Measurement of Biological Mercury Methylation in the Littoral Sediments of an Acidified and an Unacidified Lake

A Thesis

Submitted to

The Faculty of Graduate Studies

University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree of

Master of Science

By

Patricia Susan Ramlal

September, 1983

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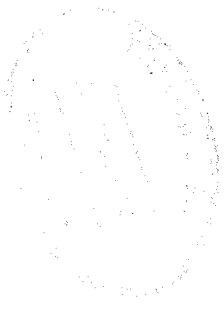

For my family

Acknowledgements

I am indebted to Dr. John W.M. Rudd for his continuous support and encouragement through the duration of my studies. I wish to thank Dr. N.E.R. Campbell for guiding me through the bureaucracy involved in being a graduate student. I would also like to thank Dr. Carol Kelly for the many helpful discussions and advice freely given. I am grateful to Dr. S.G. Lawrence, the Department of Microbiology, and the Freshwater Institute for their financial support.

I would especially like to thank Akira Furutani, whose friendship, wisdom, and enthusiasm for this project kept me going through some of its more frustrating stages.

I would like to thank B. Hauser and A. Lutz for providing chemical analyses. I am grateful to Dr. D.W. Schindler and the staff of the Experimental Lakes Area who were kind enough to provide me with support, both in the field and by their friendship. To the staff and students of the Department of Microbiology, especially Nancy Loewen and Greg Awang, who made life as a graduate student enjoyable, thank-you. A special thanks to Donna Laroque who typed the final draft of this thesis.

Finally, I cannot fully express my gratitude to my family for their never-ending patience and encouragement.

Abstract

The occurrence of elevated mercury concentration in fish of acid stressed lakes has become an issue of environmental concern. This project was intended to determine if pH-dependent processes in lake sediments were contributing to this increase in mercury concentration in fish. Epilimnetic sediments were collected from a lake with no history of acidification, Lake 239; and Lake 223, which has been receiving experimental additions of sulphuric acid since 1976. Sediments were transported to the lab where pH was adjusted over a range of 4.5-7.5. Rates of mercury methylation were monitored by adding mercury-203 as mercuric chloride at an approximate concentration of 2 µCi/2 µg mercury/g dry sediment. The samples were incubated for 12 hours, after which 203 methylmercury was extracted using an organic extraction technique. The amount of inorganic mercury available to microorganisms for methylation was measured by determining the distribution of mercury between particles and porewater in the sediment for the same pH range. The effect of acid volatile sulphide on the availability of inorganic mercury for methylation was also determined.

Results indicated that at low pH in sediments, the ability of mircoorganisms to produce methylmercury is reduced to near zero at pH values of less than 5.0. This result could be attributed to the reduced availability of inorganic porewater mercury to less than 20% of that found at ambient pH, at pH's lower than 6.0. It was also found that as sulphide was experimentally removed from sediment, the inorganic mercury became more available, suggesting that sulphide had a considerable pH-related effect on the bioavailability of mercury. In an acid stressed system 70% of the acid in precipitation is an oxide of

sulphur. The free inorganic mercury may preferentially bind to the sulphur, reducing the likelihood of immediate microbial methylation.

In another experiment the effect of aerobiosis and anaerobiosis on rates of mercury methylation was investigated. In addition to this, the microbial activity of the incubated sediments was monitored by measuring rates of carbon dioxide and methane production. These effects were investigated because as a lake is acidified, the depth distribution of oxygen in that lake increases, which will affect the types and metabolism of microorganisms at these depths. It was found that the microbial populations of the littoral sediment of L239 and L223 could produce methylmercury faster under anaerobic conditions, although the metabolic activity was similar or faster for organisms incubated in the presence of oxygen.

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HISTORICAL

Sources of Mercury to Aquatic Systems

Natural Sources

As an ore, mercury exists mainly as cinnabar, a red sulphide, primary deposits of which can occur in all types of igneous, metamorphic, and sedimentary rocks (Friberg and Vostal 1972). There are seven stable and 18 radio-isotopes of mercury with half-lives ranging from 0.4 seconds to 130 days (CRC 1967). A number of measurements of the background levels of mercury in the environment have been made and its concentration can vary a great deal. The concentration of mercury in air can range from 0.001-50 ng/cubic meter; in soils the range is 10-15 ppb, although this value can be higher if the soil has a high organic or humic content (D'Itri 1972; Goldwater 1972; Williston 1968). Precipitation has an average value of 0.33 ppb (Williston 1968), while the concentration in lakes, rivers, and oceans has been estimated at 0.02-0.7 ppb (Williston 1968).

In a comparison of natural and anthropogenic source strengths, Matheson (1979) found that although the exact quantity of natural emissions of mercury is not known, it is generally agreed that on a global basis, the levels of mercury from natural sources are higher than those from anthropogenic sources.

Anthropogenic Sources

High concentrations of mercury in waterways can usually be attributed to man's industrial disposal into the environment. The chlorine-alkali industry used large amounts of mercury as a catalyst (Hanson 1971; Friberg and Vostal 1972). It has also been used as an anti-fouling agent in paint, and a fungicide in the pulp and paper

industry as well as in agriculture. The amount of mercury released by these sources has been limited since more stringent controls have been placed on the companies involved in these processes. Production of electrical apparatus such as mercury cell batteries, fluorescent bulbs and switches, does not release much mercury during the manufacturing process, but disposal of these products can release large amounts of mercury into sewage and waste (Hanson 1971). The use of an amalgam of silver alloy mercury in dental fillings is another potential source of mercury to both the atmosphere via volatilization and by disposal of old fillings (Stopford 1979).

Sources of Mercury to the Atmosphere

Natural Sources

Elemental mercury has a high vapour pressure at ordinary temperatures, which increases its mobility in the atmosphere (Williston 1968). Williston (1968) studied background levels of mercury and found a range of 1-10 ng/cubic meter air. He also found fluctuations in concentration could occur daily or even hourly and after a smog episode, the mercury levels were elevated. It has been demonstrated that fossil fuels, depending on quality, contain varying amounts of mercury, all of which is released into the atmosphere when burned (Joensuu 1971). Joensuu took 36 samples of coal and found that concentrations of mercury varied from 0.07-33 ppm with an average values of 3.3 ppm.

Friberg and Vostal (1972) postulated that the transport of ionized forms of mercury into the atmosphere by volatilization could occur by: 1) chemical reduction into the elemental form; 2) reduction through the activity of microbes, plants, or other living organisms; or (3)

biotransformations into volatile inorganic compounds, mainly short chain alkyl mercurials.

Most of the mercury emitted to the atmosphere is in the elemental vapour form, either from natural mantle degassing or in processes involving heating of minerals to high temperatures (Matheson 1979). During the degradation of organic mercurials, inorganic elemental mercury is produced as an end product which can either be sequestered in soil or sediments or returned to the atmosphere by volatilization (Windom and Kendall 1979). Biotransformation of mercury may also occur when mercury (II) is methylated. Although methylmercury is more soluble in water than elemental mercury it is still possible for it to be volatilized (Johnson and Braman 1974). Dimethylmercury is easily volatized. In a study of Johnson and Braman (1974) it was discovered that air moving over a highly polluted area had mercury concentrations 5 times higher than normally encountered, and a considerable portion of the mercury was in the form of methylmercury.

The suggestion has been made that mercury could absorb onto fly ash particles, making it more susceptible to removal by impaction, dry deposition or washout (Matheson 1979). Mercury can travel great distances before being deposited. In cases where the quantity of mercury emitted from point source is known, only a small proportion of that mercury is deposited locally (Jernelov and Wallin 1973; Jernelov et al. 1975). It has also been reported that in areas with no known local source of mercury, the concentration of mercury in the fish has been high enough to limit human consumption (Jernelov et al. 1976; Brouzes et al. 1977). The importance of anthropogenic emissions of a number of metals has been studied in detail by Lantzy and MacKenzie (1979). They

assessed the importance of the anthropogenic flux for any metal is given by the interference factor, which is calculated as:

Total Anthropogenic Emissions/Total Natural Emissions x 100.

The interference factor for mercury was calculated as 27,500%, second only to lead of all elements measured.

The reports about mercury in rain and snow tend to be contradictory (NRC 1978). McCarthy and his co-workers (1969) found that a heavy rainstorm efficiently removed mercury from the atmosphere. However, Johnson and Braman (1974) found atmospheric concentrations of mercury to be the same before, during, and after a severe thunderstorm.

Information available from measuring mercury content of snow is also confusing. In some cases it appears mercury concentration increases at deeper snow depths possibly due to degassing or volatilization of mercury from the ground into the snow (Jonasson 1970; MacLean 1976). It was also suggested (Matheson 1979) that mercury may accumulate in the snow, and, in association with acid in snow melt, be finally conveyed to waterways. Much of the variability in results may be due to contamination of samples while they are being processed (Armstrong, pers. comm.).

Microbial Transformations of Mercury

Methylation

The possible role of microorganisms in the production of methylmercury from inorganic mercury was first investigated by Wood and his associates in 1968. They took extracts of methanogenic bacteria and observed the transfer of methyl groups from methyl cobalamine, an analogue of Vitamin B-12 (Figure 1a), to mercuric mercury. They found

that although low concentrations of mercury inhibited the production of methane, the formation of Vitamin B-12 was unaffected.

Jensen and Jernelov (1969) showed that microorganisms found in lake sediments could methylate inorganic mercury. Both mono- and dimethyl mercury were produced, and that the rate of production could be correlated to the microbial activity of the sediment. The organisms responsible for mercury methylation and the mechanisms involved has been the subject of a number of studies (Vonk and Karrs Sijpesteijn 1973; Landner 1972). Landner (1972) studies the methylating capabilities of Neurospora crassa, which does not have Vitamin B-12 involved in its metabolism, and discovered that the methylmercury produced was the result of incorrect synthesis of methionine, in which the mercuric mercury bound to homocysteine is methylated (Figure 1b). Vonk and Sijpesteijn (1973) studied a number of species of bacteria and fungi and found they could all produce methylmercury from mercuric chloride when they were incubated aerobically.

It has been proposed that mercury methylation can occur enzymatically or non-enzymatically (Bisogni 1979). In the process of non-enzymatic methylation the only step that is known for certain, is that there is a transfer of a carbanion methyl group to mercuric mercury in the presence of water (Figure 1a).

Under aerobic conditions, the methylation process involves the enzyme methionine synthetase, also known as N-5-methyl tetrahydrofolic homocysteine transmethylase. In this case, methylmercury seems to be the result of incorrect methionine synthesis (Figure 1b). Landner (1971) also studied this system using Neurospora crassa. He proposed

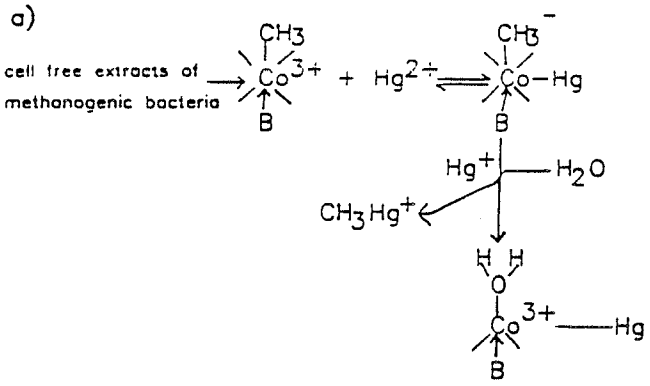
Figure 1. Proposed mechanisms for methylation of mercuric mercury (Wood et al. (1972)

a) non-enzymatic methylation

b) aerobic enzymatic methylation

c) anaerobic methylation

a)



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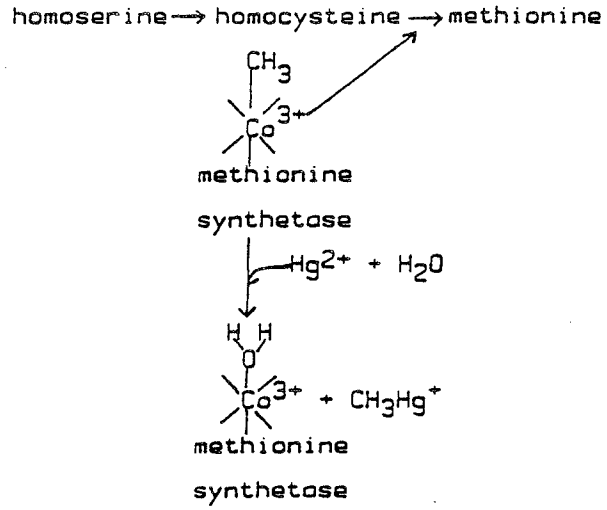

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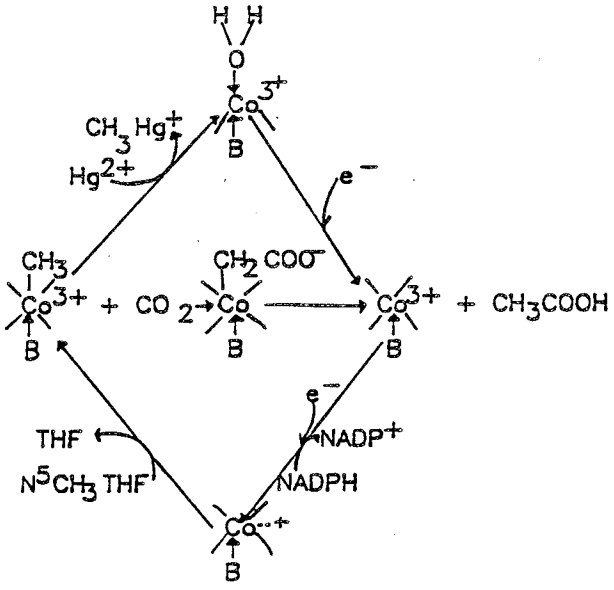


N^5CH_3 THF–N^5 METHYL TETRAHYDROFOLATE

B–5,6–DIMETHYL BENZIMIDAZOLE

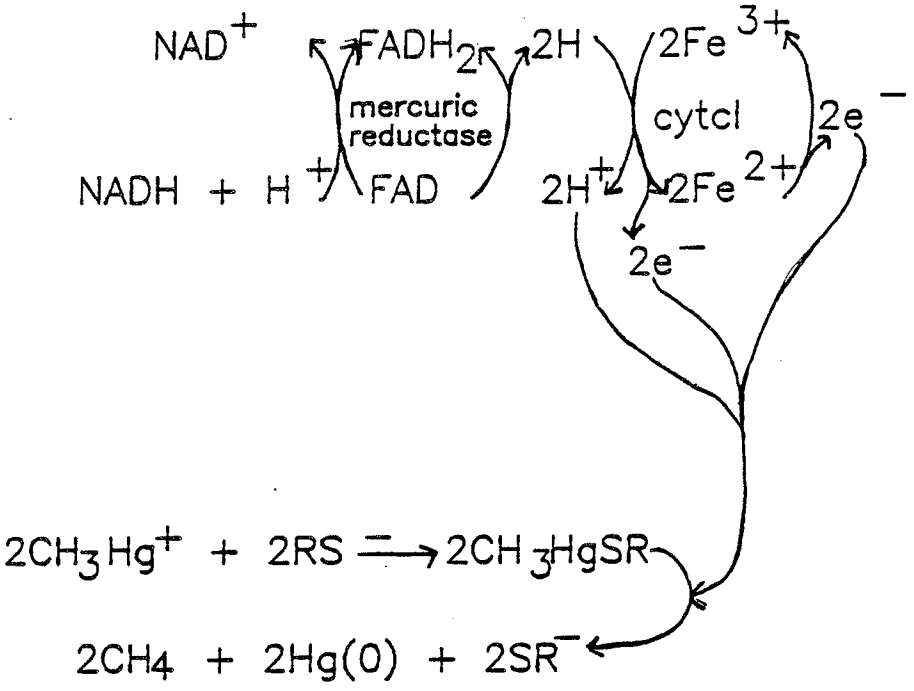
that the methyl group is transferred to the mercury atom which is complexed to homocysteine.

When organisms are incubated anaerobically, those which can produce acetic acid from carbon dioxide may also produce methylmercury via the acetate synthetase system. The normal production of acetic acid does not proceed because the mehtyl group, which normally becomes carboxylated, is instead bound to the mercuric cation. This attachment is due to the stronger binding affinity of mercury to the carbanion (Figure 1c).

Demethylation

Demethylation of methylmercury is the degradation of methylmercury to methane and inorganic mercury (Figure 2). Although the demethylation process has been observed by a number of investigators (Furukawa and Tonomura 1973; Spangler et al. 1973; Billen et al. 1974; Edwards and McBride 1975; Shariat et al. 1979; Pan-Hou et al. 1980), it is poorly understood. The process was noted by Spangler and his co-workers, when they observed loss of elemental mercury in selected cultures incubated with methylmercury in the growth medium. The mechanism of demethylation was studied by Furukawa and his associates (1973). They found that the process required the presence of the following conditions: 1) NADPH or NADH (or a system capable of producing these compounds); 2) a metallic mercury releasing enzyme containing FAD as the prosthetic group. This enzyme is now known as mercuric reductase; 3) cytochrome cI; and 4) a sulphur-containing compound such as homocysteine or reduced gluthathione could be utilized, although the oxidized form of glutathione was not suitable for the reaction.

Figure 2. Reductive demercuration of Pseudomonas K62 (Tonomura et al. 1972).



RS^- – SULPHUR CONTAINING COMPOUND

Protons and electrons are passed through the chain (Figure 2) with the organomercurial complex to produce elemental mercury and methane. The sulphur containing molecules are regenerated in this reaction. The elemental mercury which is produced is significanlty less toxic than methylmercury (Lexmond et al. 1976) and may either be sequestered in sediments or released to the atmosphere (Brosset 1981).

Kinetics of Methylmercury Formation and Decomposition

The kinetics of biological transformations of mercury is poorly understood. It is a difficult area of study because at the present time it is not possible to differentiate between the gross rates of methylmercury production and decomposition. Thus, values of kinetic studies are of net methylation rates. The net production of methylmercury could only be defined after the amount of methylmercury in the system reached equilibrium, when both methylating and demethylating organisms were working concommitantly. While the information obtained from these studies is valuable, it would be better if it could be determined whether perturbations in the natural environment were having separate or simultaneous effects on the microbial conversions of mercury. For example, information in the literature on the methylation rate by organisms under aerobic and anaerobic conditions varies considerably. It may be that oxygen is inhibiting or enhancing either methylation or demethylation, and the effects may vary depending on the chemistry and the indigenous population.

Kinetic studies on methylmercury formation were begun by Jensen and Jernelov in 1969. They found the rate of methylmercury production depended on the incubation period and on the amount of mercury

available. The amount of methylmercury in the incubation vessel after 10 days remained constant for the rest of the experimental period. This would suggest that the amount of methylmercury in the incubation vessels was at a steady state level.

Fagerstrom and Jernelov (1971) studied the rate of formation of methylmercury from mercuric sulphide, and found in aerobic, organic sediment methylmercury was produced. Yamada and Tonomura 91973) studied the same reaction using Clostridium cochlearium, T-2. After pretreating the mercuric sulphide preparation with hydrogen sulphide, they found no methylmercury was produced, which suggested to them that only mercury present as an impurity in the mercuric sulphide preparation was methylated. Furutani and Rudd (1980) found active methylation in the presence of bound sulphide.

Information about the methylation of mercury under aerobic and anaerobic conditions is diverse. Olson and Cooper (1976) studied three types of sediment from the San Fransciso Bay. They found more methylmercury was produced anaerobically, and was more stable under these conditions. They also found that the organic content of the sediment type was correlated with the amount of methylmercury produced, as was the concentration of mercury(II). Vonk and Sijpestiejn (1973) studied methylmercury production with pure cultures of bacteria incubated under aerobic conditions using mercuric chloride, and found a higher rate of production of methylmercury than that found with pure cultures incubated anaerobically.

Hamdy and his associates (1977), using pure cultures, found that methylmercury production had a cyclic pattern, and was decreased in the presence of DL-homocysteine, but was enhanced with methyl cobalamine.

Jacobs and Keeney (1974) did an *in situ* experiment located in the Wisconsin and Fox Rivers and found that the concentration of methylmercury was higher in the Wisconsin River. They attributed this to the chemical differences in the sediments especially the alkalinity and the sulphide concentrations. The Wisconsin River sediment had a pH of 5.5, 11% organic carbon, no carbonate and 280 µg S/g dry sediment. The Fox River had a pH of 6.5, 6.6% organic carbon, 1.4% carbonate, and sulphide sulphur 2,000 µg S/g dry sediment.

Genetic Research

Genetic research in the area of mercury methylation and demethylation has had more positive results than many of the kinetic studies. Olson and her co-workers (1979) undertook to determine the possible role of plasmids in the aquatic environment. Their aim was to find out if mercury volatilization was plasmid mediated, and if so, whether these plasmids were associated with methylation of mercury. Plasmids are small pieces of DNA, or minor chromosomes, often found in bacterial cells in addition to the main chromosome (Goodenough 1978). After removing one or all of the associated plasmids of one of the strains isolated, *Pseudomonas fluorescens* B69 ,they found that although the growth rates of these organisms did not change, their ability to volatilize organic mercury, or decompose an organomercurial compound, decreased to 5% of the wild type level. The enzyme involved in this transformation is mercuric reductase and is encoded on the *mer* gene carried on the plasmids. This gene has been found on the plasmids of *Eschirichia coli*, *Pseudomonas* spp., and *Staphylococcus aureus*. Plasmids are significant in the resistance to, and/or metabolism of mercury

because the mer gene has been located on transposons and is associated with sex factors, thus enhancing its chances of occurring in the environment. Transposons are plasmids which can be transmitted to bacteria through conjugation apparatus in those bacteria capable of sexual replication (Davis et al. 1973).

While Olson's (1979) work dealt mainly with the volatilization of mercury, and the degradation of organomercurials, including methylmercury, it did not examine the possible role of the mer gene in the methylation of the mercuric cation. In 1980 Pan-Hou and his co-workers published a paper dealing with the plasmid controlled biotrasnsformation of mercury by Clostridium cochlearium, T-2. They designated the parent, or unmodified strain at T-2P, and showed that although the total amount of mercury, both methylmercury and inorganic mercury, in the growth medium remained constant, the amount of methylmercury decreased to almost zero over a four hour incubation period. However, the amount of inorganic mercury in the medium increased to almost the same amount as the total mercury suggesting the transformation of methylmercury to the inorganic form. When they cured the parent strain of its single plasmid, designating it as T-2C, they found it had lost the ability to decompose methylmercury, even though it was capable of growth in the same medium as T-2P. To further prove that the ability to decompose methylmercury was plasmid mediated, they transferred a plasmid from the parent strain to the cured strain, creating T-2C* which was then capable of decomposing methylmercury. Their investigation also showed that whereas the parent strain could not produce methylmercury, the cured, or T-2C strain would produce it. From their results it may be concluded that the ability of a single strain to

methylate and demethylate mercury is dependent upon whether or not a plasmid containing the mer gene encoding for the decomposition of methylmercury is present. It would also seem likely that a single organism cannot methylate and demethylate mercury concommittantly.

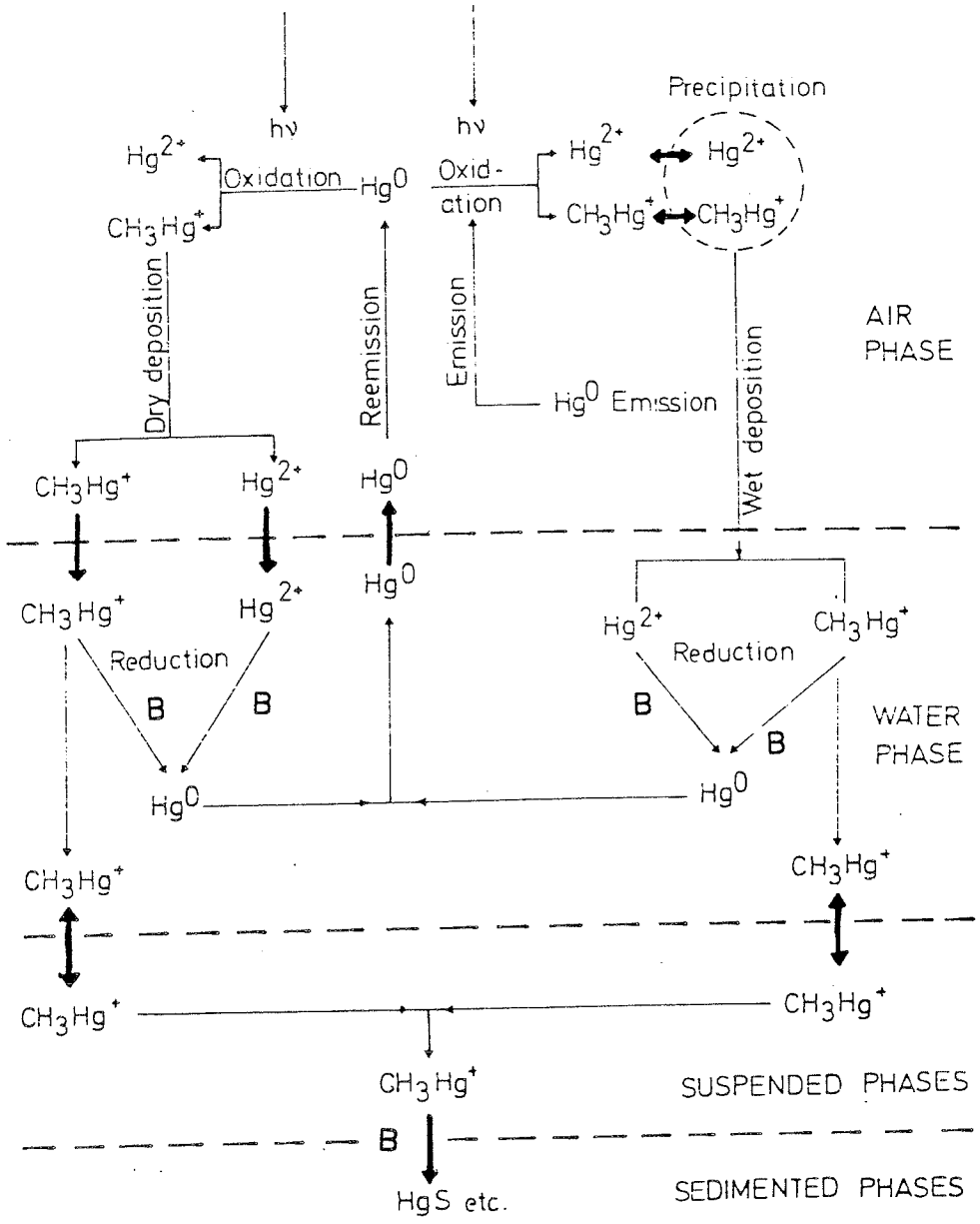
Effect of Mercury on Biota

Most "mercury-containing" substances introduced into the environment can be directly or indirectly transformed into methylmercury compounds (Figure 3) which are more toxic than other forms of organomercurials. Methylmercury is more persistent than other forms of mercury, because it has a lipophylic character which causes an increased mobility into organisms in comparison to inorganic mercury (Lexmond et al. 1976). The toxicity of mercury is caused, in part, by its interactions with S-S and S-H bonds in enzymes. This interaction induces a change in the conformation of proteins, resulting in a loss of their enzymatic activity, which in turn causes a serious distortion of cell metabolism (Lexmond et al. 1976).

Mercury is transferred through the ecosystem by accumulating in, or adsorbing onto, organisms of low trophic levels, such as phytoplankton (Windom and Kendall 1979). Microorganisms keep mercury in the water column by methylating it. This soluble form of mercury may adsorb onto the phytoplankton which are then taken up by zooplankton, and is subsequently transferred to other higher organisms. The efficiency of transfer of mercury through the food chain increases as the trophic level of the organisms increase, indicating increased retention of mercury in higher organisms (Windom and Kendall 1979).

This vertical transfer through the food chain has always been assumed to be the only mechanism of transfer. However, it has been recently demonstrated that the microflora in the intestines of fish can methylate inorganic mercury (Rudd et al. 1980). Thus, although mercury may be taken into the organisms in a less toxic form, it could be transformed by the microorganisms present in the gut, into the more toxic methylmercury.

Figure 3. The physico-chemical mercury cycle. Broad arrows indicate transitions (Brosset 1981).



B—BIOLOGICALLY MEDIATED TRANSFORMATIONS

A Brief History of Point Source Mercury Pollution

Japan

The effect of mercury discharge from anthropogenic sources into the environment received world-wide attention with the occurrence of severe health problems at Minimata Bay, Japan, in the late 1950's and early 1960's. People living around Minimata Bay whose diet consisted mainly of shellfish caught in the Bay, began to suffer from neurological disturbances which caused death or permanent disabilities. The source of these health problems was eventually traced to mercury which was contained in the waste being dumped into Minimata Bay by a factory using mercuric chloride in the production of vinyl chloride (D'Itri 1972). Japanese researchers found that during the catalytic conversion of acetylene into vinyl chloride, some of the mercuric chloride was converted to methylmercury, this was released into the Bay in the plant's effluent. This methylmercury was then accumulated by the fish and shellfish (D'Itri 1971; Tsubaki and Irukayama 1977; Nriagu 1979), which was consumed by the inhabitants of this area.

The people of Niigata area also exhibited symptoms of mercury poisoning. The main source of water was the Nagano River which was used as a dumping site for the effluent of a chemical factory (D'Itri 1972; Goldwater 1973; Tsubaki and Irkayama 1977).

In 1970 a third incident of mercury pollution was recorded in Japan. The sediments of the Jintsu and Kumano Rivers were found to contain mercury levels approximately 50% higher than those found at Niigata. The levels were attributed to the wastewater effluent of a pharmaceutical company (D'Itri 1972).

Sweden

The occurrence of mercury contamination in the Swedish environment was first noted when scientists observed a decline in the seed-eating and rodent-eating bird populations (Katz 1972). The decline in the bird population was found to be a result of birds eating the seed, or animals which had been feeding on the seed which was treated with alkylmercury compounds as dressing agents (D'Itri 1972).

In the 1960's, it was noted (Jernelov et al. 1975) that fish caught in industrialized areas contained higher concentrations of mercury than did those caught in agricultural areas. Johnels and Westermark (in Jernelov et al. 1975), also found evidence that the mercury concentration in fish eating birds had increased since the advent of the Industrial Revolution in Sweden. This mercury contamination problem was traced to the use of phenylmercuric acetate as a slimicide in the pulp and paper industry as well as the discharge of inorganic mercury into waterways by the chlor-alkali industry (D'Itri 1972). The inorganic compounds were originally believed to be biologically inert. However, Swedish investigators (Westoo 1966) showed that the mercury in fish was predominantly methylmercury even though the local releases of mercury were in the inorganic form or as phenylmercury. This finding was explained when Jensen and Jernelov (1967) found that microorganisms in lake sediments were able to synthesize methylmercury from inorganic mercury. A variety of compounds can result from microbial transformations of mercury containing compounds, but the methylated forms of mercury are highly toxic and significantly more biologically mobile than other forms of mercury (Lexmond et al. 1976).

Iraq

In Iraq there have been three major incidents of mercury poisoning. The first, in 1956, was discovered only in retrospect. In both 1956 and 1960, the Iraqi Ministry of Agriculture supplied farmers with seed grain treated with a fungicide which contained an ethylmercuric compound. In places where the food supply was inadequate, farmers supplemented their food with flour ground from treated seed grain. The diagnosis of mercury poisoning was complicated by the time delay between the distribution of the treated seed grain and the onset of the poisoning symptoms. This was due to the fact that farmers feared prosecution for using the seed grain as food (D'Itri and D'Itri 1977).

The largest epidemic of mercury poisoning occurred in Iraq in late 1971 and early 1972, after many countries had already become aware of the toxicity of mercury. It began in 1970, when an American company was allowed to dispose of stocks of treated seed either by selling the seed in the state it originally came from, or by exporting it to other countries. Almost 100,000 metric tons of grain was shipped to Iraq. The Iraqi government issued warnings "not to eat the grain" in English, and Spanish symbols for Poison were stamped onto each bag. People either could not read the warnings or ignored them because they lacked food (D'Itri and D'Itri 1977). The epidemic was reported by Bakir and his associates in 1973 when it was discovered a large number of patients were being admitted with the symptoms of mercury poisoning. Before a news blackout was put into effect, it was estimated that up to 60,000 peasants may have eaten the treated grain. The Iraqi government officially recognized 6,530 people were hospitalized and 459 people had

died from the effects of mercury poisoning (D'Itri and D'Itri 1977; Takizawa 1979).

United States

Awareness and concern over the possibility of mercury contamination in the U.S. occurred when the Alberta government instituted a ban on the hunting and eating of certain game birds because of an increase in the mercury concentrations in their flesh. As the Canadian government became aware of other sources of mercury pollution, the American government expressed concern about the effect on its inhabitants, especially where the two countries bordered on the same water sources (D'Itri 1972). In testing various sites around the country, it was found that large amounts of mercury were being discharged in the effluent of chemical companies (D'Itri 1972). Testing was carried out at a number of sites and eventually regulations were enforced controlling the amount of mercury which could be releaed into the environment.

Canada

A Canadian mercury pollution problem was first recognized in 1969, when Fimreite and his associates (1970) discovered that wildlife in southern Alberta and Saskatchewan were eating seed grain treated with mercury-containing fungicides. They found that the range of mercury content in the livers of organisms tested was 0.01-6 ppm, with an average in the Alberta wildlife of 1 ppm. Predatory birds were found to have mercury levels twice as high as the seed-eating birds and mammals, indicating a cumulative effect as the food chain level progressed.

About the same time, a study of the fish of the Saskatchewan River system, including Lake Winnipeg, Cedar Lake, the Saskatchewan and theRed Rivers (Wobeser et al. 1970; Bligh 1971), found mercury concentrations greater than 1 ppm in fish tissue from catch in several locations. These fish were deemed unsuitable for human consumption.

After the hazard of using mercury in agricultureal systems was recognized, it was decided to examine the effects of mercury in the effluent of pulp and paper mills, and in the effluent of chlor-alkali plants, both heavy users of mercury in their industrial processes. Fimreite et al. (1971) studied Lake St. Clair and the associated river system and found mercury levels greater than 0.5 ppm (the Canadian legal limit) in practically all samples of freshwater fish. In some of the fish the levels were up to 10.5 ppm. As a result of their studies fishing was banned in a number of areas.

An indepth study of mercury pollution was begun in 1973 on the English-Wabigoon River system (Armstrong and Hamilton 1973). This particular system was one of the most heavily polluted in Canada. Studies carried out by both the Federal and Provincial governments, found that the source of the mercury pollution was a chlor-alkali plant located at Dryden, Ontario which had been in operation since 1962, and had been regulating its mercury ouput since 1970. Armstrong and Hamilton (1973) surveyed the invertebrate organisms and found that the mercury concentration in the aquatic animals was related to their food selection. In most cases, omnivorous organisms and those feeding on detritus, or bottom dwelling invertebrates, had much high mercury levels than either herbivorous organisms or those feeding on zooplankton. Work since the initial study has been undertaken in order to understand the

processes involved in mercury dynamics and to find possible ways of ameliorating the mercury problem in Clay Lake and the associated waterways (Rudd et al. 1980; Parks 1976).

A mercury problem has recently been found in Southern Indian Lake, Manitoba. The mercury problem in this case is due to raising the impoundment water levels necessary for development of hydroelectric potential (Bodaly and Hecky 1979). They have found increased levels of mercury in northern pike and in commercial catches since impoundment occurred.

Recent Problems of Acidification and Mercury Pollution

Recently attention has been focused on the apparent link between low pH in lakes and an increase in the mercury content of the fish in these lakes (NRC 1981). The long range transport of mercury occurs when low grade fossil fuels such as coal, are burned releasing volatilized mercury (NRC 1981). This transport permits the deposition of mercury long distances from its source and, because of the nature of mercury, and the processes that may occur after deposition, it may be reemitted to the atmosphere (NRC 1981).

The purpose of this study was to find out what effect, it any, acidification of lake sediments had on the production of methylmercury. The rate of microbial production of methylmercury under different incubation conditions using sediment collected from an acidified and an unacidified lake was also examined.

SECTION I

INTRODUCTION

Introduction

Studies have shown that in lakes which have been affected by acidic precipitation, the fish tend to have increased levels of mercury (Jernelov et al. 1976; Brouzes et al. 1977). Lakes susceptible to acid precipitation are those with low chemical buffering capacity. The source of acid in precipitation is believed to be industrial processes and fuel combustion, especially coal (NRC 1981), the combustion of which produces oxides of sulphur and nitrogen. These compounds are then deposited as acidic precipitation and dryfall.

The combustion of coal also results in the atmospheric emission of mercury (Joensuu 1971). It has also been demonstrated that in cases where the quantity of mercury emitted from a point source is known, only a small proportion of that mercury is deposited locally (Jernelov and Wallin 1973; Jernelov et al. 1975). The combination of acid and increased mercury loading may be one explanation for an increase in the mercury levels of fish in acid lakes (Jernelov et al. 1976; Brouzes et al. 1977).

Another possibility for an increase in fish mercury levels was an increase in the mobilization of mercury from sediments. It was previously believed that an increase in the hydrogen ion concentration would cause the release of mercury(II) from particles to which it was bound (e.g. NRC 1981). It was also plausible that the microbial mercury methylating and demethylating activity was affected so that there was an increase in the methylmercury pool in the lake. A final explanation for increased mercury levels in fish is a change of the physiology of the fish, such as an increase in the mucous layer of the fish. The purpose of the experiments undertaken in this study was to determine the effect

of acidification on the ability of microorganisms to methylate mercury (II) in sediment and to determine the mechanism of these effects. To accomplish this, sediment was collected from two lakes in the Experimental Lakes Area (Brunskill and Schindler 1971). Studies were done to determine the effect of acidification on porewater mercury and on the rate of microbial methylating activity.

METHODS AND MATERIALS

Methods and Materials

Study Sites

The studies were carried out at the Experimental Lakes Area, on the Canadian Shield which lends itself to studies of lake acidification because these lakes are located in granitic rock basins of limited buffering capacity. The pH of the precipitation of this area has not decreased since the area was chosen as a study site, and as such, would not affect the results of experiments.

Recent studies at ELA has been focused on the problem of lake acidification as a result of acidic precipitation. To that end, Lake 223 (L223) has been receiving additions of sulphuric acid since 1976. Lake 223 is a typical small lake of the Precambrian Shield, with chemistry similar to lakes of the same size, in the same area (Schindler et al. 1980b). The pH of the lake has decreased from 6.79, the average value for 1976, to roughly 5.10 in 1982. The pH of the sediment from 1980 to 1982 was close to 6.5, remaining relatively constant. Activity of sulphate-reducing bacteria enhanced the buffering capacity of the lake, increasing the amount of time required to acidify the lake (Schindler et al. 1980b).

Lake 239 which has not been acidified was used as a control lake. Its chemistry and primary production compares well with that of L223 prior to acidification (Schindler, pers. comm.). The littoral sediment pH was close to 6.2, and the recent history of the lake has been well documented (Schindler et al. 1980c).

Sample Collection

Littoral sediment was collected at 4 m water depth from Lake 239, and from a 6 m depth from Lake 223. Littoral sediments were chosen for

this study because they are exposed to water of reduced pH sooner than hypolimnetic sediments (Kelly et al, submitted) in acidified lakes.

Samples were collected using a modified Eckman grab (Burton and Flannagan 1973). Approximately the top 2 cm of sediment were collected by vacuuming the surface of the sediment into a 500 ml PVC bottle using a hand vacuum pump. The samples were capped to exclude air and transported to the lab for further manipulations. One liter of sediment was collected for each experiment.

Temperature profiles were taken from surface to sediment at 1 m intervals using a thermistor (Flett Research Ltd.).

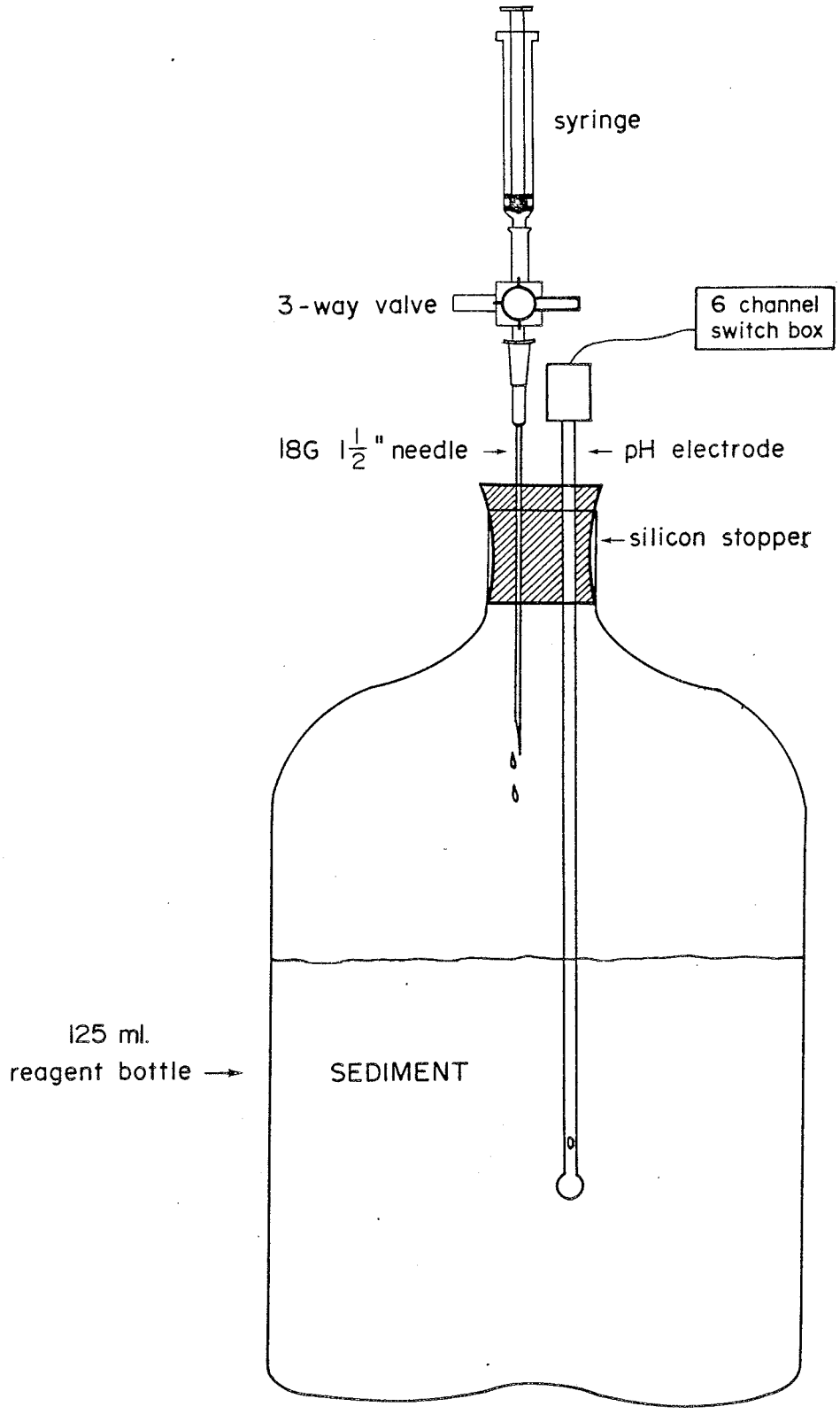
Sample Manipulation

pH Adjustment

In the field lab, 100 ml of sediment was dispensed, under deoxygenated nitrogen (Hungate 1969), into 5-125 ml reagent bottles fitted with silicon stoppers (Figure 4). A micro pH probe (Fisher Scientific) and an 18 G 1.5 inch needle attached to a 3-way valve (American Hospital Supplies) were inserted through the silicon stopper. The pH probes were attached to a pH meter (Fisher Model 630) via a 6 channel switchbox, which enabled us to monitor the pH of each of the sediment samples continuously. The probes were standardized in buffers of pH 4 and 7 (Fisher Scientific) before each experiment. The pH in four of the bottles was adjusted from 4.5-7.5 by addition of HCl and NaOH. The fifth bottle ws not adjusted, and remained at the natural pH of approximately 6.3.

After dispensing the sediment into the 125 ml reagent bottles, the pH was monitored, and readjusted over approximately a 2 hour period until it had stabilized at the predetermined value.

Figure 4. Apparatus used to adjust and monitor pH of sediment samples.


syringe
3-way valve
6 channel switch box
18G 1½" needle
pH electrode
silicon stopper
125 ml. reagent bottle
SEDIMENT

Sample Incubation and Extraction

After this period, 2-10 ml aliquots were drawn into each of two wet 50 cc glass syringes (American Hospital Supplies) which had been flushed with nitrogen. Another 5 ml of nitrogen was added to each syringe to facilitate proper mixing. Two extra samples of the same size and treated with nitrogen were killed with 1 ml of 4N HCl and used as blank controls. Each sample/syringe received an addition of radioactive mercury (II) as $^{203}HgCl_2$ (New England Nuclear) to an approximate dose of 2 µCi/2 µg Hg/g dry weight sediment. The syringes were sealed with 18G 1.5 inch needles inserted in rubber stoppers, they were shaken vigourously for 1 minutes.

Samples were incubated for 12 or 48 hours at <u>in situ</u> temperature, and then killed with 1 ml of 4N HCl (Furutani and Rudd 1980). The contents of the syringe was transferred to a 250 ml glass reagent bottle. The $^{203}Hg\text{-}CH_3Hg^+$ produced during the incubation period was extracted using the procedure of Furutani and Rudd (1980), and collected in the benzene phase of the final extraction. Aliquots of the benzene phase were counted to 2% error in PCS fluor (Amersham) using a Beckman 7000 Liquid Scintillation Counter (LSC). The extraction efficiency and the replicability of this method has been tested elsewhere (Furutani and Rudd 1980) where it was shown that 95-100% of the methylmercury was recovered. The mean percent coefficient of variation for these samples was 9.9 ± 10.2% for 14 replicates.

The calculation for determining the rate of methylmercury formation as dpm/g dry weight sediment/hour was as follows (samples were normalized to 2 µCi/2 µg Hg/g sediment):

$$R = (s-b)d(2\ \mu Ci/a)(2\ \mu g\ Hg/m)/wt \quad \text{(equation 1)}$$

where:

R = rate of mercury methylation as dpm/g sediment/hour

s = sample activity as dpm

b = activity of killed blank control as dpm

d = dilution factor from extraction procedure

a = actual activity of the sediment as µCi/g sediment

m - actual µg Hg/g sediment

w = sample dry weight in grams

t = incubation period in hours

Chemical analyses of the sediment were done for carbon, nitrogen, phosphorus, and sulphur (Stainton et al. 1977), and total mercury (A. Lutz). X-ray diffraction was performed by M. Capel.

Porewater Experiments

The effect of pH of 203-mercury concentration in the sediment porewater was also determined. This was of interest because it is necessary for the mercury to be dissolved before it can be biologically methylated.

Radio-labelled mercuric chloride was added to pH-adjusted sediment in the same concentration as was used for the measurement of the rate of mercury methylation. The samples were shaken for one minute, then allowed to equilibrate for one hour prior to centrifugation. The samples were centrifuged for 45 mintues at 3000 r.p.m. The supernatant volume was measured, filtered through a 0.45 µ membrane filter (Millipore) and a 500 µl subsample of the filtrate was counted in a liquid scintillation counter (Beckman). The mercury which passed through the 0.45 µ filter was operationally defined as dissolved porewater mercury.

The amount of dissolved 203-Hg in the sediment porewater was calculated as follows:

Porewater radioactivity = (p-fb)(Vt/Vp)/Vc (equation 2)

where:

porewater radio-activity is as dpm/ml

p = activity of the filtered porewater sample as dpm

fb = background activity of the fluor as dpm

Vt = total volume of the sediment sample used as ml

Vp = volume of the porewater as ml

Vc = volume of filtered porewater counted in the LSC as ml

Acid Volatile Sulphide Experiments

The porewater radio-activity was also measured in sediments in which the acid volatile sulphur (AVS) was removed. This was done to determine if sulphur in the sediments would have an effect on the availability of 203-mercury, and if so, whether or not changes in the sediment pH would alter this effect. The AVS in sediments is defined as those sulphide-containing compounds, such as some forms of FeS; which are volatilized as hydrogen sulphide at very low pH. Other forms of bound sulphide, such as pyrite are more stable at the low pH levels (Howarth and Teal 1979). The AVS was removed and measure by combining methods developed by Jorgenson (1978) and Howarth and Teal (1979) (C. Kelly, pers. comm.). Sediment was put into reaction vessels, enough acid was added to reduce the pH to 1 in one case, and concentrated HCl was added to the vessels in another case. Concentrated HCl is normally used to remove AVS from sediments in this procedure (Kelly, pers. comm.) The AVS was removed by bubbling the sediments with deoxygenated

nitrogen, and trapped in zinc hydroxide prepared by mixing 18 ml of 2.6% zinc acetate with 4.5 ml of 6% NaOH (Howarth and Teal 1979). After the sediment had been bubbled for 2 hours the AVS was measured and the sediment was pooled according to the conditions of AVS removal, i.e. sediment pH reduced to pH 2 or treatement with 6N HCl.

The sulphide in the zinc acetate traps now in the form of zinc sulphide was solubilized by sulphuric acid addition. Then 0.003 M iodine was added to each trap in 5 ml aliquots until the solution was straw coloured. This solution was poured into 250 ml Erlenmeyer flasks containing 50 ml of distilled water. Starch solution was added, and the entire volume was titrated with thiosulphate of a known concentration.

$$AVS = [(E-A)/2]\ N_{S_2O_3^=} \quad \text{(equation 3)}$$

where:

AVS = acid volatile sulphide as µmoles

E = amount of standard thiosulphite required to titrate a trap from a water blank as ml

A = actual amount of thiosulphate used in the titration as ml

$N_{S_2O_3^=}$ = normality of the thiosulphate as µeq/ml

Following the removal of AVS the pH of the sediment lowered to pH 2 was raised to its original lake pH (approx. 6.3). The sediment treated with concentrated HCl was washed with lake water and centrifuged at 8000 r.p.m. for 20 minutes, three times to remove the acid. The sediment was resuspended in lake water to bring it back to its original volume and the pH was raised to its original value.

To determine the effect AVS had on porewater mercury concentration at natural and adjusted pH's, treated and unaltered sediment was

distributed in five lots: (1) unadjusted sediment; (2) sediment with AVS removed at pH 2; (3) sediment with AVS removed at pH 2, FeS added (Baker Reagent Grade); (4) sediment AVS removed with concentrated HCl; and (5) sediment AVS removed with concentrated HCl, FeS added. The FeS (Baker) was added to the bottles in the same concentration in which it was removed from the sediment (Table 1). Each of these five lots of sediment were redistributed into 5-125 ml reagent bottles and the pH was adjusted as previously described. The porewater mercury(II) measurement was carried out as before using radio-labelled mercuric chloride. The experiment was done using sediment from L239 and L223. X-ray crystalography was done on the reagent FeS to ensure that it had not all been converted to pyrite.

RESULTS

Results

The results of chemical analysis of the sediment showed that the sediments layer used from both lakes were comparable. The particle size was small enough to pass through a line adapter of a 50 cc glass syringe. The organic carbon and total nitrogen was determined using 5 replicates from each lake. The sediment from L239 had an organic carbon content of 51.6 mg organic C/g dry sediment, and the total nitrogen was 4 mgN/g dry sediment. The sediment collected from L223 had 50.2 mg organic C/g dry sediment and 4.9 mgN/dry sediment.

The average amount of total natural mercury in the L239 sediment was 0.37 + 0.07 µg Hg/g dry sediment (n = 4), in the L223 sediment the average total mercury was 0.30 + 0.17 µg/g dry sediment (n = 4).

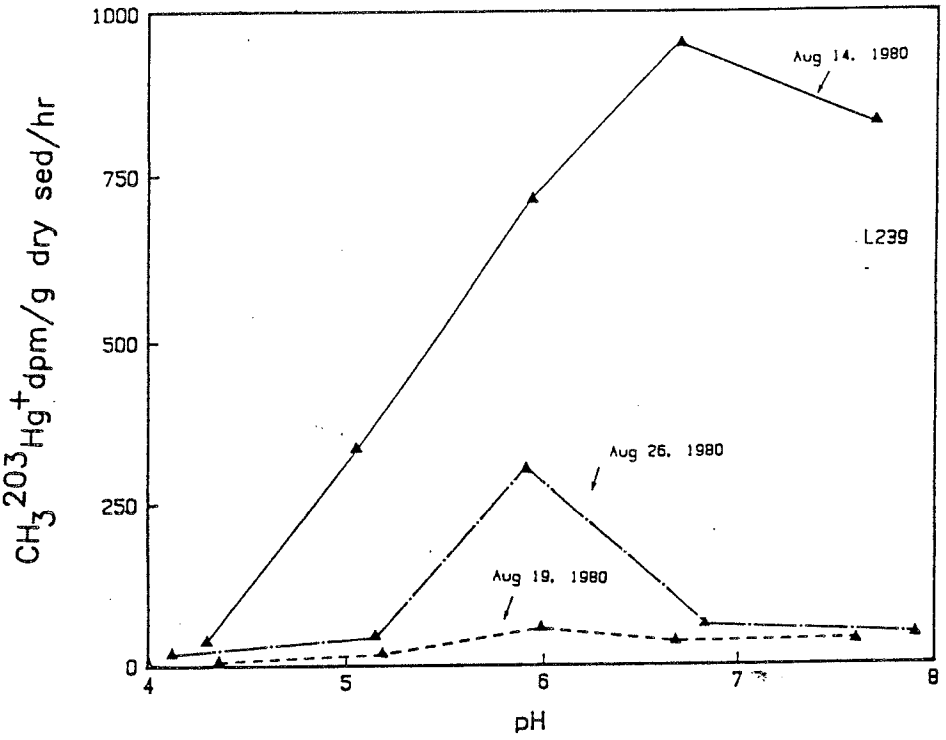
The amount of acid volatile sulphur in the sediment of L223 was higher by an order of magnitude from that found in L239, although the total sulphur in the sediments was similar (Table 1).

The preliminary studies done in 1980 (Figure 5), using pH adjusted sediment from L239 and L223, indicated rates of biological mercury methylation decreased as pH decreased. The rate of methylmercury formation did not continuously increase as pH increased, instead the rate decreased at pH's greater than ambient pH (6.2 and 6.5 respectively). The overall rate of methylation decreased dramatically between the sampling dates of August 14 and August 19, but increased slightly by August 26. These experiments were repeated on several occasions during 1981 and 1982 (Figures 6 and 7) with similar results, although the decrease in mercury methylation rates was not always observed. In 1982, sediment collected from L239 on June 4th and L223 on June 15th had low methylation rates although incubated for 48 hours.

Table 1. AVS and total sulphur in L223 and L239 sediment.

Lake	Treatment	Acid Volatile Sulphide (μmol/g dry sed.)	Total Sulphur (μmol/g dry sed.)
L239	sediment pH 2	0.13	43.67
	6N HCl	0.38	62.38
L223	sediment pH 2	1.74	68.62
	6N HCl	2.84	37.43

Figure 5. Rates of 203-methylmercury formation in L239 and L223 sediment under different pH conditions during 1980.


1000
750
500
250
0
4
5
6
7
8
pH
$CH_3{}^{203}Hg^+$dpm/g dry sed/hr
Aug 14, 1980
Aug 26, 1980
Aug 19, 1980
L239

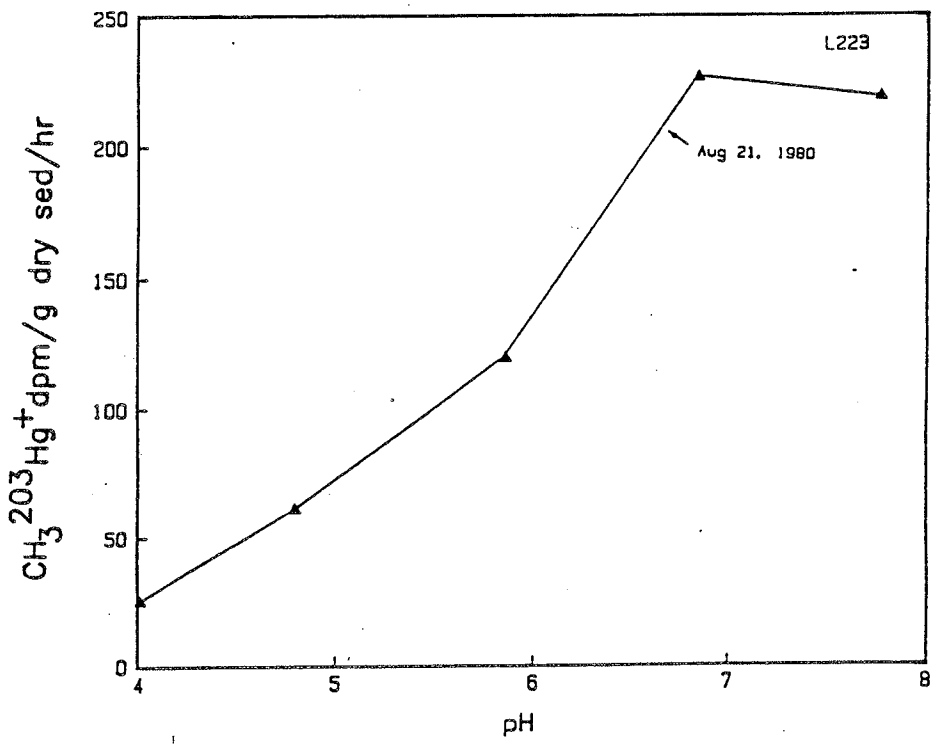
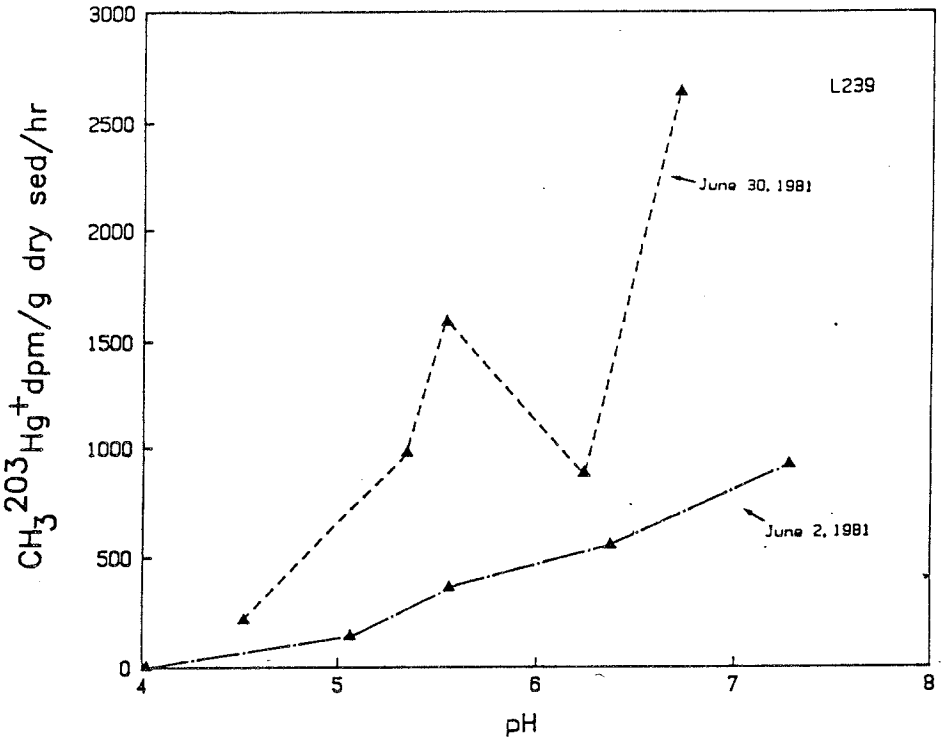

250
200
150
100
50
0
4
5
6
7
8
pH
$CH_3{}^{203}Hg^+$dpm/g dry sed/hr
L223
Aug 21, 1980

Figure 6. Rates of 203-methylmercury formation in L239 and L223 sediment under different pH conditions during 1981.


L239
June 30, 1981
June 2, 1981
$CH_3{}^{203}Hg^+$ dpm/g dry sed/hr
pH
0
500
1000
1500
2000
2500
3000
4
5
6
7
8

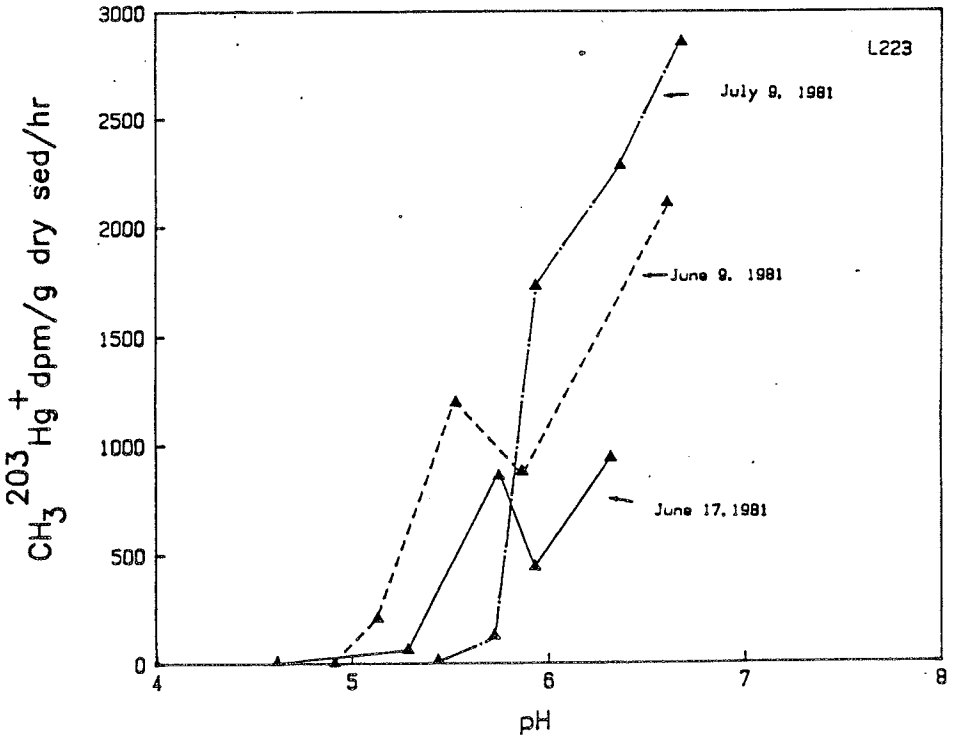
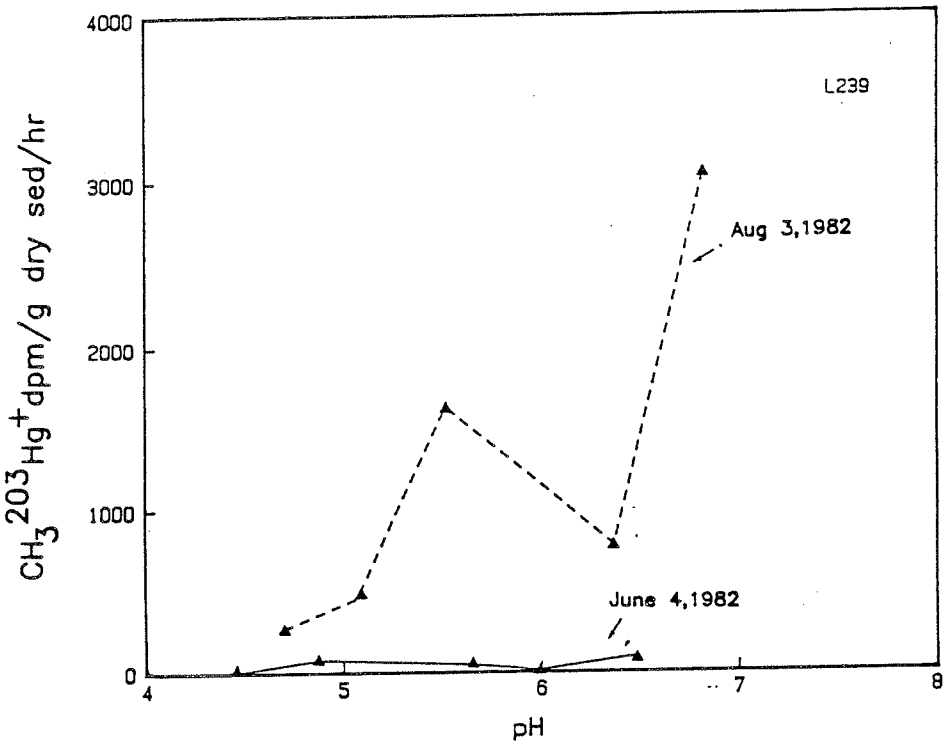
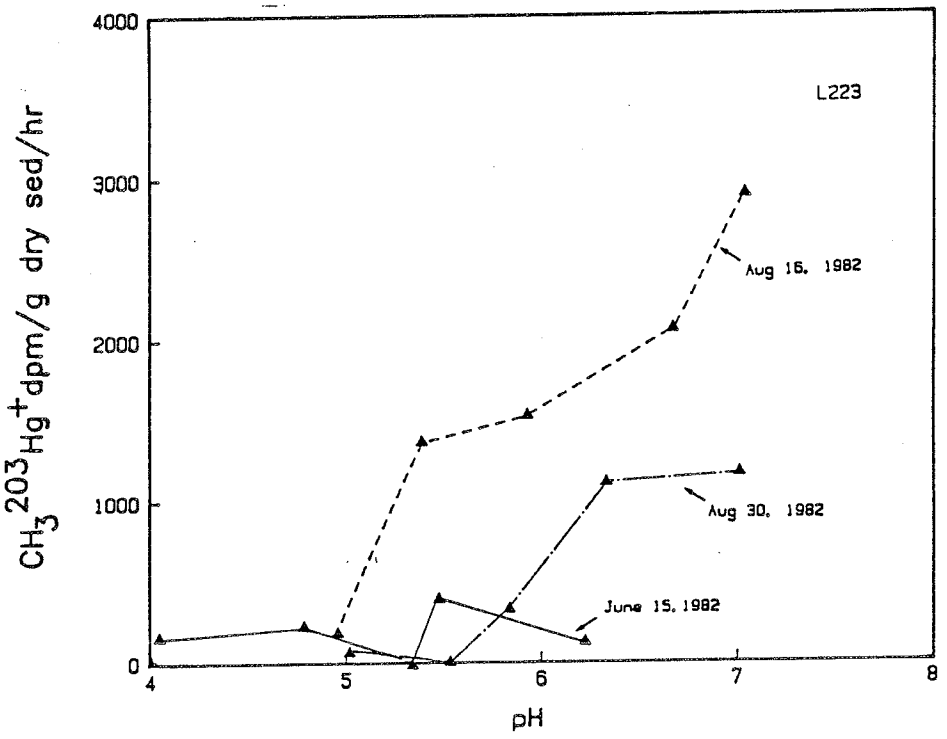

L223
July 9, 1981
June 9, 1981
June 17, 1981
$CH_3{}^{203}Hg^+$ dpm/g dry sed/hr
pH
0
500
1000
1500
2000
2500
3000
4
5
6
7
8

Figure 7. Rates of 203-methylmercury formation in L239 and L223 sediment under different pH conditions during 1982.


L239
Aug 3,1982
June 4,1982
$CH_3{}^{203}Hg^+$dpm/g dry sed/hr
pH
0
1000
2000
3000
4000
4
5
6
7
8

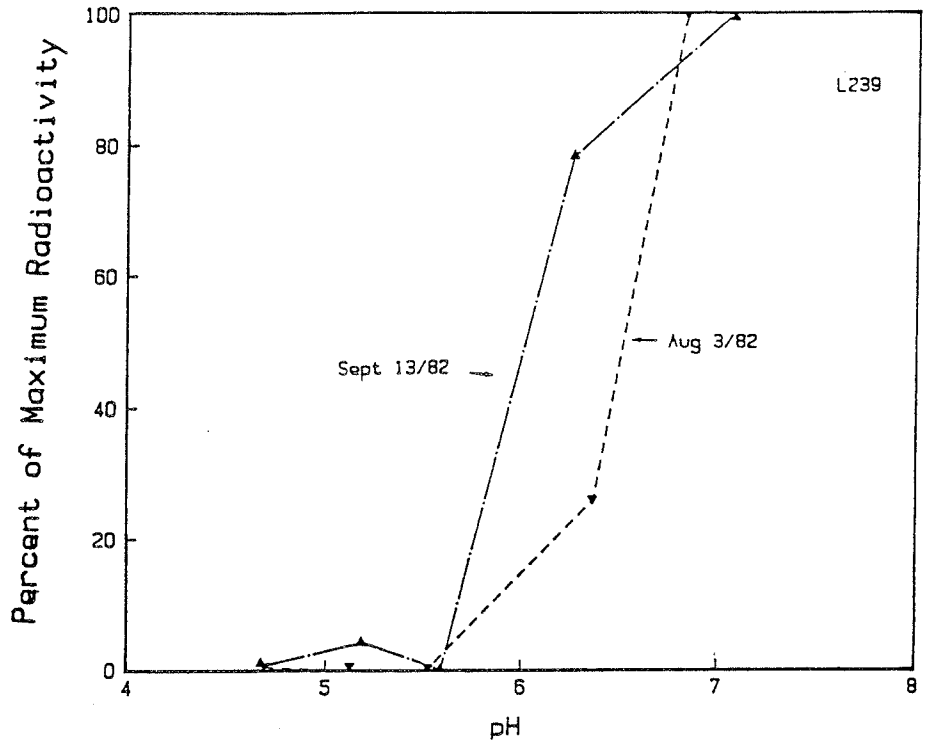

L223
Aug 16, 1982
Aug 30, 1982
June 15, 1982
$CH_3{}^{203}Hg^+$dpm/g dry sed/hr
pH
0
1000
2000
3000
4000
4
5
6
7
8

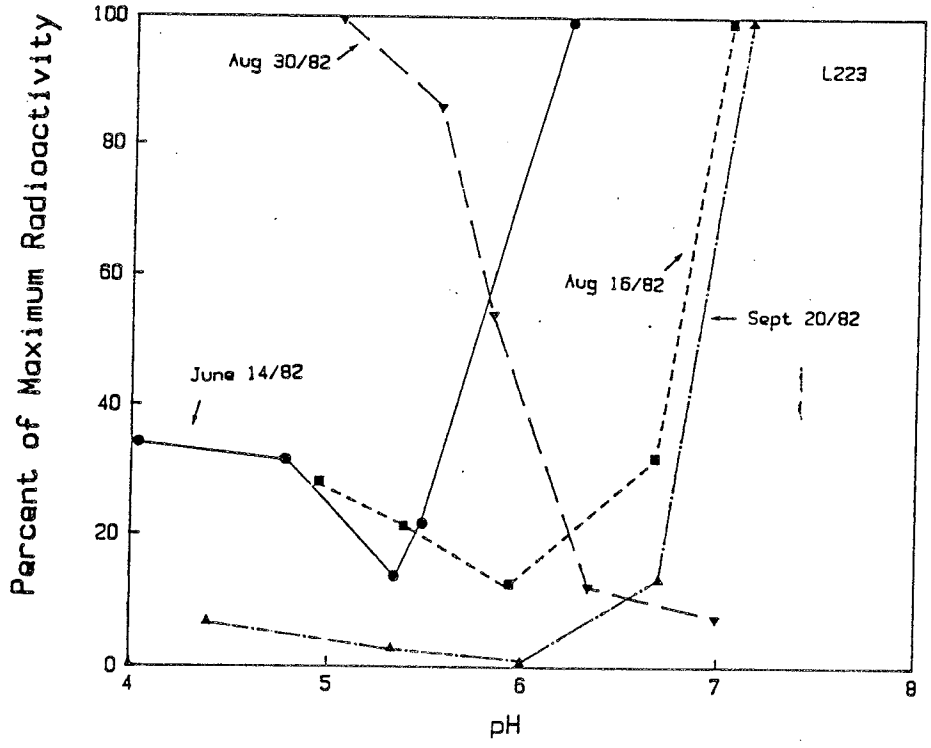
This low production was believed to be due to low microbial activity of sediments during the early stages of the ice-free season.

It was then decided to study the availability of inorganic mercury in sediment porewater, to determine if the methylation rate was affected by the amount of dissolved mercury or by the microbial activity of the sediment. The results (Figure 8) show that in both L239 and L223 there was less 203-mercury in the porewater at low pH. The only time this did not occur was on August 30, 1982 using sediment collected from L223.

The next series of experiments were designed to examine the possibility that bound sulphur released at reduced pH in the sediment might reduce the availability of 203-mercury in the porewater. Sediment collected from L239 on September 13, 19182 was apportioned into subsamples and subjected to 5 different manipulations. The first manipulation determined the solubility of inorganic mercury (Hg^{++}) in porewater under conditions normally used to determine the effect of pH on mercury methylation. It was found to be the same as in previous experiments (Figure 8). The curve indicates less porewater mercury at low pH. In the next two subsamples, the AVS was removed from the sediment by acidification of the sediment to pH 2 and then stripping of the AVS from it. In the first of these two, the amount of soluble mercury increased at pH above and below ambient. In the second of these two, when FeS was added as an artificial substitute to replace the previously removed sulphide at the concentration at which the AVS was removed, the porewater mercury was less than when there was no FeS was added. In addition to this, the total amount of porewater mercury decreased in sediment to which FeS had been added (Table 2). The final two manipulations were made using sediment in which the AVS had been

Figure 8. Porewater radio-activity of 203-mercury pH adjusted sediment from L239 and L223.


Percent of Maximum Radioactivity
100
80
60
40
20
0
4
5
6
7
8
pH
L239
Sept 13/82
Aug 3/82


Percent of Maximum Radioactivity
100
80
60
40
20
0
4
5
6
7
8
pH
L223
Aug 30/82
Aug 16/82
Sept 20/82
June 14/82

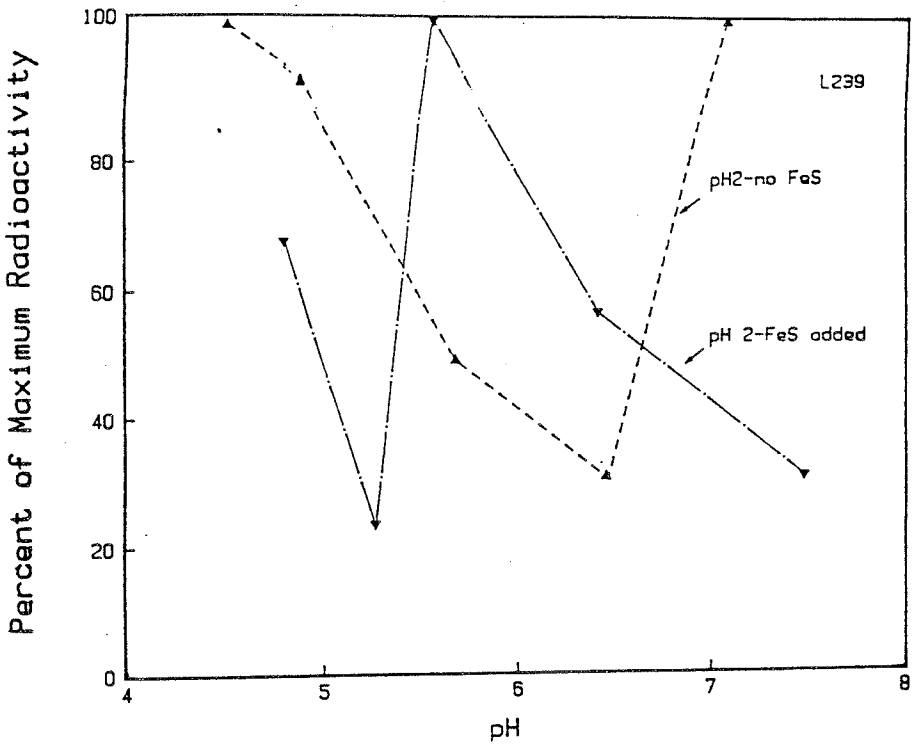
removed from the sediment using 6N HCl. Again, one trial was made using sediment to which FeS had been added in the same concentration as that of the removed AVS. These results (Figure 9) indicate that the porewater solubility of mercury was low at low pH in both situations but the absolute concentration of labelled mercury was two orders of magnitude higher than the natural system (Figure 8, Table 2).

The experiment was repeated using sediment collected from L223 (Figures 8-10). The results are similar to those found using sediment from L239. The sediment in which the AVS had not been removed had reduced porewater mercury at low pH. In the sediment with AVS removed at pH 2, the porewater of 203-mercury was high at pH 4.7 after which it was low and relatively constant. When FeS was added back to the sediment the 203-mercury concentration at low pH decreased, the concentration being greatest at pH 6.5. After the AVS had been removed with concentrated HCl, the results showed the same trend as found in the similar situation using L239 sediment. Again the absolute concentration of porewater 203-mercury was two orders of magnitude higher than that found in sediment which had not been treated with 6N HCl (Figure 8).

Table 2. Maximum values of porewater ^{203}Hg activity.

Lake	Treatment	Date	pH	Maximum Activity (dpm/ml)	Percent of Total 203-Hg Added
L239		Aug 3/82	6.83	7,362	2.4
		Sept 13/82	7.07	5,563	1.6
L223		June 14/82	6.22	11,321	6.4
		Aug 26/82	7.04	3,242	2.3
		Aug 30/82	5.02	890	0.6
		Sept 20/82	7.14	56,176	17.5
L239	pH 2; no FeS	Sept 13/82	7.07	896	0.3
	pH 2; add FeS	Sept 13/82	5.55	797	0.2
	6N HCl; no FeS	Sept 13/82	6.59	317,704	90.2
	6N HCl; add FeS	Sept 13/82	6.59	222,544	63.2
L223	pH 2; no FeS	Sept 20/82	4.69	9,431	2.9
	pH 2; add FeS	Sept 20/82	6.52	7,976	2.5
	6N HCl; no FeS	Sept 20/82	6.57	316,711	98.6
	6N HCl; add FeS	Sept 20/82	6.37	171,538	53.4

Figure 9. Porewater radio-activity of 203-mercury in pH adjusted sediment from L239 after the removal of acid volatile sulphide.


Percent of Maximum Radioactivity
100
80
60
40
20
0
4
5
6
7
8
pH
L239
pH2-no FeS
pH 2-FeS added

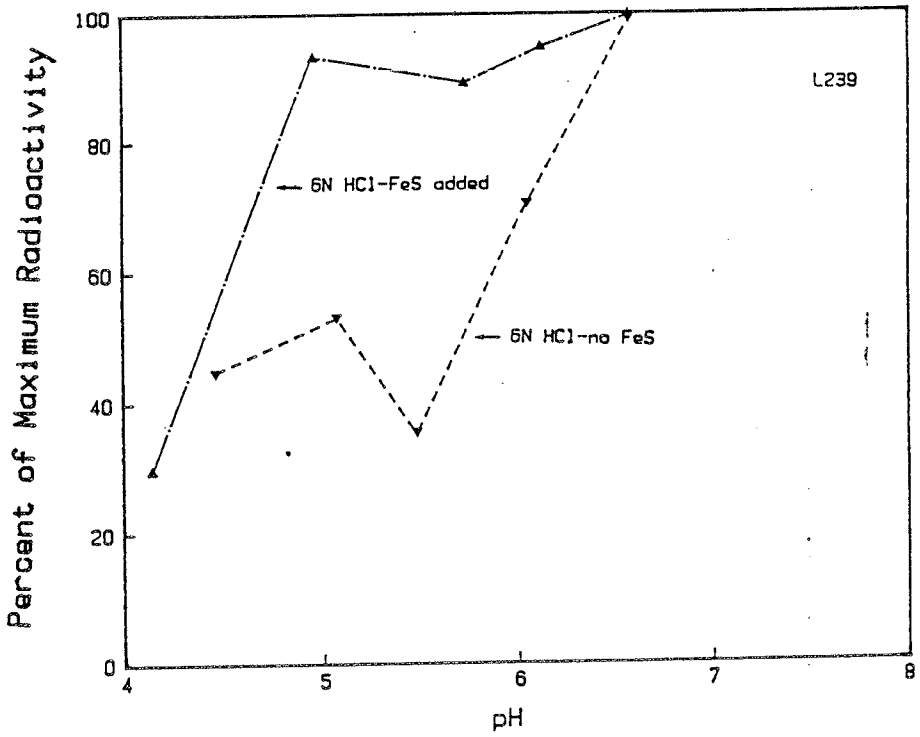
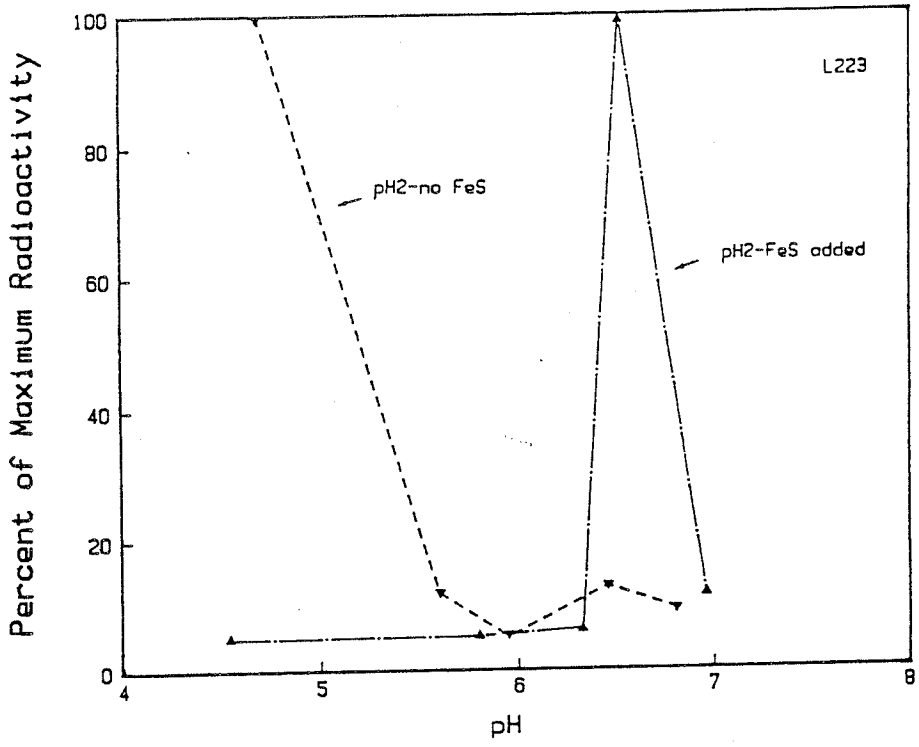
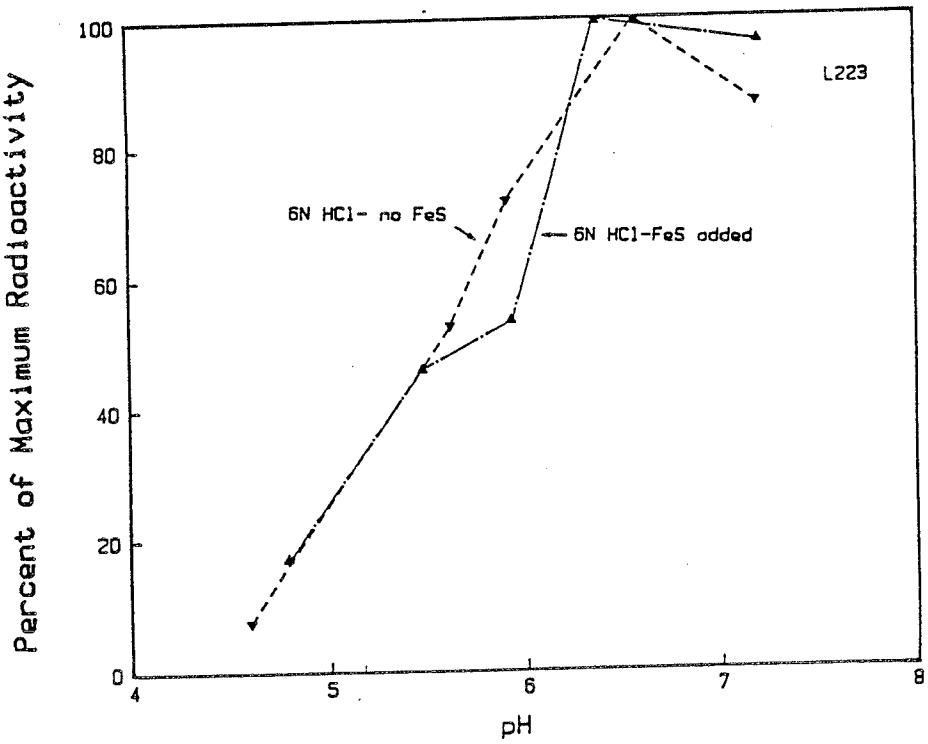

Percent of Maximum Radioactivity
100
80
60
40
20
0
4
5
6
7
8
pH
L239
6N HCl-FeS added
6N HCl-no FeS

Figure 10. Porewater radio-activity of 203-mercury in pH adjusted sediment from L223 after the removal of acid volatile sulphide.


Percent of Maximum Radioactivity
100
80
60
40
20
0
4
5
6
7
8
pH
L223
pH2-no FeS
pH2-FeS added


Percent of Maximum Radioactivity
100
80
60
40
20
0
4
5
6
7
8
pH
L223
6N HCl- no FeS
6N HCl-FeS added

DISCUSSION

Discussion

The results of this study show that in two lakes, one acidified and the other unacidified, under conditions of low pH in the sediment, microorganisms methylate mercury at a lower rate than they would at natural or neutral pH.

This study also suggested that the reason for the reduction in mercury methylation at low pH could have been because of increased association of mercury and sulphur.

Previous studies have also shown that pH affected mercury distribution between particles and the aqueous phase of sediment. Miller and Akagi (1979) found that the methylmercury production in the sediments was not influenced at a pH range of 5-7. They also found the amount of methylmercury in the overlaying water increased as the pH decreased. From this they inferred that it was alterations in the pH which affected the distribution of mercury between the sediment and water, leaving the methylation process relatively unaffected. Our studies would indicate the pH also affected the amount of methylmercury produced in the sediment and released to the overlaying water. This can be seen from the result of the methylation experiments (Figures 5-7) in which the rate of methylmercury formation decreased as the pH decreased. The reason for the effect was apparently due to a decrease in porewater mercury which is the only form of mercury available for methylation (de Simone et al. 1979).

It has been shown by others, that unlike most heavy metals, mercury is not mobilized from the sediment at low pH (Hakanson 1974; Schindler et al. 1980b) with the exception of the results found by Zischke and his co-workers (1983).

Schindler et al. (1980a) found results similar to our lab results under field conditions. Using 10 m diameter enclosures in L224, sealed to the sediment, and acidifying the water with sulphuric acid, they found that the concentration of ^{203}Hg decreased as the pH decreased. Hakanson (1974) showed in lab studies that 97% of the mercury adsorbed and deposited with particles at pH 5, whereas only 32.3% was deposited with materials at pH 9. From this he concluded there is an increased probability for sedimentation of mercury in acid waters.

Zischke and his co-workers (1983), using experimental channels (ambient channel, pH 8; acidified channel, pH 5), found although the maximum amount of mercury added to their channels was 0.1 µg/l, after two months the amount of mercury in the ambient channel was 0.3 µg/l and in the acidified channel the total mercury was 0.6 µg/l. It seems possible that the amount of mercury in their channels is related to factors other than pH, may be due to atmospheric deposition.

The role of reduced sulphur compounds on mercury dynamics in sediment porewater was investigated, that is, an attempt was made to explain the reduction of mercury methylation at reduced pH. The amount of sulphide in the sediment affected the concentration of mercury in the porewater. After the AVS was removed from the sediment at pH 2 (Figures 9-10) and the sediment pH was readjusted, the amount of available 203-mercury increased above that found previously (Figure 8). In this system, the introduction of FeS caused a decrease in the available mercury. The sediment in which the AVS was removed using 6N HCl, had a 100-fold increase in the solubility of 203-Hg(II) (Table 2), both in the presence and absence of FeS, although the trend was to a low mercury concentration in porewater at low pH. It is likely the drastic

treatment of the sediment with the 6N HCl solubilized substances to which the mercury was bound, causing it to remain in the porewater. In general, however, for acidified sediment from both L239 and L223, the presence of sulphur decreases the amount of mercury available for methylation.

There is also a possibility that the reduced rate of methylmercury production at low pH could have been due to a decrease in the microbial activity.

Fagerstrom and Jernelov (1972) found the rate of methylation of mercury was well correlated with general microbial activity and is enhanced by the same conditions as would enhance microbial activity. This is substantiated by Bieger and Jernelov (1979) who conclude that the efficiency of mercury methylation depends on the metabolic activity of the microorganisms as well as the concentration and availability of mercury. It seemed plausible that as the microbial activity of the sediment was not disturbed by acid addition, nor was the concentration of mercury different at the different pH values used in the experiments, there must be a process affecting the availability of mercury at low pH.

This has been investigated elsewhere (Furutani et al., in prep.; Kelly et al., submitted), where it was reported that the microbial activity, as measured by methane plus carbon dioxide production, was not inhibited in sediments of low pH, but methylation was reduced (Furutani et al., in prep.). These studies also show that the microbial activity was not altered by the additions of chloride ions which would be introduced into the system with hydrochloric acid.

The results of this study suggest that sediment mercury methylation is reduced at low pH. Stimulation of sediment mercury methylation is

not a likely explanation for high levels of mercury in acidified lakes. However, two other possibilities concerning mercury methylation in these systems also exist.

Nagase and his co-workers (1982) investigated the non-biological methylation of mercury by fulvic and humic acids acting as methyl donors in the formation of methylmercury. They found that in fulvic acid solutions, methylmercury production was highest at pH 4 and decreased when the pH was above or below that level.

The liberation of organic acids does not apear to have happened in L223 where the ambient pH of the sediment is still above 6, although the pH of the epilimnetic water is around 5 (Schindler, pers. comm.). However, the presence of organic acids in some acid lakes may account for the increased levels of mercury in the fish of acid lakes. Landner and Larsson (1972) found high mercury levels in the fish of a number of acid lakes studied, in their study they also reported that all of the lakes had high humic content. A second mercury methylation related possibility which has not been explored is the effect of pH and or elevated aluminum concentration on mercury methylation in fish slime.

Tsai et al. (1975), using acetate and phosphate buffer systems found the uptake of mercury was increased at low pH in both test species: Pimephales promelas (fathead minnow) and Notropis atherinoides (emerald shiner). They discovered the addition of less than 10 ppm of sodium sulphide increased the uptake of mercury by the fathead minnow. They also found high mercury in fish mucous at pH 5. Fish mucous is a potential site for microbial methylation of mercury (Jernelov 1972). Jernelov (1972) found the microbial community in fish slime was subject to seasonal changes. In his study he reported that the bacteria in the

fish slime methylated mercury rapidly during late winter and early spring after which the bacterial community methylated mercury poorly. Fish in acid lakes produce more slime, especially around the gills (Klaverkamp, pers. comm.). It may be that a strong spring acid pulse into the lake, coupled with an influx of other metals such as aluminum stimulates slime production and hence methylation during early spring. The combination of increased mucous production, as well as the more efficient retention of mercury in slime, may be another explanation for high mercury concentration in fish of acid lakes. This hypothesis is supported by the results of an experiment in which mercury-203 accumulation by crayfish under acid conditions was studied (Chang et al. 1982). Crayfish are in intimate contact with sediment and they have no mucous layer (Malley, pers. comm.). In Chang's study it was found that in a number of cases low pH (5.0 and 5.4) appeared to retard the accumulation of mercury-203 in crayfish tissues.

The results of the experiments undertaken in this study indicate it is unlikely that the bacteria in acidified sediment of lakes play a major role in contributing to the high mercury content in the fish of these lakes. The release of some bound sulphur at reduced pH may be important in reducing the amount of free mercury, thus limiting methylmercury production.

From these studies the rate of methylmercury production appears to be a function of the amount of available mercury. In sediments the available mercury is controlled by the pH of the sediment. The concentration of available mercury decreases as the pH of the sediment decreases. This relationship seems to be a linear one until the

sediment pH is less than 5.5, when there is almost no mercury available in the sediment porewater for microbial methylation.

Although the validity of the link between high mercury content and low pH in lakes is not questioned, the explanation for elevated fish mercury levels at low pH is likely to be due to other processes than sediment methylation. These elevated concentrations could be due to increased input to the lake either from atmospheric or drainage sources, changes in fish physiology, or a decrease in the rate of degradation of methylmercury.

SECTION II

INTRODUCTION

Introduction

A number of studies (Vonk and Sijpesteijn 1973; Bisogni and Lawrence 1975; Olson and Cooper 1976; Berdicevsky et al. 1979) have been conducted to determine whether or not microbial methylation in the natural environment was basically an aerobic or an anaerobic process. Bisogni and Lawrence (in Jernelov and Martin 1975) found the highest rates of mercury methylation occurred in aquatic sediments incubated aerobically. Olson and Cooper (1976), on the other hand, found that methylation was fastest in sediments incubated anaerobically.

Lexmond and his co-workers (1976) suggested that while anaerobic methylation could occur under laboratory conditions, it was unlikely that this would happen in the sediment. He proposed that inorganic mercury in anaerobic sediments would most likely be bound to sulphide, making it biologically unavailable for microbial methylation.

Information available on the biochemical processes (Wood et al. 1972) indicate that is is possible for methylmercury formation to occur both aerobically and anaerobically, although the pathways involve different enzyme systems. It is now known that intact methanogenic bacteria do not methylate mercury although cell free extracts containing methylcobalamine will methylate (Figure 1).

The experiments conducted in this study were designed to determine what effect, if any, the presence or absence of oxygen, would have on the ability of microorganisms to methylate mercury. Samples were also collected to determine how these different incubation conditions affected the micorbial activity of the sediment being investigated, and consequently the effect of microbial activity on the microbial mercury methylating activity.

METHODS AND MATERIALS

Methods and Material

Sample Aquisition and Incubation

The sediment samples from both lakes, L239 and L223, were collected and transported to the field lab as previously described. Once the sediment was in the lab, the contents of the PVC bottles were pooled in a 1500 ml Erlenmeyer flask. The flask was slushed with deoxygenated nitrogen (Hungate 1969) prior to sediment addition and was continously flushed during sediment manipulations. Ten ml aliquots of sediment were drawn into wet 50 cc glass syringes and stoppered with 18G-1.5 inch needles, inserted into rubber stoppers, so that no gas phase was left in the syringe.

For the mercury methylation experiments, nine syringes were divided into three lots of three syringes. This meant there were duplicate samples and a killed control sample for each of the incubation conditions used. The incubation conditions used in 1981, were the same for every experiment. One set of syringes received 5 ml of deoxygenated nitrogen, and was shaken at 150 r.p.m. (Junior Orbit Shaker) for the duration of the experiment. The second set of syringes were incubated with 5 ml of room air in the head space, and shaken as before. The final set of syringes also contained 5 ml of room air, but were not shaken except for three minutes after the addition of the radio-isotope, $^{203}HgCl_2$.

In 1982, in some cases the incubation conditions were slightly different. Two sets of syringes were incubated under static conditions, one set containing 5 ml of deoxygenated nitrogen in the head space, the other containing 5 ml of room air. The third set of samples were shaken at 150 r.p.m. Sediment sampled prior to July were incubated with room

air in the head space, sediment sampled during July and August were incubated with nitrogen in the head space.

The sample blanks were killed with 1 ml of 4N HCl. Inorganic mercury as mercury-203 mercuric chloride was added to each sample so that the concentration in the sediment was approximately 2 µCi/2 µg mercury/g dry sediment. The results were corrected for isotope and mercury concentration differences in the final calculations. The samples were incubated at in situ temperatures in water basins in the incubator to prevent gas leaks from the syringes. Incubation of glass syringes in water basins prevents gas from escaping from the dead volume between the syringe and the syringe barrel.

Sample Extraction Procedure

After incubation, samples were killed with 1 ml of 4N HCl and the contents of the syringes were injected into 250 ml glass reagent bottles. The syringes were rinsed twice with 50 ml of distilled water which was subsequently added to the reagent bottle. The mercury-203 methylmercury was extracted from the samples using an organic extraction method (Furutani and Rudd 1980).

The extraction procedure used for the extraction of methylmercury was developed by Furutani and Rudd (1980) and is summarized as follows:

1) a 2 ml aliquot of 0.5 M copper sulphate and 10 ml of 3 M sodium bromide in 11% sulphuric acid was added to each sample. The samples were shaken by hand for 3 minutes and left standing until the floc had settled and the aqueous phase had cleared.
2) 60-90 ml of the aqueous phase was transferred to a 125 ml separatory funnel and 20 ml of glass distilled toluene (Caledon) was added.

These samples were shaken again for 3 minutes and allowed to stand until the aqueous and organic phases had separated.

3) The aqueous phase was drawn out of the funnel and discarded. The toluene was dried by adding approximately 0.5 g of anhydrous sodium sulphate. The toluene was transferred to a 125 ml Erlenmeyer flask which contained another 0.5 g of anhydrous sodium sulphate.
4) A 10 ml aliquot of the toluene was added to a 20 ml screw cap test tube containing 5 ml of 0.0025 M sodium thiosulphate in 20% ethanol. The test tubes were capped and shaken for three minutes.
5) Three ml of the aqueous phase was pipetted into a 5 ml sintered glass-stoppered test tube which contained 1 ml of 3 M potassium iodide and 1 ml of benzene. The samples were shaken for 3 minutes and, after separation, a 500 µl subsample of the benzene was added to 14 ml of PCS scintillation counting cocktail.

Samples were counted to 2% error using a Beckman 7000 Liquid Scintillation Counter. The extraction efficiency of this method was found to be 97-100% (Furutani and Rudd 1980).

Measurement of Microbial Activity

The activity of the micorbial community in the sediment samples was monitored by measuring the rate of carbon dioxide and methane production. This activity was monitored in sediment samples incubated under the same conditions as the samples used to measure mercury methylation. The samples were initially set up in three sets of four samples each. One sample of every set was killed with 3 ml of 100% formalin. The gases generated in the other three samples were stripped

after 0, 24, and 48 hours respectively. The gas in the sample containing formalin was stripped after 48 hours.

Samples were stripped of dissolved methane and carbon dioxide by acidifying each sample with 1 ml of 4N HCl and adding 40 ml of deoxygenated nitrogen (Hungate 1969) to each sample. The samples were then shaken for 10 minutes at 150 r.p.m. The gas in the syringe was injected into 6 ml serum vials fitted with serum stoppers and containing distilled water with 100 µl of 4N HCl. The liquid in the vial was displaced by the gas being injected. The vial was flushed with the gas. Samples could be stored in the vial up to five days without loss although analyses was usually done with three days of the experiment. The samples were analyzed for carbon dioxide using a Lira Infrared Analyzer (Mine Safety Appliances Co.). Carbon dixoide standards were prepared and stored in the same manner as the samples. Using N/50 sodium bicarbonate (British Drug House), concentrations of 0, 500, 1000, 2000, and 3000 µmoles carbon dioxide/l were prepared. Ten ml aliquots were added to wet 50 cc glass syringes, 40 ml of reduced nitrogen was also added and the standards were acidified with 1 ml of 4N HCl. The standards were shaken for 10 minutes, stripped and stored in serum vials. The amount of carbon dioxide was determined using a standard curve.

The methane in the serum vials was measured by injecting 0.2 ml of gas into a flame ionization detector of a gas chromatograph (Varion 3700). Standards of methane were prepared a concentrations of 1:1000 and 1:1500. Methane concentration was calculated as (from Stainton et al. 1977):

Sample (μmoles/l) = chart units x scale factor/0.968 (equation 4)

where:

scale factor for standard 1:1000 = A/(chart units x attenuation)

scale factor for standard 1:1500 = 2A/(chart units x attenuation)

A = (273/273 + temp C) x (atm pressure/std pressure) x 44.64 μmoles/l

The total microbial activity, i.e. the amount of carbon produced from each sample, was calculated by adding values for methane and carbon dioxide production. Rate of production was calculated by determining the slope of the line generated from the total carbon produced during the incubation period.

RESULTS

Results

The results of mercury methylation in 1981 (Table 3) indicate that the presence or absence of oxygen does affect its production. The sediment collected from L239 and shaken with room air throughout the incubation period, had poor rates of methylation. The rate was considerably higher when the samples contained nitrogen in the head space and were shaken during incubation, with the exception of low rates of methylation on June 3, 1981. The fastest rates occurred for sediment samples not shaken, but containing room air in the head space.

The sediment collected from L223 during 1981 (Table 3) had faster rates of methylmercury production than those found in L239 although the trends were similar. Production was fastest for those samples incubated anaerobically and slowest for the samples shaken and containing room air during incubation.

In 1982 measurements of the rate of methylmercury production in sediment from L239 and L223 were repeated a number of times from May 16th-August 16th. The incubation conditions were not always the same for each date sampled (Table 4). For the dates sampled in 1982 in both lakes, the lowest rates of mercury methylation occurred in the samples containing oxygen and shaken. These rates were less than 50 dpm/g dry sediment/hr. Samples which had air in the head space but were not shaken during incubation methylated mercury more slowly than occured in 1981 (Tables 3-4). Rates were rapid for static samples with nitrogen in the head space. High rates were also found in samples containing nitrogen and shaken during incubation. The rates of production usually increased throughout the summer as the incubation temperature increased (Table 5). Temperature does not seem to be the only factor causing the

Table 3. Effect of oxygen on the rate of microbial mercury methylation in sediment from Lake 239 and Lake 223 during June 3-August 5, 1981.

Lake	Incubation Condition	Rate of Mercury Methylation (dpm/g dry sediment/hr)		
		June 3	June 22	August 5
L239	Shaken with nitrogen	11	1012	2317
	Shaken with air	90	22	113
	Static with air	292	1423	4562
		June 15	July 6	August 4
L223	Shaken with nitrogen	2485	1225	5589
	Shaken with air	109	19	21
	Static with air	1243	248	4048

Table 4. Effect of oxygen on microbial mercury methylation in Lake 239 and Lake 223 from may 19-August 16, 1982.

Lake	Incubation Condition	Rate of Mercury Methylation (dpm/g dry sediment/hr)		
		May 19	June 21	July 19
L239	Static with nitrogen	62	159	2174
	Shaken with nitrogen	-	-	4183
	Static with air	12	169	1246
	Shaken with air	0	48	-
		June 3	August 16	
L223	Static with nitrogen	81	3182	
	Shaken with nitrogen	-	995	
	Static with air	97	1871	
	Shaken with air	12	-	

methylation rate to increase as there is an order of magnitude difference between the methylation rates in L239 measured in late June of 1981 and 1982 (Table 5).

In some cases, in addition to measuring the mercury methylation rate, the microbial activity of the sediment was also measured. This was done by monitoring the production of carbon dioxide and methane for 48 hours (Table 6). The rate of total carbon production is expressed for each incubation condition used at each of the dates samples. The lowest carbon production occurred in samples incubated under anaerobic, static conditions, in both lakes, although the rate of methylmercury production was high in these samples. Highest production of carbon usually occurred in those samples containing room air in the syringe head space and shaken throughout incubation.

Table 5. Incubation temperatures used for samples during 1981 and 1982.

Lake	Date	Temperature (C)	Anoxic Rate of Methylation (dpm/g dry sed/hr)
L239	June 3, 1981	15	11
	June 22, 1981	16	1012
	Aug 5, 1981	20	2317
	May 19, 1982	11	62
	June 21, 1982	16	159
	July 19, 1982	20	2174
L223	June 15, 1981	15	2485
	July 6, 1981	18	1225
	Aug 4, 1981	21	5589
	June 3, 1982	11	81
	Aug 16, 1982	20	3482

Table 6. Microbial activity in sediments of Lake 239 and Lake 223 during 1982.

Lake	Incubation Condition	Microbial Activity (μmol C/g dry sediment/hr)	
		June 21	July 19
L239	Static with nitrogen	6.6	7.0
	Shaken with nitrogen	-	7.0
	Static with air	10.4	8.9
	Shaken with air	15.0	-
		August 16	
L223	Static with nitrogen	7.7	
	Shaken with nitrogen	7.6	
	Static with air	8.8	

DISCUSSION

Discussion

The results of this series of experiments show that methylmercury production is fastest when samples are incubated anaerobically or when the contact with oxygen is minimized. During both 1981 and 1982, the rate of methylmercury production increased as the incubation temperature increased, although the increase in production could also be due to change or an increase in the availability of carbon sources. The microbial activity as measured by carbon production was fastest when the samples were shaken with room air in the head space. These aerobic shaken samples also had the lowest rate of production of methylmercury.

The information available in the literature is divided as to whether oxygen enhances or limits production of methylmercury. Vonk and Sijpesteijn (1973) studied a number of pure cultures of bacteria and fungi, and found that methylmercury was produced by all of the organisms studied when grown aerobically. They found cultures of Escherichia coli and Aerobacter aerogenes produced methylmercury anaerobically, but at a lower rate. Bisogni and Lawrence (1973) found high methylation rates in sediments incubated aerobically. They also found that as the microbial activity of the sediment increased so did the rate of methylmercury formation.

Jernelov and Lann (1973) studied the effect of shaking on the ability of mercury contaminated sediments to produce methylmercury. It was discovered that shaking enhanced the production of methylmercury. They found aeration increased methylation by a factor of 25 in sediments collected from a eutrophic lake and by a factor of 9 in sediments from an oligotrophic lake. These results are in direct contrast to those

found at ELA where it was found that aeration limited and/or inhibited the production of methylmercury.

Olson and Cooper (1976) found methylmercury production was faster and the end product was more stable in anaerobic sediments. Jernelov (1972) examined a number of field and lab experiments and decided that under lab conditions, the production of methylmercury would be fastest when the organisms were grown anaerobically, but in field experiments, the opposite is true. He explains this by the presence of sulphides in anaerobic sediments which would bind to inorganic mercury, rendering it unavailable for biological methylation. This theory does not hold true for the sediments collected for study from ELA. The sulphide in the sediments (Table 1) did not limit the available mercury when the sediment was incubated at the in situ pH. Possibly the amount of other cations in the sediment, especially iron, were in such an excess compared to the mercury that its availability was not affected. Similar results to these were found in studies undertaken at Clay Lake, Ontario (Furutani and Rudd 1980), where it was shown that the presence of sulphur in the sediment did not reduce the production of methylmercury. Fagerstrom and Jernelov (1971) found that the rate of methylmercury formation was 100-1000 times slower under aerobic conditions when mercuric sulphide was used as a substrate. However, if mercuric sulphide does not precipitate in anaerobic sediments with high iron content then this does not seem to be a reasonable explanation for slow anaerobic methylation rates. McEntire and Neufeld (1975) explained the differences in rates as a result of different concentrations of mercury. They decided that at high concentrations of mercury, the methylation reaction proceeds non-enzymatically, requiring anaerobic

conditions, therefore the rate of methylmercury formation is fastest under anaerobic conditions. This theory does not seem to be a possibility for the results found in this study as all of the samples received the same amount of inorganic mercury.

An explanation of the results of the experiments undertaken in this study would seem to be a compilation of the varied proposals. The microbial activity was fastest in the sediment which had the lowest rate of mercury methylation. Olson and Cooper (1976) proposed that low aerobic methylation may in fact be due to an increase in the activity of bacteria capable of demethylation. At the present time it is not possible to measure separately the processes of methylation and demethylation in environmental samples., but it is most likely that an explanation for the variety of results found by different investigators would be shown if both processs could be studied concommittantly. By using radioisotopes to measure microbial production and decomposition of methylmercury, the chance of population changes in the microbial community is reduced. This possibility is reduced because shorter incubation times are required for measureable results.

These methods would also limit the effect of using high concentration of mercury required to produce detectable amounts of methylmercury.

CONCLUSIONS

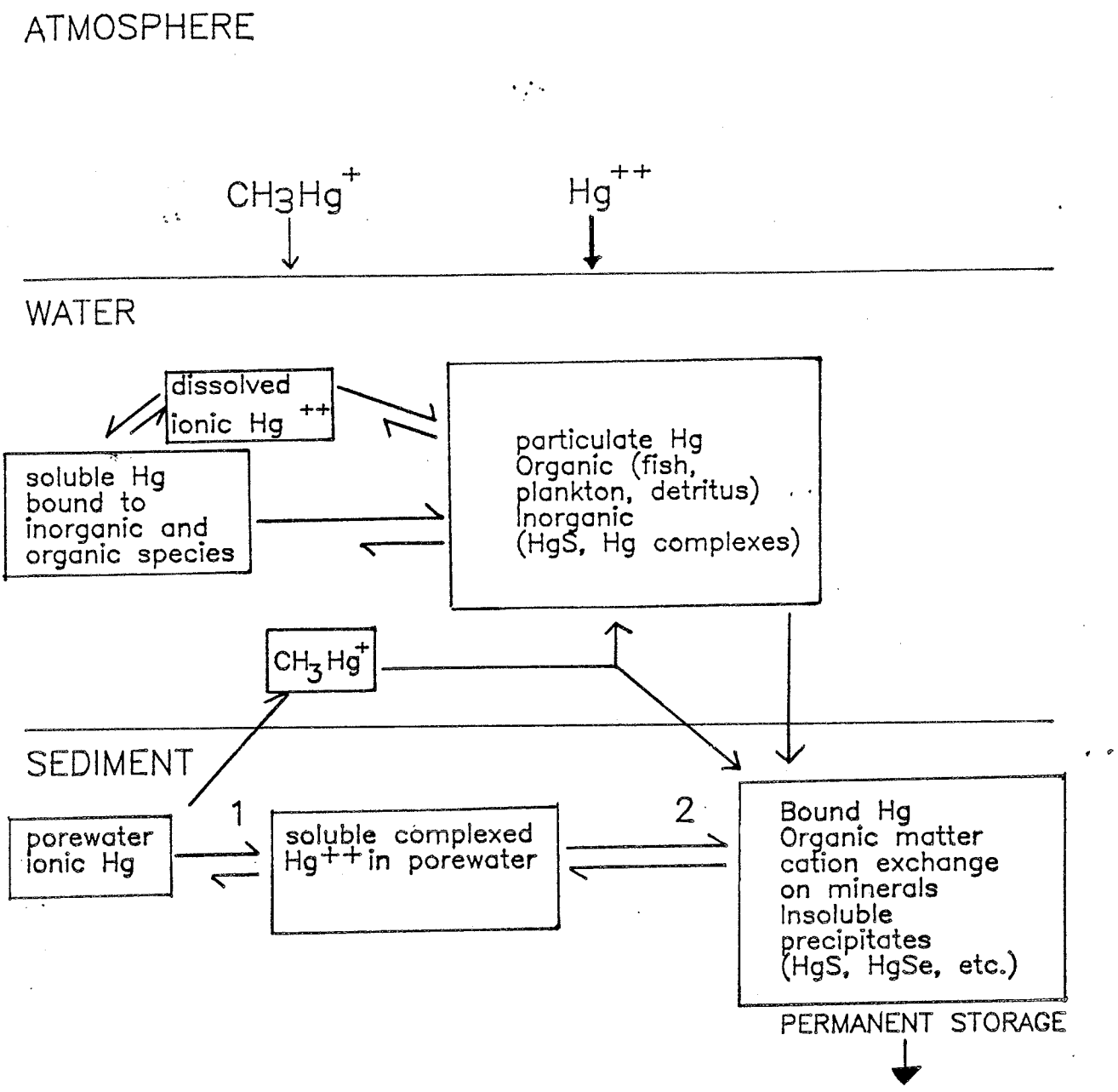
Conclusions

The experiments studying the effect of lake acidification on the ability of microorganisms to methylate mercury indicate that increased methylating activity in the sediments is not a likely explanation for the increase of mercury content in fish of acid stressed lakes. The results of these studies show in fact, that inorganic mercury(II) is less available for methylation in an acidified lake where there is sulphide available; and indeed, that in acidified sediments the rate of methylation is usually low or nonexistant at pH less than 5.

A simple model (Figure 11) illustrates some of the interactions of mercury in freshwater. This model combines information from outside sources (Brosset 1981; Jackson et al. 1980) as well as that found in these studies. In this model sediment interactions are affected by the presence of acid. The amount of ionic mercury in the porewater decreases as the pH of the sediment/porewater decreases. It may be bound to insoluble organic matter, or inorganic precipitates (Reaction 2, Figure 11) or it may complex with soluble material and form insoluble precipitates (Reaction 1, Figure 11). It is possible that the presence of acid in the sediment causes the release of previously bound compounds, such as metal hydroxides, or metal sulphides, leaving the anions free to bind to mercury(II), subsequently making it insoluble and thus unavailable for microbial methylation processes.

Experiments undertaken in this study to determine the effect of incubation conditions on methylmercury production indicate that the rate of microbial methylmercury production is greatest when the sediment is incubated anaerobically or under conditions where the contact between the sediment and the air interface in the incubation vessel is limited.

Figure 11. A proposed model for the mercury cycle in acidified and unacidified lakes.


ATMOSPHERE
CH_3Hg^+
Hg^{++}
WATER
dissolved ionic Hg ++
soluble Hg bound to inorganic and organic species
particulate Hg
Organic (fish, plankton, detritus)
Inorganic (HgS, Hg complexes)
CH_3Hg^+
SEDIMENT
porewater ionic Hg
1
soluble complexed Hg^{++} in porewater
2
Bound Hg
Organic matter cation exchange on minerals
Insoluble precipitates (HgS, HgSe, etc.)
PERMANENT STORAGE

It seems that the ability to methylate mercury is related to a number of factors such as organic content, pH, and the presence of sulphide in the sediments, and the metabolic activity of microorganisms capable of demethylating methylmercury in environmental samples.

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