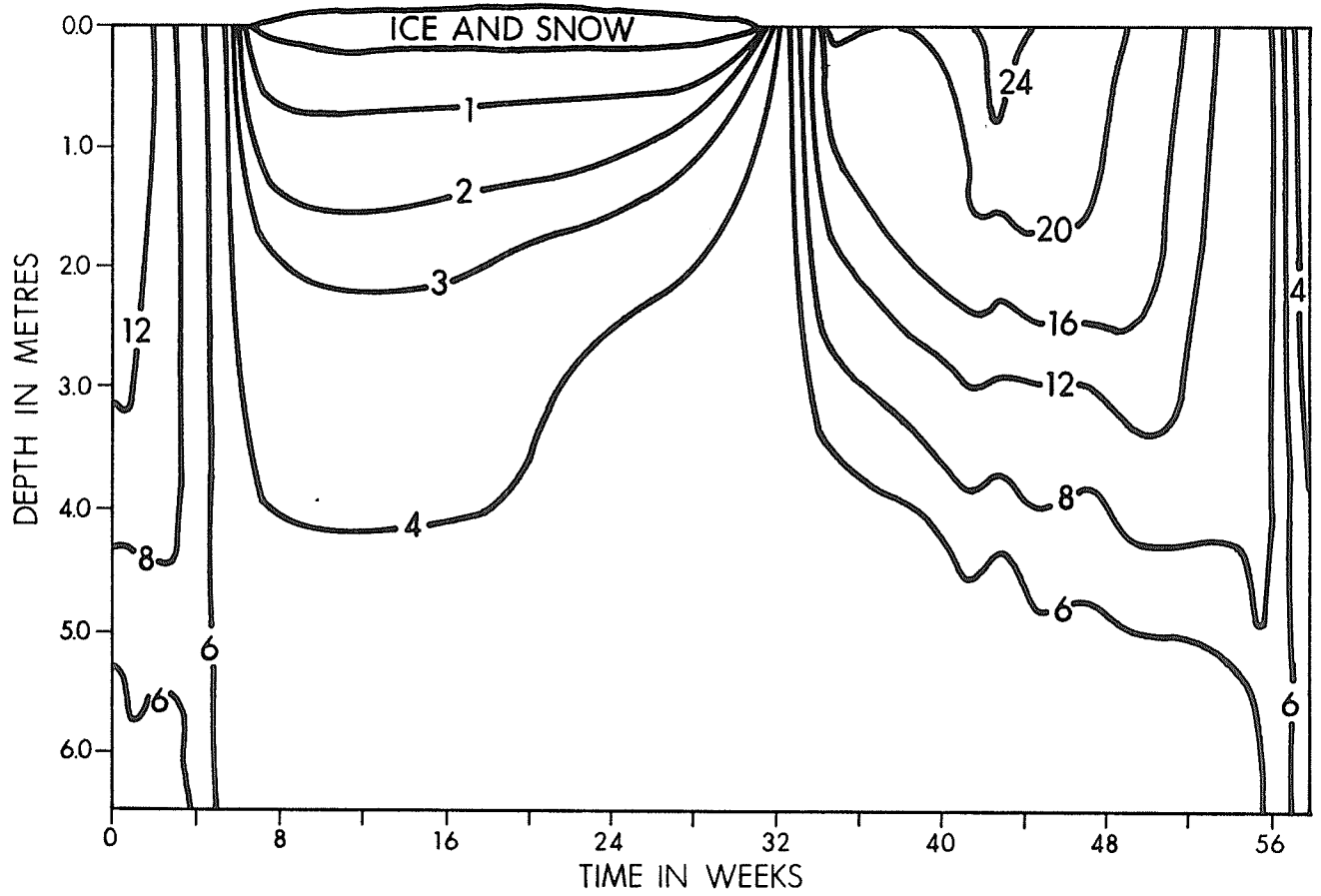

ICE AND SNOW
1
2
3
4
6
8
12
16
20
24
4
6
6
0.0
1.0
2.0
3.0
4.0
5.0
6.0
DEPTH IN METRES
0
8
16
24
32
40
48
56
TIME IN WEEKS

Figure 32. Isotherms for lake site I (adjacent) over the period 16 Sept., 1979 to 26 Oct., 1980. Values are given in degrees centigrade.


ICE AND SNOW
1
2
3
4
12
8
6
6
24
20
16
12
8
6
4
6
DEPTH IN METRES
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0
8
16
24
32
40
48
56
TIME IN WEEKS

or the entry of highly oxygenated waters during a storm event. In tubes 2 to 4 (figs. 33b, 34 a & b) there was a sudden massive incursion of highly oxygenated water at approximately week 20, just before the onset of fall overturn. This incursion also occurred in tube 1 (fig. 33a) but not to as great an extent as in the other tubes. There was no sign of this incursion in tube 5 (fig. 35a), but there was some indication of the entry of oxygenated water near the end of overturn, at about week 24. With the onset of fall overturn there was a sharp, steady drop in the depth of the less than 7 mg O_2/l isopleths, along with a concurrent rise towards the surface of the isopleths above this level, in tubes 2 to 4. In the case of tube 1, only the zero concentration isopleth sank to the bottom while the others either stayed roughly at their summer depths (i.e. the 2 mg O_2/l isopleth) or rose to the surface (4 mg O_2/l and above). In tube 5 all of the isopleths rose towards the surface with the zero concentration isopleth reaching a minimum depth of 2 metres. As mentioned earlier there was a slight intrusion of oxygenated water into the surface layers of this tube near the end of overturn. In all, this portion of figure 35a bears little resemblence to the other figures.

Dissolved ogygen isopleths from the lake (fig. 35b) show the same general patterns of high and low oxygen concentrations as do the tube isopleths, differing only in degree. In spring the high concentrations of dissolved oxygen were much higher than in the tubes and occurred at a depth of almost 3 metres as opposed to only 1 - 2 metres in the tubes. Higher concentrations of dissolved oxygen were observed throughout the

Figure 33. Dissolved oxygen isopleths for the two control field tubes (#1 - fig. 33a: #3 - fig. 33b) for the period 8 May, 1980 to 29 Oct., 1980. Values are given in mg O_2/l.

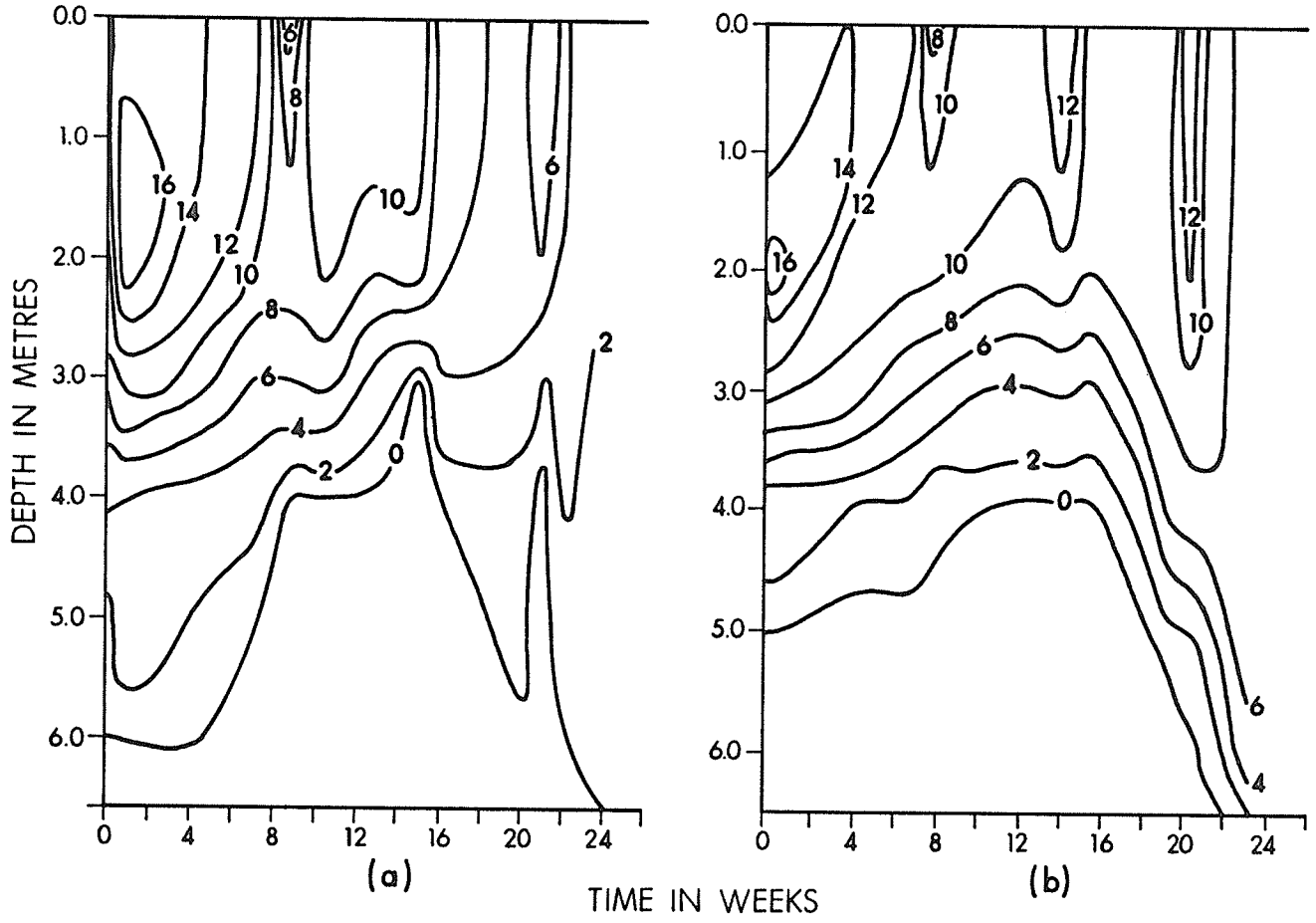

DEPTH IN METRES
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0
4
8
12
16
20
24
(a)
(b)
TIME IN WEEKS

Figure 34. Dissolved oxygen isopleths for field tubes #2 (fig. 34a, acid additions only) and #4 (fig. 34b, Cd additions only) for the period 8 May, 1980 to 29 Oct., 1980. Values are given in mg O_2/l.

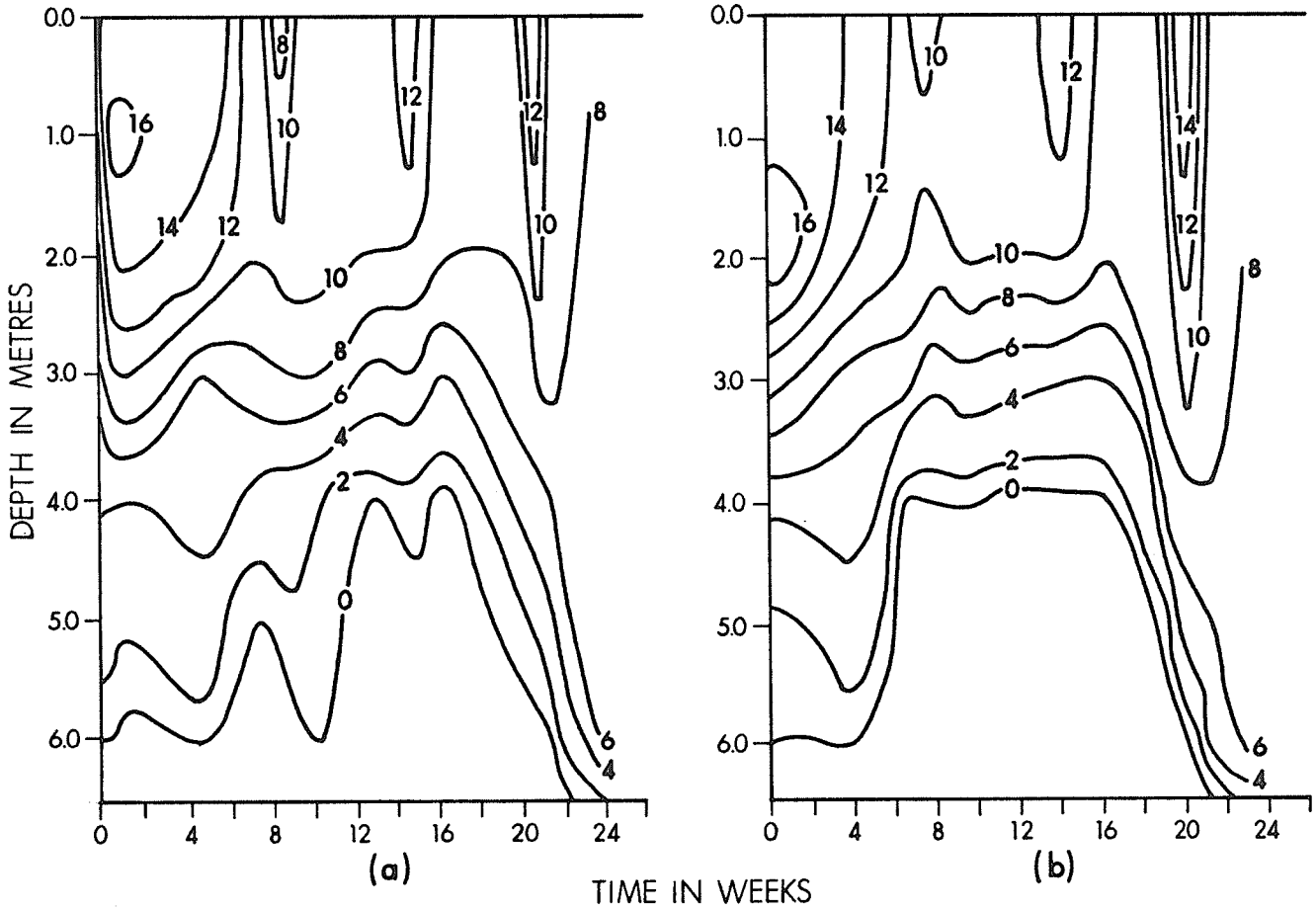
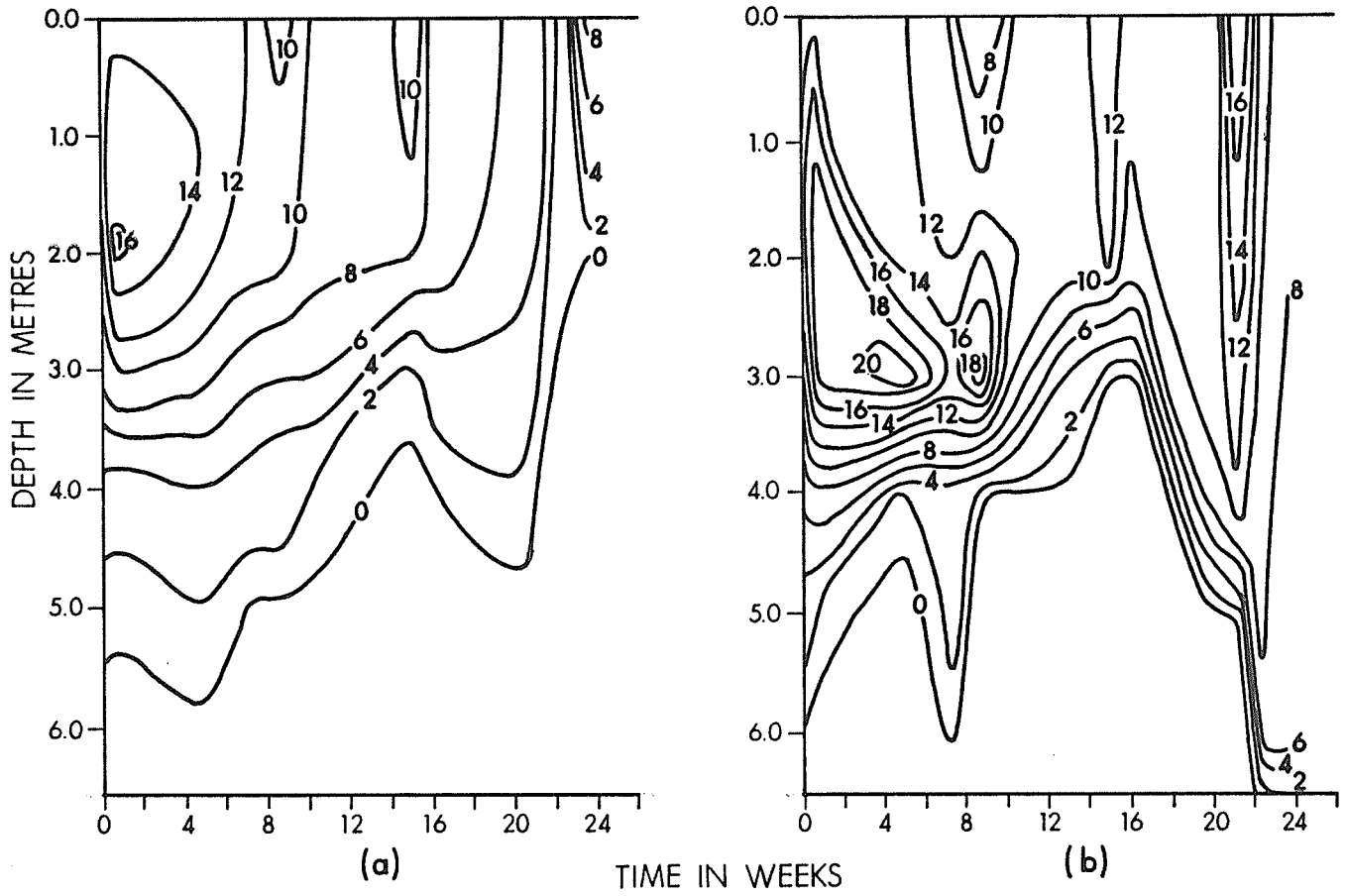

DEPTH IN METRES
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0
4
8
12
16
20
24
(a)
(b)
TIME IN WEEKS

Figure 35. Dissolved oxygen isopleths for field tube #5 (Cd and acid additions, fig. 35a) and lake site I (adjacent, fig. 36b) for the period 8 May, 1980 to 29 Oct., 1980. Values are given in mg O_2/l.


DEPTH IN METRES
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0
4
8
12
16
20
24
(a)
(b)
TIME IN WEEKS

summer in the lake and the transition region from zero oxygen to high oxygen concentration was much narrower than those seen in the tubes. During fall overturn the increase in the depth of high oxygen levels was slightly more rapid in the lake, especially at weeks 20 to 22 when a large incursion of oxygenated water occurred. In general, the lake and the tube isopleths paralleled one another fairly well, excepting only tubes 1 and 5 as discussed earlier, but the lake exhibited higher oxygen concentrations than were found in the tubes. The lower oxygen levels found in the tubes as compared to the lake are another indication of the interference by the tube material with the circulation of water in the tubes.