INVESTIGATIONS CONCERNING THE BIOLOGICAL METHYLATION OF MERCURY WITH SPECIAL REFERENCE TO THE MERCURY POLLUTED ENGLISH-WABIGOON RIVER SYSTEM OF NORTHWESTERN ONTARIO

By

DAVID ROBERT WRIGHT

A Thesis

submitted to

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University of Manitoba

In partial fulfillment

of the requirements for the degree of

Master of Science

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TO MOM AND DAD,

AND THE FAMILY

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THE ROAD NOT TAKEN

Two roads diverged in a yellow wood,
And sorry I could not travel both
And be one traveler, long I stood
And looked down one as far as I could
To where it bent in the undergrowth;

Then took the other, as just as fair,
And having perhaps the better claim,
Because it was grassy and wanted wear;
Though as for that the passing there
Had worn them really about the same,

And both that morning equally lay
In leaves no step had trodden black.
Oh, I kept the first for another day!
Yet knowing how way leads on to way,
I doubted if I should ever come back.

I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I -
I took the one less traveled by,
And that has made all the difference.

- Robert Frost

ABSTRACT

A laboratory flow-through system utilizing plexiglass "microbasins" was developed to study the basic kinetics of the mercury methylation process in sediments. Experiments were conducted with sediment with a low mercury content from Lake 239 (Experimental Lakes Area) and mercury polluted sediments from the English-Wabigoon River system, Northwestern Ontario.

Methylation appeared to follow saturation kinetics in that net methyl mercury release from sediments increased with increasing concentration of mercury. An apparent exponential increase in net methyl mercury production in the range of up to 15-20 µg Hg/gm sediment (dry wt.) was observed whereas no further rise in methyl mercury release was found at sediment mercury concentrations greater than 20 µg Hg/gm. Microbasin data also suggest that a methylating-demethylating equilibrium exists within mercury contaminated sediments.

Upon the addition of tryptic soy broth (TSB) to microbasins, increased methyl mercury production was observed. The addition of 35 mg TSB/ℓ to only the water overlying sediments within microbasins and not directly to the sediments themselves, was more effective in

increasing the rate of methyl mercury production than mixing 1 or 4 gm of nutrient directly with the sediments. It is suggested that most of the methyl mercury released from sediments is produced at or near the sediment-water interface.

The release of methyl mercury from microbasins at 4°C was found to be 50-70% of the methyl mercury production observed in similar sediments at 20°C. Since water temperature in Canadian Shield Lakes is at or near 4°C for a large part of the year, low temperature methylation may account for most of the methyl mercury found in aquatic organisms.

A laboratory dialysis cylinder system was developed and utilized to examine the possibility of water column methylation, and first results indicate that methyl mercury can be so formed. Although the production of methyl mercury was very low in dialysis cylinder experiments, it may be significant in Clay Lake for example, simply because of the large volume of water within the lake.

It is concluded that the microbasin and dialysis cylinder systems can be effective tools in laboratory and field (in situ) investigations concerning methylation, in providing understanding of the process and in guiding attempts to control it and ameliorate the contamination of biota.

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INTRODUCTION

INTRODUCTION

Many lake and river systems throughout the world contain high levels of mercury (1, 2, 3). The mercury is almost entirely in sediments and is present as the result of a high natural abundance of the metal and/or some human related activity (4, 5, 6). Fish and other aquatic life present in such contaminated systems often have elevated levels of mercury (7, 8, 9, 10). While mercury in the watercourse is present predominantly as inorganic bivalent or elemental mercury, an organo-mercurial neurotoxin (methyl mercury) is the form usually identified as being present in aquatic organisms (7, 9, 10, 11).

The net production of methyl mercury is biologically controlled (9, 12, 13, 14) and it is thought that the important site for methyl mercury production is in mercury laden sediments (15, 16). Although pure culture studies have shown that specific microorganisms native to sediments have the potential to carry out a methylation of inorganic mercury (17, 18, 19), it has not been conclusively demonstrated that they are in fact the organisms responsible for the observed <u>in situ</u> production. From such investigations it is difficult to predict what effect the alteration of physical and chemical factors in sediment will have in terms of the degree of methyl mercury enrichment in

organisms in nature.

The research project described in this thesis was initiated with the intent of providing fundamental information concerning the biological methylation of mercury with particular reference to the mercury polluted English-Wabigoon River system.

HISTORICAL REVIEW

HISTORICAL REVIEW

Mercury in the Environment

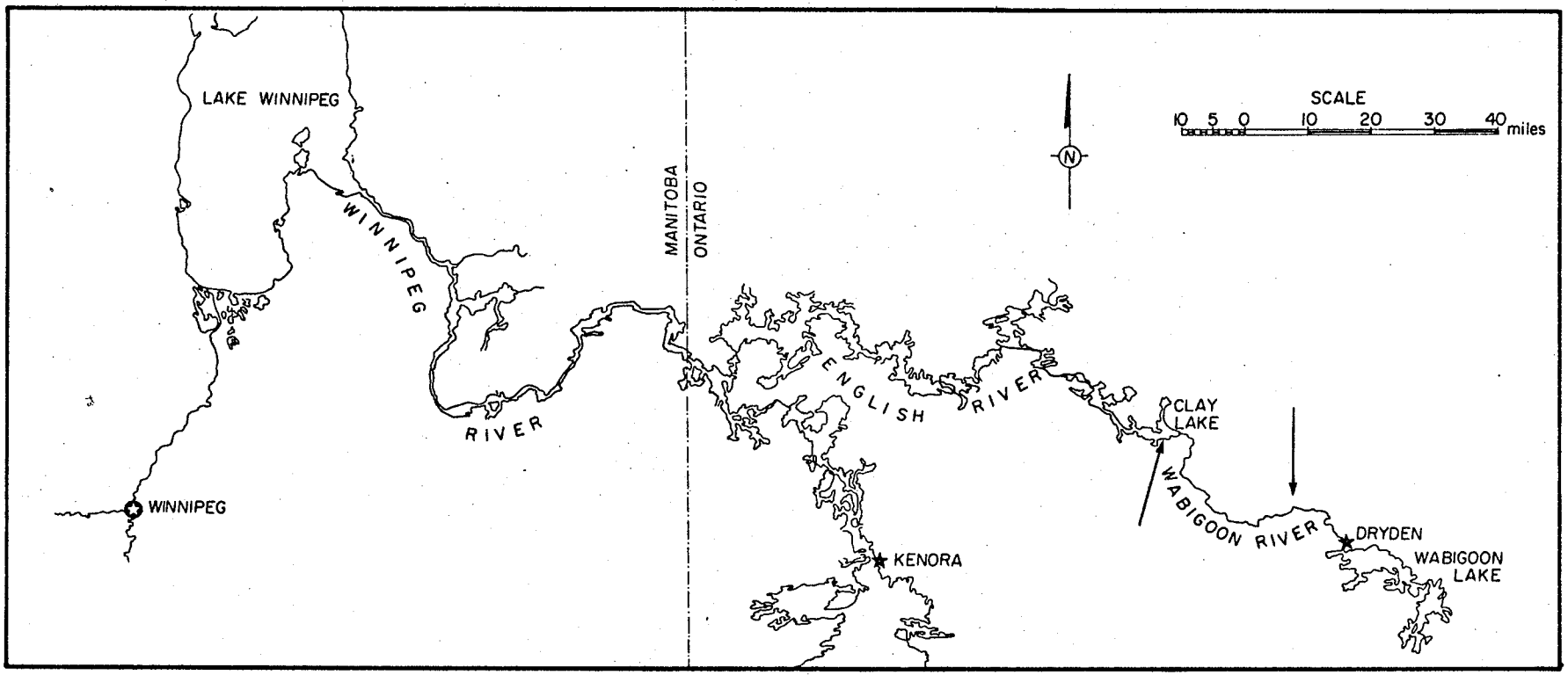
Mercury enters the environment as a result of both natural processes (rock weathering, volcanoes, etc.) and human related (anthropogenic) activities. Gavis and Ferguson (5) estimate that 1.6×10^{10} metric tons of mercury have been released through geological time as a result of rock weathering and that another 800 tons are being released from rock into the environment through weathering each year. They further claim (5) that as a direct result of human activity, 1.2×10^{5} metric tons of mercury have entered the environment. Although the yearly loss value for human related industrial activities is difficult to estimate, the mercury release from the burning of coal alone is set at approximately 4.6×10^{3} metric tons per year (4, 20) and about 1×10^{4} metric tons of mercury are mined yearly (1970 data) for industrial applications (5). Anthropogenic mercury losses may seem small relative to that from natural weathering (10^{5} vs 10^{10} tons, respectively) but one must remember that the human losses have occurred over an infinitesimally shorter period of time than have the natural losses. Moreover, the amount of mercury mined and used in industry has been

increasing steadily (5) and so mercury losses probably will increase as well. Already, there are in excess of 3000 uses of mercury in industry (3) and even if one were to believe that direct mercury loss from industrial applications is small, over the long term virtually all mercury mined and utilized by industry will be returned to the environment in one form or another.

It should be noted that the greatest problem with industrial mercury losses is not so much the size of the loss relative to mercury from natural sources but rather that the industrial outfalls are point source losses which result in the extreme contamination of a localized region. Fimreite (21) estimates that as of 1970 the chlor-alkali industry, which produces chlorine (gas) and sodium hydroxide (caustic soda) and is the largest Canadian mercury user, was losing 200,000 lb. of mercury per year to the Canadian environment. One plant in particular, the Dow chlor-alkali installation in Sarnia, Ontario, apparently was discharging up to 200 lb. metallic mercury per day (3). The mercury losses are primarily by way of plant waste effluents which are usually fed directly into lake and river systems.

The English-Wabigoon River system is an excellent example of point source mercury pollution. In 1910 the town of Dryden was incorporated at the western end of Wabigoon Lake where the Wabigoon River originates (Fig. 1).

Figure 1. Map of English-Wabigoon River system as it flows from Wabigoon Lake to Lake Winnipeg. (arrows denote sampling locations)


LAKE WINNIPEG
WINNIPEG
RIVER
MANITOBA
ONTARIO
ENGLISH
RIVER
CLAY
LAKE
WABIGOON RIVER
DRYDEN
WABIGOON
LAKE
KENORA
WINNIPEG
N
SCALE
10 5 0 10 20 30 40 miles

In 1913 a pulp and paper mill was established in Dryden and from that time to the present the Wabigoon River has been burdened with large discharges of organic wastes from the plant, and these discharges have been greatly in excess of Ontario Ministry of the Environment (formerly Ontario Water Resource Commission) objectives for at least the last 20 years (22). However, in addition to the Wabigoon River's serious problems with organic pollution, a chlor-alkali plant adjacent to the pulp and paper mill began operation in March 1962 and it is estimated that approximately 9,000 - 11,000 kg of mercury were released into the river during 1962-1970 (23). Today the plant uses a process which no longer requires mercury and so the mercury releases have been halted. However, fish caught in Clay Lake have been found to contain up to 16 µg Hg/gm (24) (well above the federal health guideline of 0.5 µg Hg/gm) and are among the most heavily mercury contaminated fish in North America (25).

The Mercury Problem is Recognized

The threat of mercury as a public health hazard did not emerge until the 1950's when a number of Japanese fishermen and their families were struck by a strange neurological disorder. During 1953-1960 there were 121 officially documented cases in the villages around Minamata Bay in the Kumamoto prefecture of Japan. The illness, called Minamata Disease, was characterized by

neurological disturbances accompanied by structural deterioration of the brain. There was a second breakout of the disease in the Niigata prefecture during the early 1960's in villages along the Agano River.

Prior to the human cases a number of house cats in the Minamata Bay region showed symptoms similar to those later to develop in humans and it was noted that a number of the cats actually committed suicide by jumping off cliffs (3). Researchers at Kumamoto and Niigata Universities showed that the symptoms of Minamata Disease could be reproduced in experiments where cats were fed fish and shellfish which had elevated levels of methyl mercury (26). The source of the mercury was found to be a vinyl chloride plant whose effluent was directed into Minamata Bay. At first it was thought that only inorganic mercury was present in the plant's effluent and (in 1960) Kurland et al (27) hypothesized that inorganic mercury could be transformed into the organic methylated form as a result of microbial activity. However, it was later found that approximately 1% of the mercury released from the industry was as methyl mercury and so the suggestion of Kurland and his coworkers was not taken seriously. Many poor people in the Minamata region relied upon seafood from the bay for almost all of the protein in their diet, and fish and shellfish in Minamata Bay had accumulated up to 50 and 85 µg Hg/gm, respectively (3). A similar industrial site was responsible for the Niigata incident.

At the same time as Japanese scientists were working on the Niigata mercury problem, Swedish researchers found that there was a great decline in the numbers of certain species of birds in Sweden. Berg et al (28), while studying the problem, found that many seed eating species contained elevated levels of total mercury when compared to museum specimens. They found a direct correlation between the mercury levels in seed eating birds and the introduction of fungicides containing methyl mercury used as seed coatings after World War II. However, Johnels and Westermark (as cited in (16)), also through the comparison of museum specimens, found that the level of total mercury in fish eating species of birds had been increasing constantly since the industrial revolution in Sweden (1860-1880). The overall effect of the increase in mercury levels in both seed eating and fish eating birds was that predatory birds such as the white tailed eagle (Haliaetus albicilla), which feed upon fish as well as terrestrial birds and animals, were beginning to vanish from the Swedish landscape. In 1966 a ban upon the use of alkyl-mercury seed coatings was levied and mercury levels in terrestrial orientated birds began to decrease (16).

A large scale Swedish investigation into mercury sources found that the pulp and paper industry was losing large amounts of phenyl mercuric acetate (PMA), a slimicide, to the freshwater environment. As well, chlor-alkaline factories which used a mercury electrode process

were discharging inorganic mercury to Swedish lakes and rivers. Certain freshwater fish, particularly pike Esox lucius), were found to contain elevated levels of mercury (as cited in (29)) and in 1966 a ban was put upon the sale for human consumption of mercury contaminated fish in Sweden. Westoo (11) contributed a very puzzling piece of information to the mercury investigation when she showed that the mercury present in fish was predominantly in the methyl mercury form whereas all of the known mercury outfalls were either as inorganic mercury or PMA. It was left to Jensen and Jernelov (as cited in (16)) who, in 1967, opened a whole new field of microbiological investigation when they showed that microbial methylation of mercury could occur in mercury contaminated lake and river sediments.

The contamination of waterways with mercury was not, however, recognized as being a problem in other industrialized countries around the world in spite of the Japanese and Swedish revelations concerning mercury pollution. Many North Americans became aware of the danger of a potential mercury health hazard only after the journal Environment presented an overview of the mercury experiences in Japan and Sweden (30, 31, 32). Novick (30), for example, pointed out that there was almost no information whatsoever concerning the status of mercury in food items and the environment in North America. Abelson (33), in an editorial in Science, joined in the call for more research

into mercury pollution.

Authorities in Canada became aware of the Canadian mercury problem through the work of two independent investigations. Wobester et al (34) found levels of up to 11.2 µg Hg/gm in fish from the Saskatchewan River. In the Lake St. Clair region, Fimreite et al (7) found up to 10.5 and 17.4 µg Hg/gm in fish and birds, respectively. Commercial fishing in both areas was immediately halted. The existence of the mercury problem in North America had finally been recognized.

Today many lakes and rivers in Canada are known to contain fish with mercury levels in excess of the 0.5 µg Hg/gm federal guideline (35). Tam and Armstrong (35), in 1972, noted that most mercury contaminated waterways (and those containing fish with the highest mercury levels) were associated with chlor-alkali plants located on Canadian rivers and lakes. The English-Wabigoon River system, for example, received its mercury from a chlor-alkali plant located at Dryden, Ontario.

There has been no conclusive proof at this time as to what direct effect the pollution of Canadian waters with mercury has had upon the health of those people living within a close proximity to the contaminated lake and river systems. In the case of the Indian people who live along the English-Wabigoon River system, however, the sociological difficulties which have arisen are well documented (36, 37).

Microbiology and Biochemistry of Methylation

Jensen and Jernelov, in a series of experiments (1967-1969), showed that mercury could be methylated in freshwater sediments as a result of biological activity (9). This was an important extension of Westoo's finding in 1967 (as cited in (38)) that methyl mercury could be formed in liver homogenates from inorganic mercury. Also, the biological methylation of mercury in sediments, as demonstrated by Jensen and Jernelov, provided a rational for Westoo's observation in 1966 that fish could be enriched with methyl mercury even when all known mercury outfalls were either as inorganic mercury or phenyl mercuric acetate (11).

At about the same time as the Swedish discoveries concerning methyl mercury formation were taking place, Wood and his coworkers were investigating the transfer of methyl groups from cobalt (III) to mercury (II) in cell-free extracts of the methanogen MOH (18). It had already been shown by Halpern and Maher (39) that mercury (II) reacts with the alkyl vitamin B_{12} analogue methylpentacyano-cobaltate to form methyl mercury. Wood et al (18) used methyl-cobalamine (CH_3-Co-5,6-dimethylbenzimidazolylcobamide) as the methyl donor in their experiments as it was known to be an excellent substrate in the formation of methane by MOH. They found that the reaction forming methane was strongly inhibited when low concentrations of mercury (II) were

added to the MOH extract, but that the formation of reduced vitamin B_{12} continued. Further examination revealed methyl mercury and dimethyl mercury to be sole reaction products (18). This led Wood to suggest that methylcobalamine may serve, both enzymatically and non-enzymatically, as an alkylating agent for mercury and other metals and metalloids in the environment (18, 40).

A number of investigators studied the non-enzymatic methyl transfer from cobalt (III), in corrinoids, to mercury (II) during 1970-1971 (41, 42) and their findings were analogous to those of Halpern and Maher (39). Imura et al (42), for example, found that methyl mercury was quickly generated from inorganic mercury when methyl-cobalamine and mercury (II) were mixed together in neutral aqueous solution. Bertilsson and Neujahr (41) obtained similar results but also pointed out that cobalt corrinoid compounds had not yet been isolated from certain methane bacteria. Also, they found (41) that upon the addition of the thiols mercaptoethanol and homocysteine to their aqueous mercury methylating system, the rate of methylation decreased by more than 60%. This was presumably as a result of the thiols binding to the mercuric ion thus making it unavailable for acceptance of the methyl group. It would not be surprising to find that free methylcobalamine is rare in nature (16) and Bertilsson and Neujahr (41) suggest that the significance of non-enzymatic methylation by methyl corrinoid compounds is not clear. It is also

difficult to predict, because of the abundance of thiol groups in cells, what the potential for methyl mercury formation is when methylcobalamine and inorganic mercury are intimately associated within a living cell.

Wood and his coworkers conducted further experiments regarding the methylation of mercury (II) and in 1973 they proposed a biochemical mechanism for the alkylation of metals and metalloids (43). Wood points out that there are three methylating coenzymes for alkyl transfer reactions (40). Of these, S-adenosyl-methionine and the N^5-methyltetrahydrofolate derivatives carry out alkyl transfers to nucleophiles only and therefore are not directly responsible for metal alkylation. Only the methyl corrinoid derivatives can achieve displacement, of the methyl group from the cobalt to mercury (II), as the required carbanion (40). Wood claims that methylcobalamine is an important alkylating agent in N^5-methyltetrahydrofolate-homocysteine transmethylase (B_{12}-dependent) in liver and in all microorganisms capable of synthesizing methyl corrinoids (38, 40).

Landner (44) investigated the formation of methyl mercury by the fungus Neurospora crassa because methyl corrinoids are not known to be involved in its metabolism. The reaction mechanism for methyl mercury formation should, therefore, be different than that examined in MOH by Wood. Research carried out by Miller and Harmon (45) had suggested that in the bacterium Staphylococcus the

loci for methionine synthesis and mercury resistance were associated in one complex gene. Landner, therefore, examined the possible connection between methionine biosynthesis and mercury resistance. Landner isolated mutant strains of N. crassa and found that methyl mercury formation increased with an increase in mercury resistance and he claimed that this implied a detoxification of mercury through methylation (44). Landner also proposed a mechanism for the formation of methyl mercury in N. crassa (44) which he believed to involve an "incorrect" synthesis of methionine. However, there has been serious criticism of Landner's proposed mechanism (17, 46).

Vonk and Kaars Sijpesteijn in 1973 examined the methylation of mercury in 5 bacterial and 3 fungal species (33) in order to see if the ability to methylate mercury was a general microbial property. All of the organisms that they screened could methylate mercury to approximately the same extent (17). As well, the bacteria tested were methylating mercury under aerobic conditions and this was one of the first occasions upon which methylation by aerobic bacteria had been shown.

In one of the organisms Vonk and Kaars Sijpesteijn worked on (17), Aerobacter aerogenes, a large increase in methyl mercury production was observed upon the addition of vitamin B_{12} to the culture. A similar observation was made by Yamada and Tonomura (19) regarding cultures of Clostridium cochlearium. However, when Vonk and Kaars

Sijpesteijn added vitamin B_{12} to mercury methylating cultures of Escherichia coli, no increase in methylation was seen (17). Since A. aerogenes and C. cochlearium are known to contain methyl corrinoids and E. coli is known not to contain methyl corrinoids (47), these observations tend to support Wood's view (38, 40) of methylation by way of methyl-vitamin B_{12} compounds in certain microorganisms.

Bertilsson and Neujahr (41) have argued that the methylation of mercuric ion must involve transfer of the methyl group as a carbanion and so a satisfactory methylation mechanism for non-corrinoid containing microorganisms has yet to be proposed. The mechanism and kinetics of methylation and corrinoid dependent enzyme reactions has been the subject of a number of review articles (5, 6, 48, 49, 50).

Many investigations have been carried out to determine the effect of chemical and environmental parameters upon methylation. Jensen and Jernelov (as cited in (51)) were the first to show that the rate of methylation corresponded to general microbial activity. Metabolic activity, and therefore methylation, can be increased by increasing nutrient levels (51, 52, 53) or temperature (51, 53, 54). Generally speaking, a rise in the concentration of inorganic mercury available for methylation also results in increased methyl mercury production (9, 52, 54).

There has been no agreement as to whether aerobic or anaerobic conditions result in optimal methylation. Initially it was thought that methyl mercury formation required anaerobic conditions, but the work of Landner (44) and Vonk and Kaars Sijpesteijn (17) showing aerobic methylation to be very significant, has indicated that anaerobiosis is not a prerequisite to the production of methyl mercury. Indeed, Vonk and Kaars Sijpesteijn suggest that the ability to methylate mercury should be considered a common property of most, if not all, microorganisms (17).

There has been some question as to whether mercury is available for methylation under reducing conditions where sulfide is present. This is because of the very low solubility of mercuric sulfide (solubility product Ksp = 10^{-53}(55)). Fagerstrom and Jernelov (55) found the rate of formation of methyl mercury from HgS to be only about 0.1% of that when non-sulfide mercury was available. However, their experiments employed the addition of pure HgS as a starting material and to date there has been no conclusive proof that mercury which enters the environment in a non-sulfide form will, under reducing conditions, be effectively sequestered through sulfide addition.

Another factor which may be important in methylation is pH. Jernelov (as cited in (51)) suggested that under neutral and acid conditions (mono) methyl mercury is

formed whereas dimethyl mercury is produced under alkaline conditions. Dimethyl mercury is volatile and so would, upon formation, escape to the atmosphere where it would be broken down to elemental mercury through the action of ultra-violet light (51). The elemental mercury so produced would enter the global mercury cycle (51). However, additional experiments are required to conclusively demonstrate that pH does in fact have this effect in nature.

Langley (56) and Shin and Krenkel (54) studied methyl mercury production in undisturbed and artificial sediments through the use of fish as bioaccumulators. Their experiments were designed such that fish were incubated in water overlying mercury contaminated sediments. The effect of chemical and environmental factors upon the methylation capacity of the sediments was determined through the analysis of the fish for methyl mercury content. The results of the Shin and Krenkel study in particular are interesting and their results (54) support the general observations made by other investigators in that conditions encouraging microbial activity result in an increased formation of methyl mercury.

While most research concerning methylation has been carried out in the laboratory, a few investigators have examined methyl mercury production under natural conditions. Jacobs and Keeney (14) studied the methylation of mercury in sediments from the Fox and Wisconsin Rivers in Virginia. They found that under natural conditions more methyl mercury

was produced from phenylmercuric acetate than from mercuric chloride. This was contrary to the findings of Matsumura et al (57) who failed to observe any methyl mercury production from phenylmercuric acetate. Jacobs and Keeney suggest that the methylation rate is related to the chemical composition of the sediments; the river sediment with more organics and less sulfur had a higher methyl mercury yield (14). Olson and Cooper (58, 59) found mercury to be methylated in mercury polluted sediments in San Francisco Bay. Beckert et al found methyl mercury to be produced in the soils of mercury contaminated agricultural plots (60), and Rowland et al (61) found that methylation could even occur through the action of the intestinal flora in rats.

Tonomura and his coworkers, in a series of experiments (62, 63), showed that organo-mercurals could be enzymatically decomposed by cell-free extracts of a mercury resistant pseudomonad which had been isolated from a heavily mercury contaminated soil. Benzene, ethane, methane and elemental mercury were end products when phenylmercuric acetate, ethylmercuric phosphate and methylmercuric chloride, respectively, were added to the cell-free extracts. A significant contribution to methylation microbiology was made by Spangler et al (14, 64) when they extended Tonomura's observations to natural systems and showed that numerous microorganisms common in the environment excercised their capacity to convert methyl mercury to

elemental mercury. This demethylating activity resulted in the loss of elemental mercury to the atmosphere. The discovery of demethylation was important as it explained why methyl mercury levels were not found to be constantly increasing in mercury contaminated sediments. Methylation and demethylation work in concert and provide a constant level of methyl mercury in contaminated sediments. Jacobs and Keeney found this to be the case in their field experiments dealing with the Fox and Wisconsin Rivers (14), for example. As well, Billen et al found demethylation in sediments of the Sambre River in Belgium (12) and it now appears that the ability to convert methyl mercury to volatile elemental mercury is a common microbial activity. Therefore, to measure the total methyl mercury content of a sediment is uninformative as the amount of methyl mercury present may well have no direct relationship to actual methyl mercury production (40). Most mercury methylation experiments should in fact be considered as indicating net rather than gross methylation because they ignore demethylation activity.

Although there are a number of review papers concerning the microbiology of methylation (5, 6, 50, 51, 52), the study of microbe-metal interactions is still in its infancy. Clearly, an examination of the pertinent scientific literature demands that further research be carried out to more fully examine the chemical and physical factors controlling the methylation process as well as the rates of methyl mercury formation, release, and subsequent

uptake and enrichment in aquatic organisms. Furthermore, all potential methylation sites within lakes and rivers must be identified, examined, and evaluated as to their significance relative to the entire lake and/or river system's total net methyl mercury production.

There are a wide variety of conversions that mercury can undergo in nature (1, 3, 6, 65) and even though methyl mercury is the important form in terms of uptake and enrichment of mercury in organisms is concerned, one must not lose sight of the fact that the various conversions of mercury are all intertwined into an overall mercury cycle (40, 66, 67). The movement of mercury from one compartment in the cycle to another is not unidirectional but is instead controlled by a dynamic equilibrium involving many different chemical and biological transformations (Fig. 2). It is obvious that knowledge concerning the interactions between microorganisms and metals is basic to our understanding of the cycling of metals and metalloids in the environment.

Figure 2a. Generalized global mercury cycle (adapted from (27)).

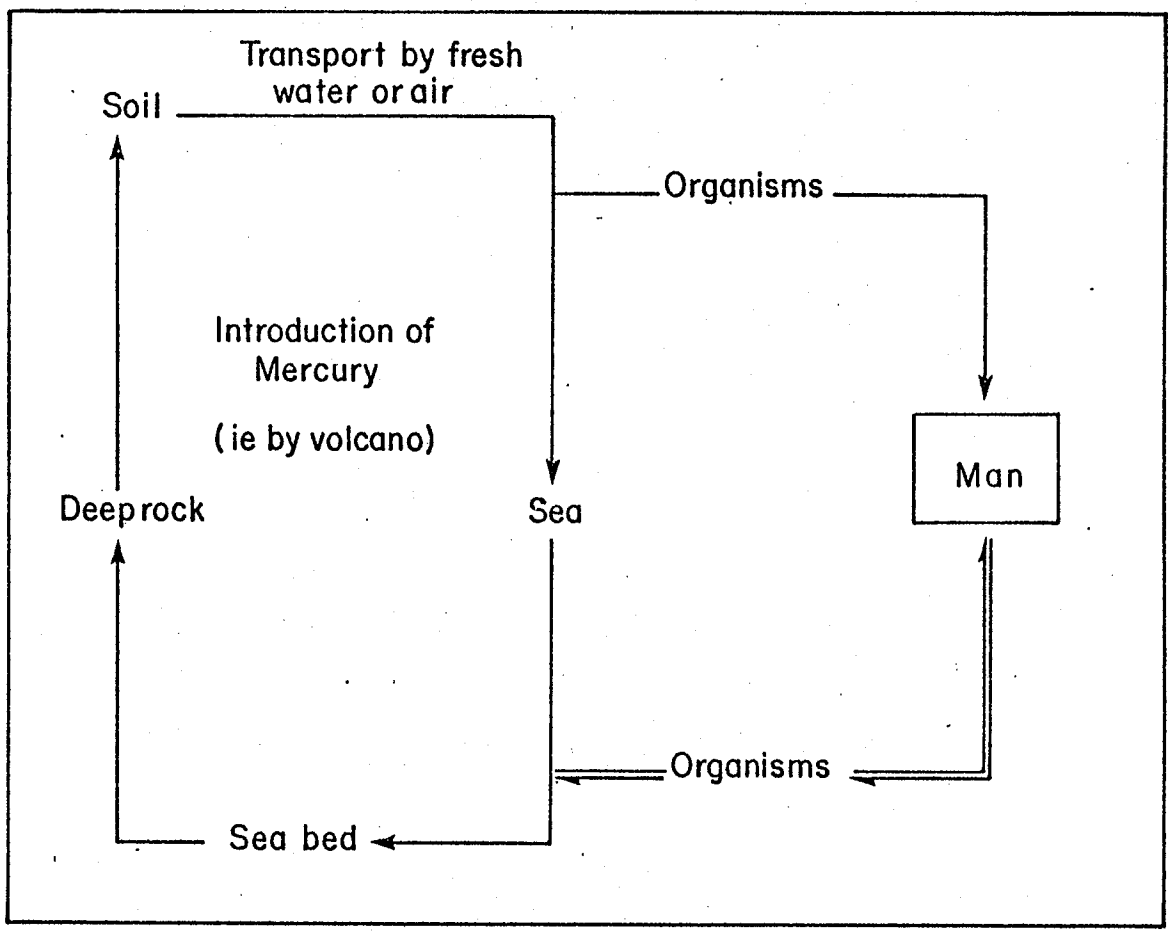
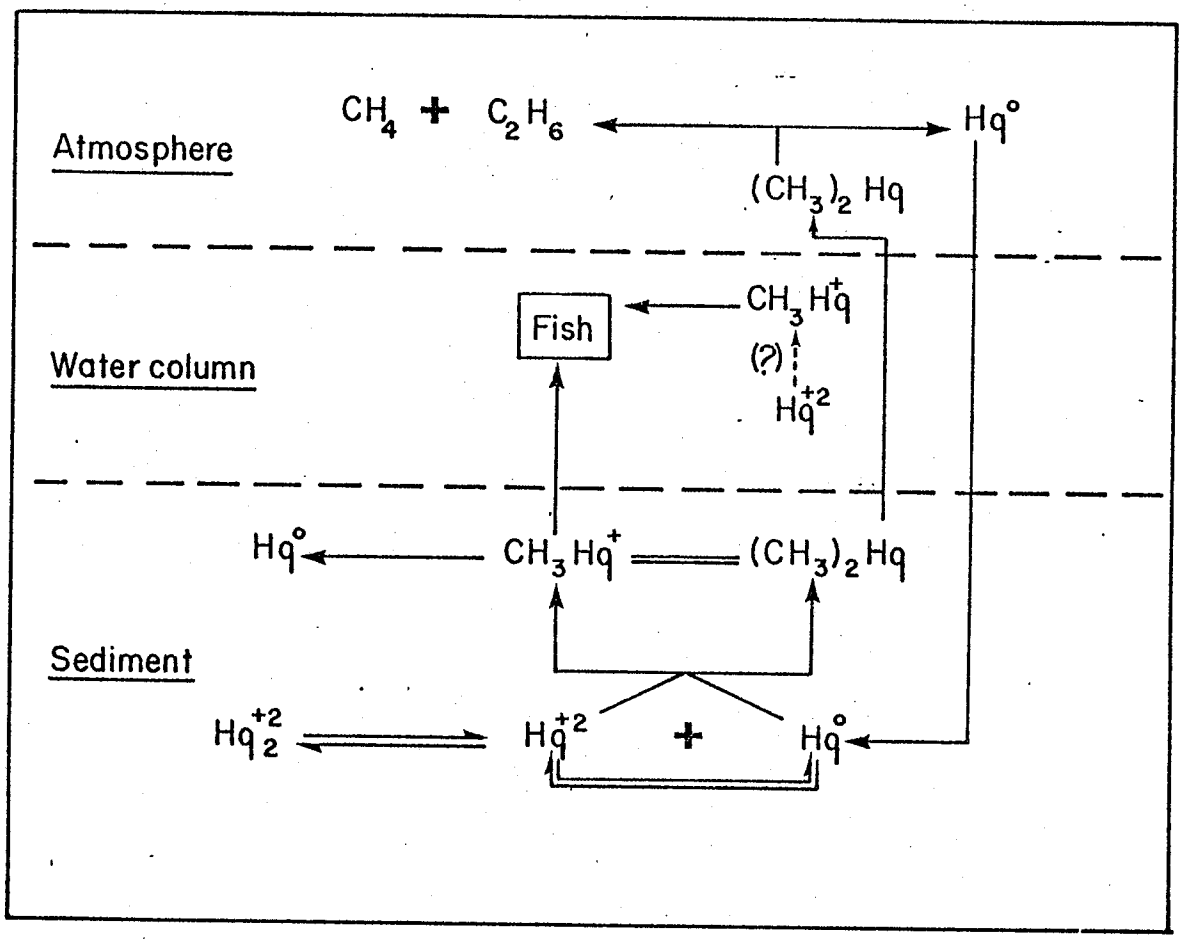

Transport by fresh water or air
Soil
Organisms
Introduction of Mercury
(ie by volcano)
Man
Deep rock
Sea
Organisms
Sea bed

Figure 2b. Microbiology and chemistry of mercury in the aquatic environment (adapted from (27)).


Atmosphere
Water column
Sediment
$CH_4 + C_2H_6$
Hg^0
$(CH_3)_2Hg$
Fish
CH_3Hg^+
(?)
Hg^{+2}
Hg^0
CH_3Hg^+
$(CH_3)_2Hg$
Hg_2^{+2}
Hg^{+2} + Hg^0

MATERIALS AND METHODS

MATERIALS AND METHODS

Sediments Used

Mercury contaminated sediments were collected from the Wabigoon River and Clay Lake (50° 04'/93° 30') during May to October, 1977 (Fig. 1). Most sediments were collected with a modified Ekman dredge (68). The sediments were previously characterized (23, 69). Only sediments from the top 6-8 cm of the lake and river bed, where most of the mercury is located (23, 69), were collected. Samples were transported to the laboratory in polyethylene-lined 2 gallon pails. After digestion of sediment samples by the Dow Chemical (aqua regia) procedure (70), total mercury was determined by atomic absorption spectrophotometry (AAS) using the instrumentation procedure reported by Armstrong and Uthe (71). For Clay Lake and Wabigoon River sediments collected, total mercury values were 3 and 4 µg Hg/gm (dry wt.), respectively.

Sediments with a low background level of mercury (approx. 100 ng Hg/gm, dry wt.) were obtained from Lake 239 (L239) in the Experimental Lake Area (ELA) of Northwestern Ontario (49° 40'/93° 44').

Microbasin Design and Operation

Rectangular plexiglass boxes ("microbasins") were

Figure 3. A view of a microbasin containing Wabigoon River sediment. The removable end of the basin, used during the basin set-up process, is shown in the figure. During basin operation L239 water flowed over the sediments and was released from the basin by way of the outflow port (upper right). Also shown in the figure is the gas phase, which was trapped at the top of the basin, after its production and release from the sediments. The masking tape on the top of the basin covers holes through which sampling of sediments was carried out. The overall basin dimensions were 100 x 10 x 20 cm.

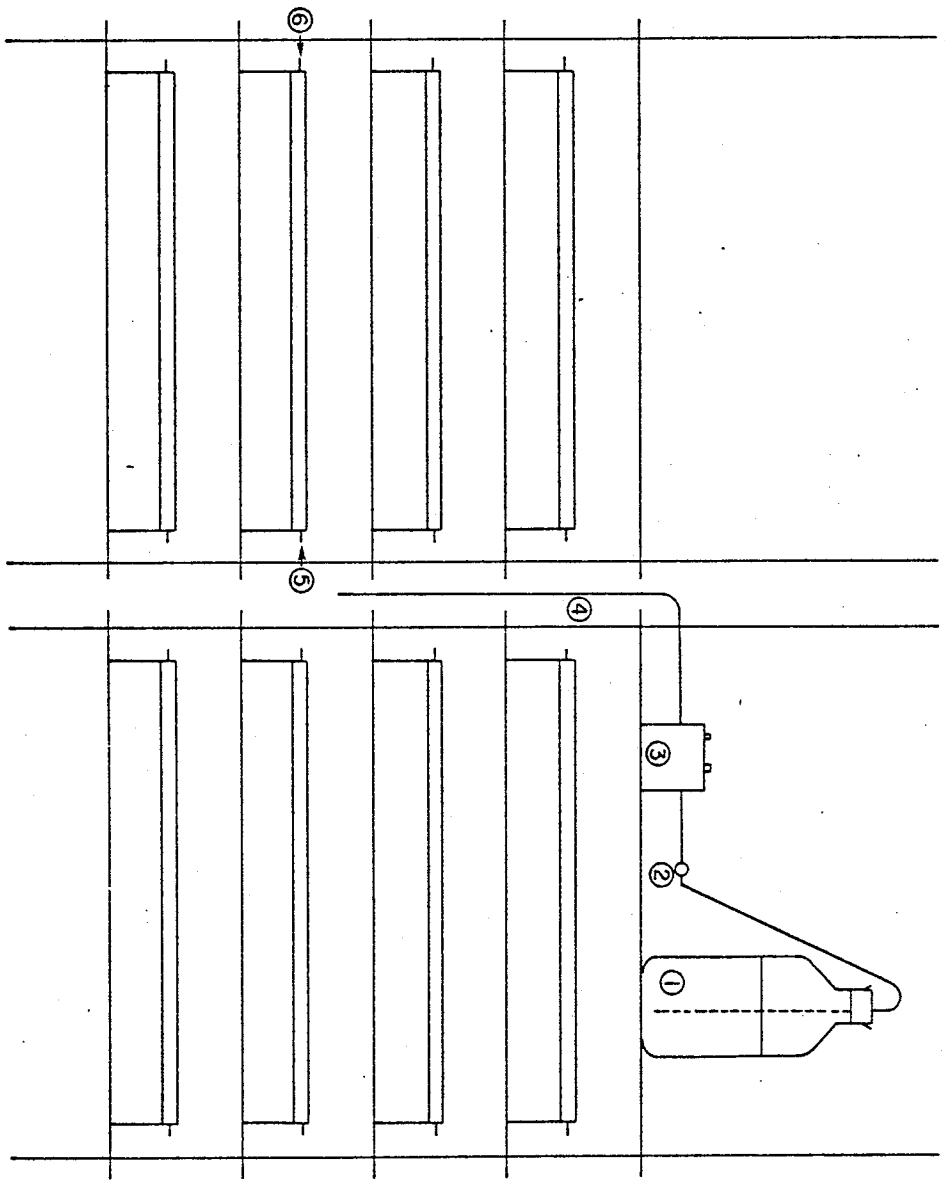
utilized in the study (Fig. 3). One end of a microbasin was removable and it was through this opening that sediments were introduced into the basin. In experiments requiring higher levels of mercury than background levels, the sediment was enriched by adding $HgCl_2$ solution (0.05 mg Hg/ml) during filling. When the microbasin was filled to 80% capacity, the basin end was screwed on and sealed with RTV 108 silicone rubber adhesive (General Electric, Waterford, N.Y.). The microbasin was shaken by hand for 5 minutes and then allowed to settle for 1 day. Sampling for analysis was carried out through small holes on top of the basin.

After the settling period, unfiltered water (from L239, ELA) was pumped over the surface of the sediment in the basin at the rate of 1 ℓ/24 hours (approx. 0.7 ml/min) resulting in a residence time within a microbasin of about 60 hours. Bssins were set up in groups of up to 10 microbasins (Fig. 4) and water from a reservoir was partitioned through a glass manifold (1 entrance, 12 exits) by way of a Manostat cassette pump (Manostat, New York, N.Y.).

All methylation experiments (except for those at 4°C) were carried out in a controlled environment room maintained at 20°C.

Methyl mercury, produced as a function of methylating and demethylating activities in the microbasin sediments, was released in the basins' effluent. The microbasin effluent was collected and analyzed for methyl

Figure 4. Schematic diagram of the microbasin flow-through system. 1) L239 water reservoir; 2) manifold; 3) cassett pump; 4) water line to a basin; 5) microbasin inflow; 6) microbasin outflow.


⑥
⑤
④
③
②
①

mercury content which was indicative of the net methyl mercury release from the sediments within the basin. Effluent from each microbasin was collected in a 1ℓ bottle so that the amount of water flowing through the basin could be monitored daily; Volumetric flasks (1ℓ) were utilized to collect effluent, over 24 hours, for analysis. The loss of methyl mercury from the volumetric flasks over the 24 hour collection period was found to be < 10%.

Aeration Experiment

The sampling holes in the top of one microbasin, which were sealed with masking tape during normal basin operation, were utilized for an aeration experiment. Clean air was forced with a Cole-Parmer peristaltic pump (Cole-Parmer Instrument Co., Chicago, Ill.) through one sampling hole, over the surface of the water layer in the basin, and out a sampling port at the other end of the microbasin. The air flow rate was approximately 50 cm^3/ minute.

Low Temperature Experiments

Low temperature methylation experiments were carried out with microbasins immersed in a waterbath. The temperature in the plexiglass waterbath was maintained at 4 ± 1°C.

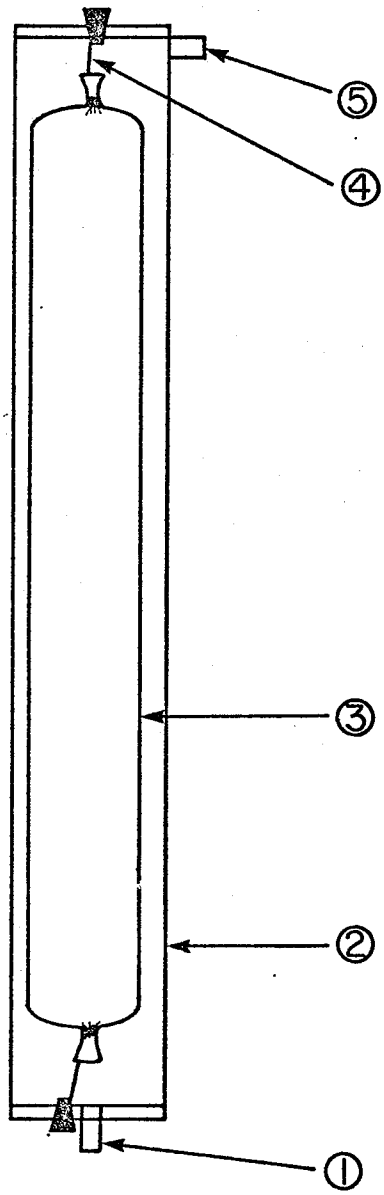
Microbasin Nutrient Enrichment Experiments

Two separate series of nutrient enrichment experiments were carried out using tryptic soy broth (Difco). In the first experimental series, TSB (tryptic soy broth) was added directly to the sediments during the microbasin set-up process with the result being 1 or 4 gm TSB/microbasin (approx. 16 ℓ sediment). In a separate series of experiments, the TSB was not added directly to the sediments but instead the addition was made to the L239 water flowing through the microbasins and was approx. 35 mg TSB/basin/day.

Dialysis Cylinder Design and Operation

Dialysis cylinders, used in a preliminary attempt to test for methylation of mercury in microbasin effluent, were constructed of 5 cm diameter (approx. 4 cm I.D.) plexiglass tubing (Fig. 5). Effluent from an aerated Wabigoon River 4 µg Hg/gm sediment basin (1 ng Hg/ml in effluent) was placed into a 4.5 x 60 cm piece of Spectapor dialysis tubing (Spectrum Medical Indust., Los Angeles). The tubing had previously been washed with heavy metal cleaning solution (132908) and sulfur cleaning solution A + B (132906) (Spectrum Medical Indust.) so as to remove heavy metal and sulfur residues. One end of the dialysis cylinder was unscrewed and the dialysis bag was placed

Figure 5. Schematic diagram of a dialysis cylinder. 1) L239 water inflow from cassett pump; 2) plexiglass dialysis cylinder; 3) dialysis bag containing microbasin mercury-containing effluent; 4) string suspending dialysis bag within dialysis cylinder: the string is attached to a cork stopper; 5) dialysis cylinder effluent outflow.


⑤
④
③
②
①

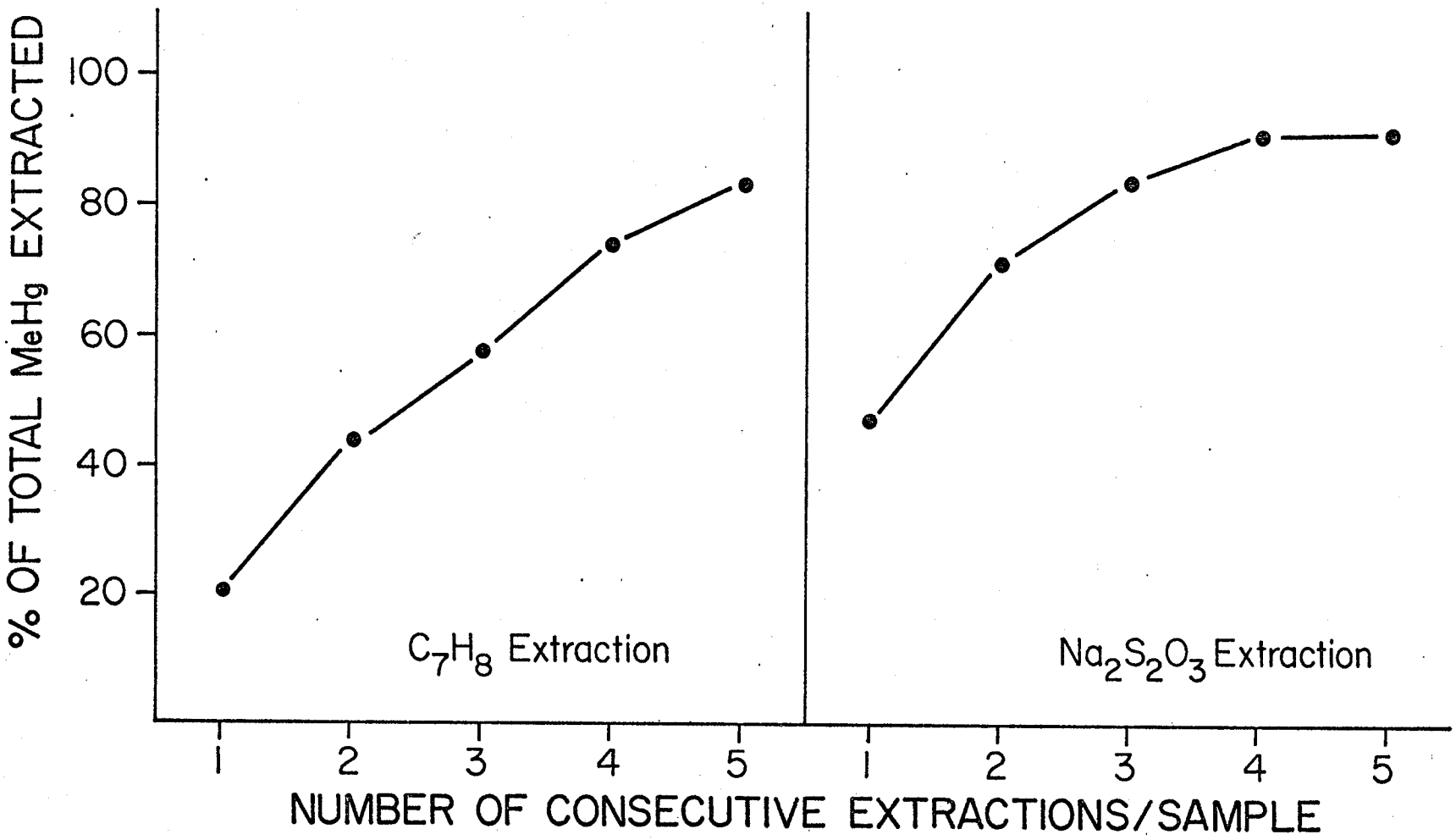
within the cylinder. The bag (with an approx. volume of 400 cm^3) was suspended in the cylinder by way of a string at each end of the bag. The ends of the cylinder were sealed with RTV 108 silicone rubber adhesive and secured with screws. The dialysis cylinder was mounted in a vertical position and water was forced through a hole in the bottom of the cylinder, over the surface of the dialysis bag, and out a hole in the top of the cylinder. The flow rate was 1 ℓ /24 hours. Dialysis cylinder effluent was collected (over 24 hours) and extracted for methyl mercury.

Two experiments were carried out using the dialysis cylinder system. In one experiment, L239 water was passed through the cylinder. In the other, the L239 water used was enriched with (approx.) 35 mg TSB/ ℓ .

Methyl Mercury Analysis

Effluents (1 ℓ samples collected over 24 hours) were extracted in a 2 ℓ separatory funnel by the method of Uthe et al (72). The funnel was shaken on an Equipoise Heavy-Duty Shaker (265 cycles/min.) upon which a 2 ℓ separatory funnel cradle had been secured. The results were corrected for a 67% extraction efficiency (measured by extracting methyl mercury spiked L239 water). An extraction efficiency profile is shown in Fig. 6.

Figure 6. A methyl mercury extraction profile. The method used (66) extracts methyl mercury from water into toluene (C_7H_8), from toluene into sodium thiosulfate ($Na_2S_2O_3$), and from sodium thiosulfate into benzene. Four and three consecutive extractions of toluene and sodium thiosulfate, respectively, were used throughout this study. The overall extraction efficiency, the product of the toluene and thiosulfate extractions, was about 67%.

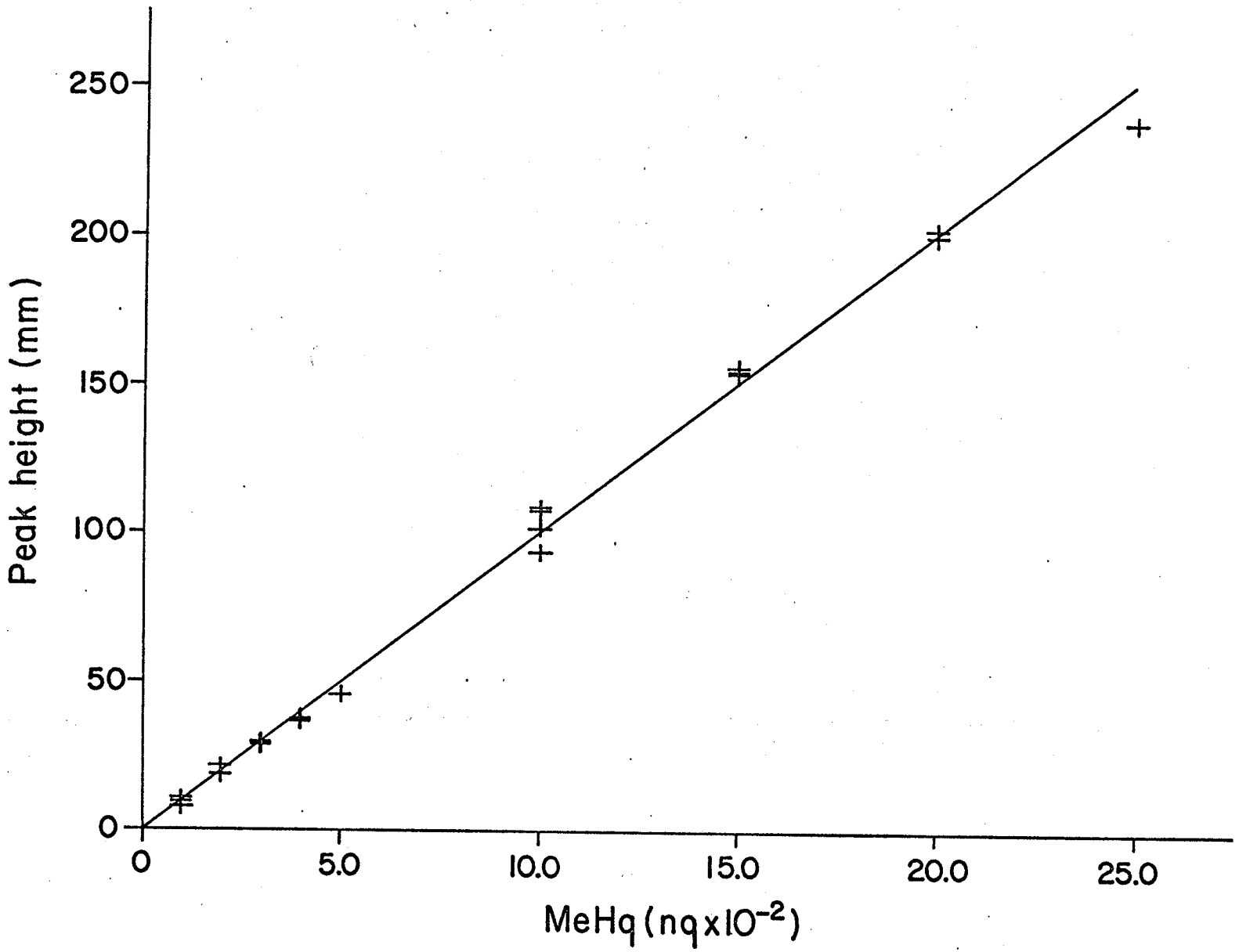

% OF TOTAL MeHg EXTRACTED
100
80
60
40
20
C_7H_8 Extraction
$Na_2S_2O_3$ Extraction
1
2
3
4
5
1
2
3
4
5
NUMBER OF CONSECUTIVE EXTRACTIONS/SAMPLE

Analysis was by GLC with electron capture detector (72). The detector response to injection of methyl mercury standards is shown in Fig. 7.

All glassware used in the analysis for methyl mercury was washed with H_2SO_4 (conc.), $Na_2S_2O_3$ (3M), and rinsed thoroughly with distilled water.

Toluene and benzene used in analysis was pesticide grade from Caledon Laboratories Ltd. (Georgetown, Ont.). Methyl mercury (95%) was obtained from Alfa Inorganics (Danvers, Ma.) and all other chemicals were Baker "analyzed" (Phillipsburg, N.J.) or the equivalent thereof.

Figure 7. Methyl mercury standard injection curve for the GLC electron capture detector.


250
200
150
100
50
0
Peak height (mm)
0
5.0
10.0
15.0
20.0
25.0
MeHq (nq x 10⁻²)

RESULTS

RESULTS

All results, except for those specifically identified as being from dialysis cylinders, are from experiments carried out using the microbasin flow-through system.

Effect of Mercury Concentration on Net Methyl Mercury Production

Microbasin experiments within a given series, although not all carried out simultaneously, showed reproducible and predictable results. The release of methyl mercury as a function of time from Clay Lake and Wabigoon River mercury laden sediments (Figs. 8 and 9) characteristically demonstrated lag, increase, maximum, decrease and level-off phases. The net production of methyl mercury in a particular microbasin characteristically reached a maximum after about 4 weeks. This was preceded by an initial lag in methyl mercury release during the week immediately following the setting up of a basin. After the period of maximum methyl mercury release, the net production of methyl mercury was observed to drop to a steady state (level-off) value which was characteristic of a particular sediment mercury concentration.

Figure 8. Net release of methyl mercury from mercury contaminated Clay Lake sediment as a function of time showing 1) lag; 2) exponential; 3) maximum; 4) decrease; and 5) level-off phases.

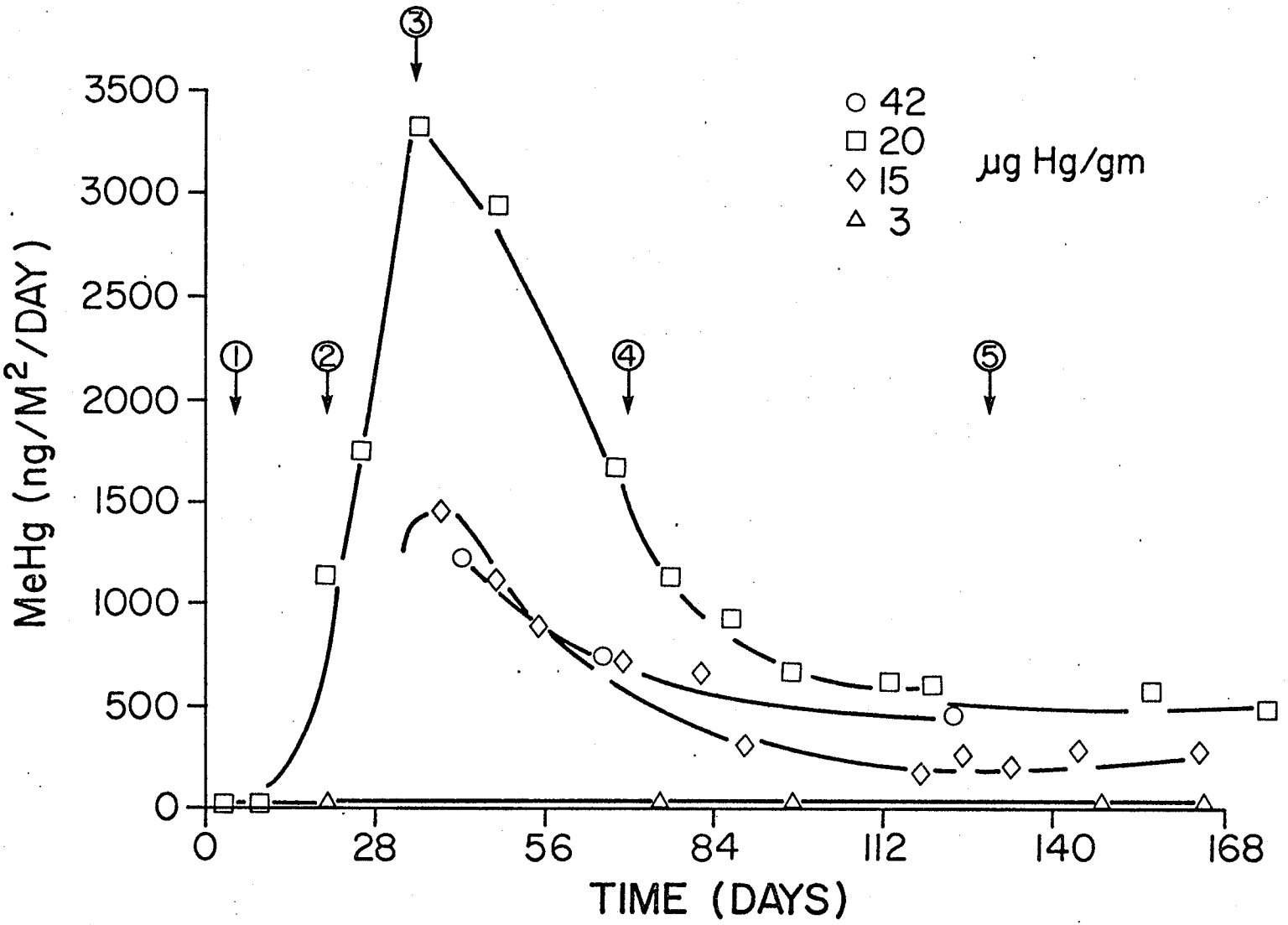
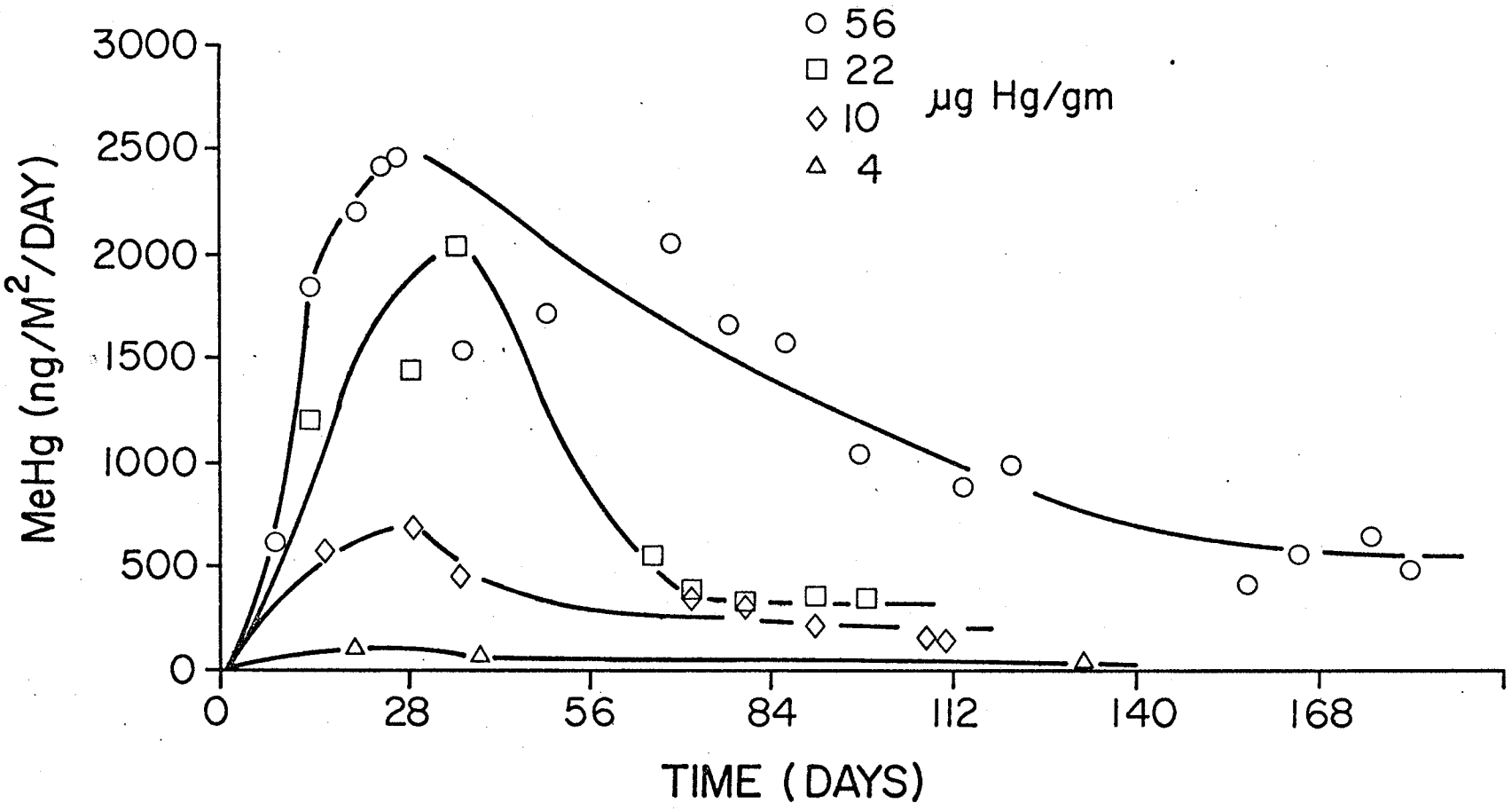

3500
3000
2500
2000
1500
1000
500
0
MeHg (ng/M²/DAY)
0
28
56
84
112
140
168
TIME (DAYS)
① ② ③ ④ ⑤
○ 42
□ 20
◇ 15
△ 3
µg Hg/gm

Figure 9. Net release of methyl mercury from mercury contaminated Wabigoon River sediments as a function of time. Characteristics (i.e. lag, etc.) same as in Fig. 8.

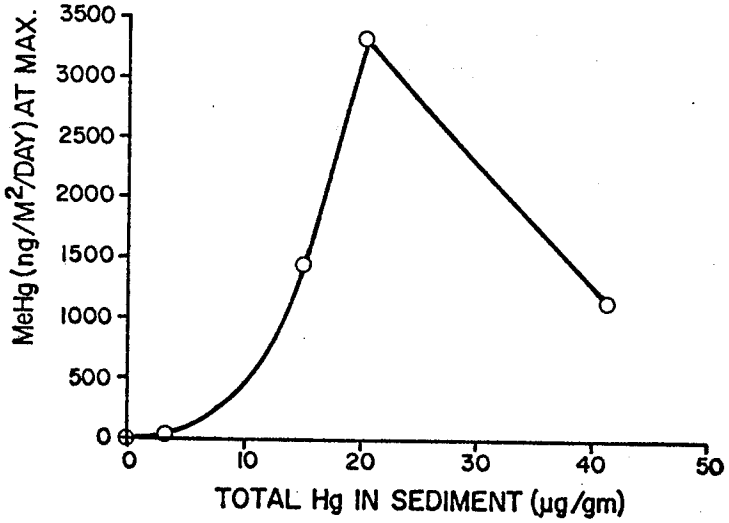

○ 56
□ 22
◇ 10
△ 4
µg Hg/gm
MeHg (ng/M²/DAY)
3000
2500
2000
1500
1000
500
0
0
28
56
84
112
140
168
TIME (DAYS)

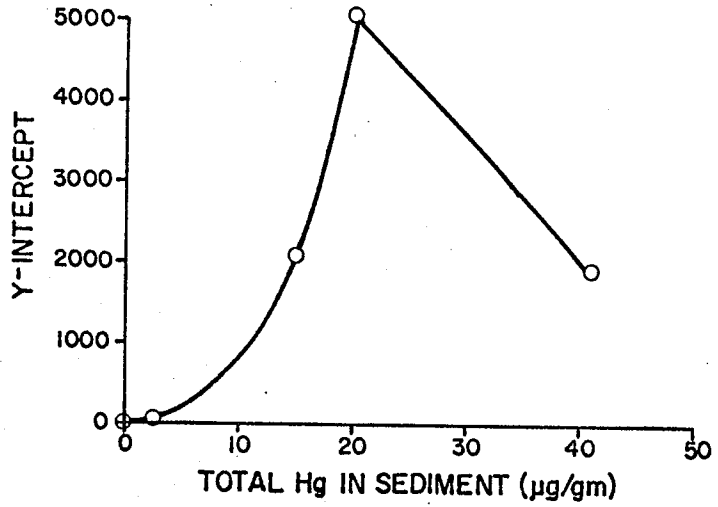
In Fig. 10 a comparison of Clay Lake microbasin performance characteristics (from Fig. 8) are shown as a function of sediment mercury concentration. It can be seen that any of a variety of plotting techniques produces curves which are very similar. Regardless of the method of treatment, these data suggest that a linear increase in the total mercury concentration in Clay Lake sediment resulted in an exponential rise in net methyl mercury release. At least, methylation increases more steeply than mercury concentration in sediment, up to approximately 20 μg Hg/gm. Similar results were obtained in Wabigoon River microbasin experiments (Fig. 11).

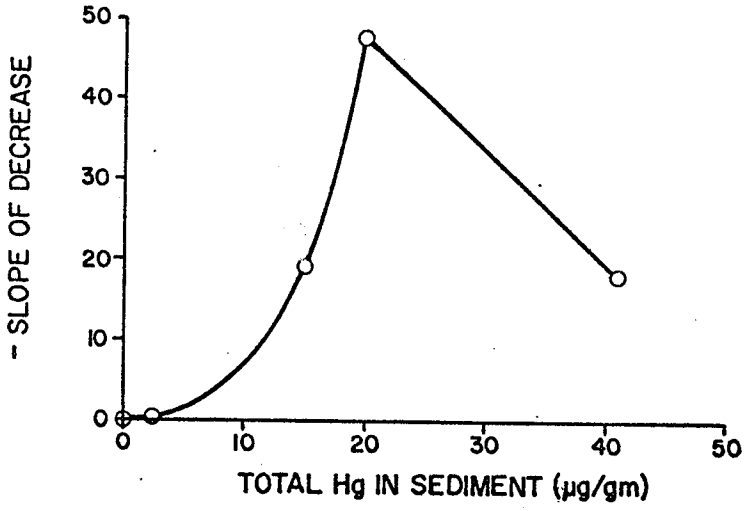
Effect of Aeration

In the headspace of a microbasin there exists a region where gas evolved from sediment in the basin accumulates and is effectively trapped (Fig. 2). It could well be imagined that a microbasin is an anaerobic system as the only source of oxygen is the oxygen dissolved in water flowing through the basin. With the low flow rate used in these studies the contribution of oxygen from this source is minimal. To see what effect aeration of the microbasin headspace would have upon the net release of methyl mercury observed in basin effluent, a Wabigoon River microbasin was set up in the normal manner except that room air (activated charcoal filtered) was pumped through the headspace of the basin.

Figure 10. Plotting characteristics from Fig. 8 (i.e. level-off values, etc.) as a function of total sediment mercury concentration.


MeHg (ng/M²/DAY) AT MAX.
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
TOTAL Hg IN SEDIMENT (µg/gm)


Y-INTERCEPT
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
TOTAL Hg IN SEDIMENT (µg/gm)


- SLOPE OF DECREASE
50
40
30
20
10
0
0
10
20
30
40
50
TOTAL Hg IN SEDIMENT (µg/gm)

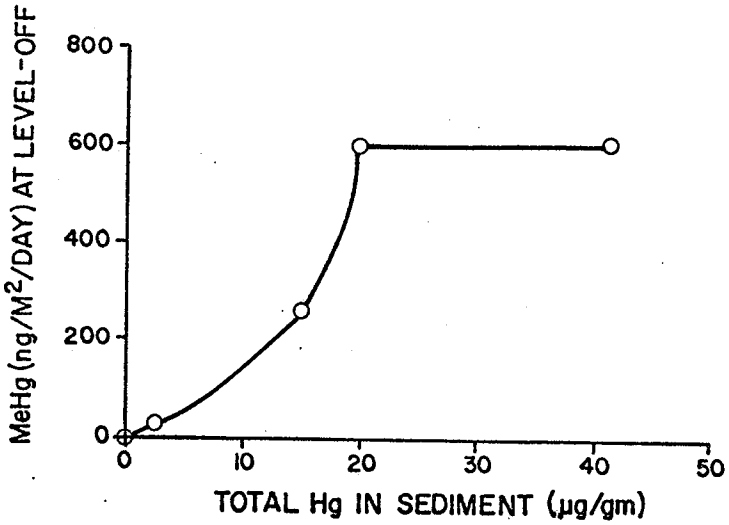
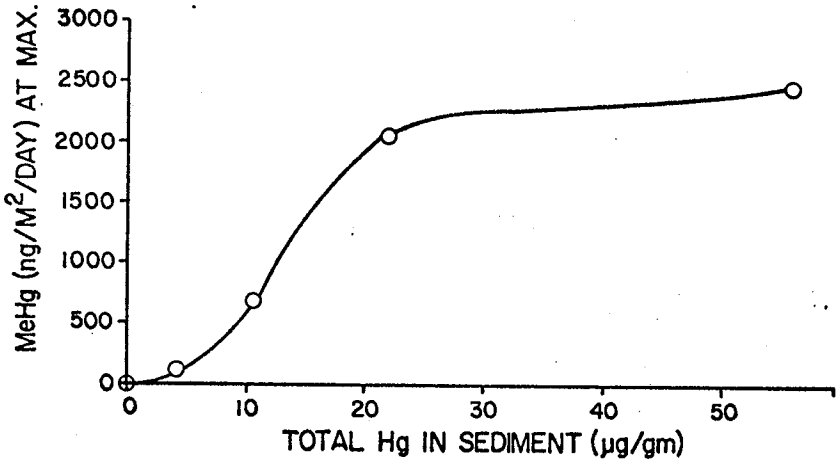
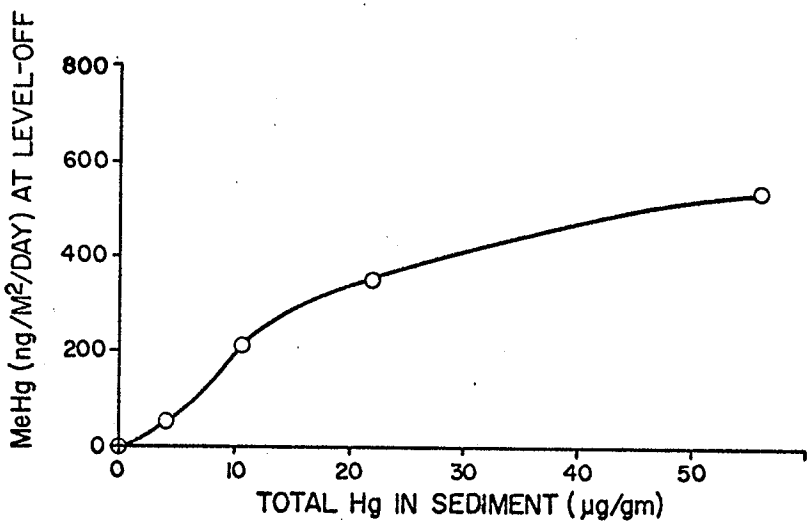

MeHg (ng/M²/DAY) AT LEVEL-OFF
800
600
400
200
0
0
10
20
30
40
50
TOTAL Hg IN SEDIMENT (µg/gm)

Figure 11. Plotting characteristics from Fig. 9 (i.e. level-off values, etc.) as a function of total sediment mercury concentration.


MeHg (ng/M²/DAY) AT MAX.
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
TOTAL Hg IN SEDIMENT (µg/gm)


MeHg (ng/M²/DAY) AT LEVEL-OFF
800
600
400
200
0
0
10
20
30
40
50
TOTAL Hg IN SEDIMENT (µg/gm)

There was no observable difference in the net methyl mercury release in the aerated microbasin relative to a non-aerated basin (Fig. 12) at a similar sediment mercury concentration. The aerated basin's effluent was, however, much more turbid than that of the non-aerated basin. Also, the aerated basin showed a higher release of total mercury than did the non-aerated basin (Table 1) which is probably a reflection on the observed higher activity of benthic animals (primarily oligochetes) in the aerated microbasin. Although these benthic invertebrates were present in all basins, over the long term their survival and activity was observed to be greatly enhanced in the aerated microbasin. The presence, absence, or apparent activity of these animals had no demonstrable effect on the level of methyl mercury released from (4 μg Hg/gm) Wabigoon River sediment.

Effect of 4°C Incubation Upon Net Methyl Mercury Release

The net production of methyl mercury in microbasins incubated at 4°C is shown in Fig. 13. The net methyl mercury release rates observed at 4°C are somewhat reduced from those obtained from similarly contaminated sediments in microbasin 20°C experiments. However, as can be seen in Table 2, the release of methyl mercury from microbasins incubated at 4°C is still (approx.) 50-70% of the net methyl mercury production rates observed in 20°C microbasin experiments.

TABLE 1. Total mercury in effluent from microbasins containing Wabigoon River sediment.

µg Hg/gm sediment	ng Hg/ml effluent
56 (nonaerated)	0.413
23 "	0.458
10 "	0.238
4 "	0.317
4 (aerated)	1.144

Figure 12. Net methyl mercury release from mercury contaminated Wabigoon River sediment (4 µg Hg/gm) in aerated and nonaerated microbasins, as a function of time.

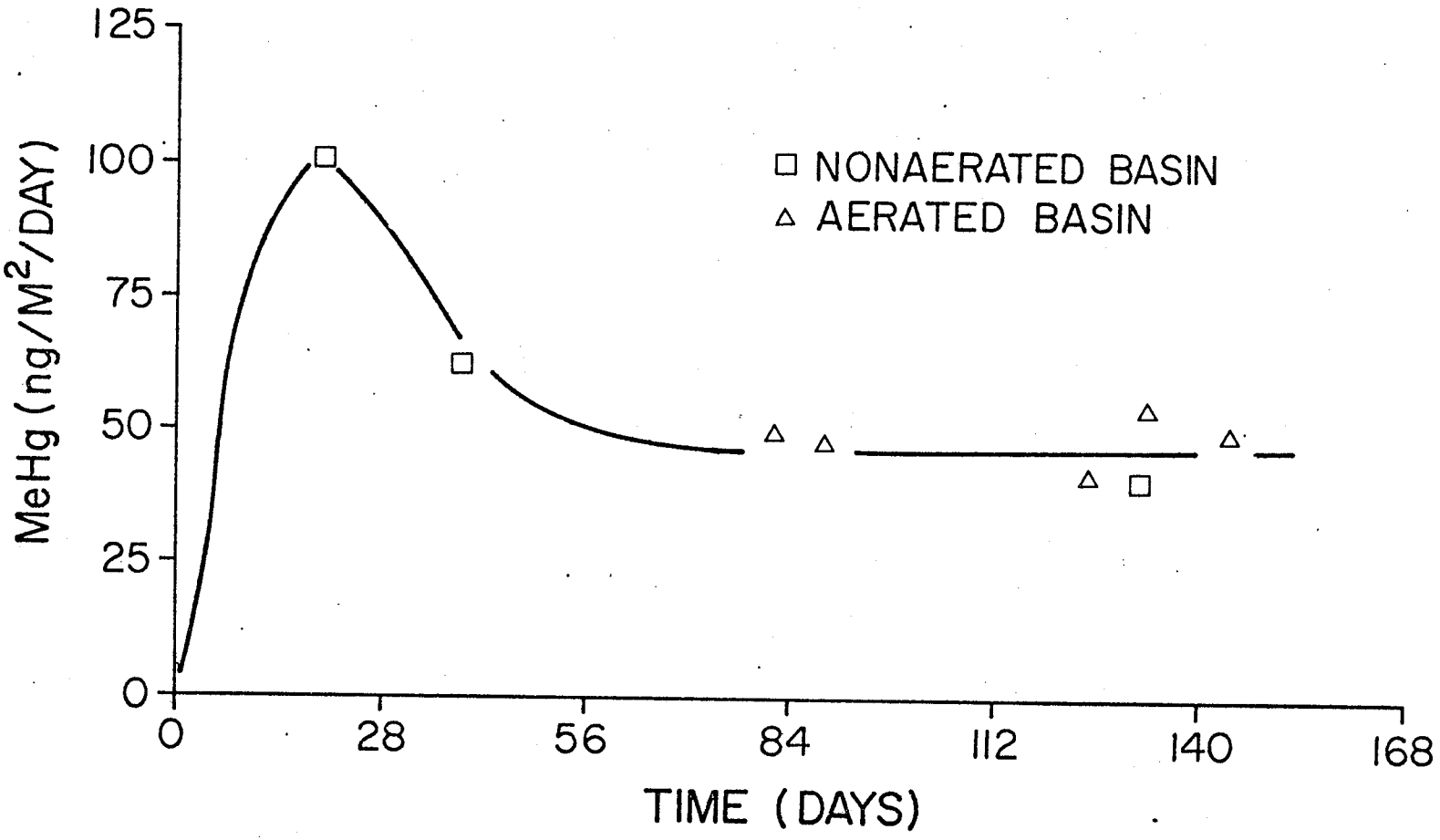

125
100
75
50
25
0
MeHg (ng/M²/DAY)
□ NONAERATED BASIN
△ AERATED BASIN
0
28
56
84
112
140
168
TIME (DAYS)

Figure 13. Net release of methyl mercury at $4^{o}C$ as a function of time from Wabigoon River and Clay Lake sediments.

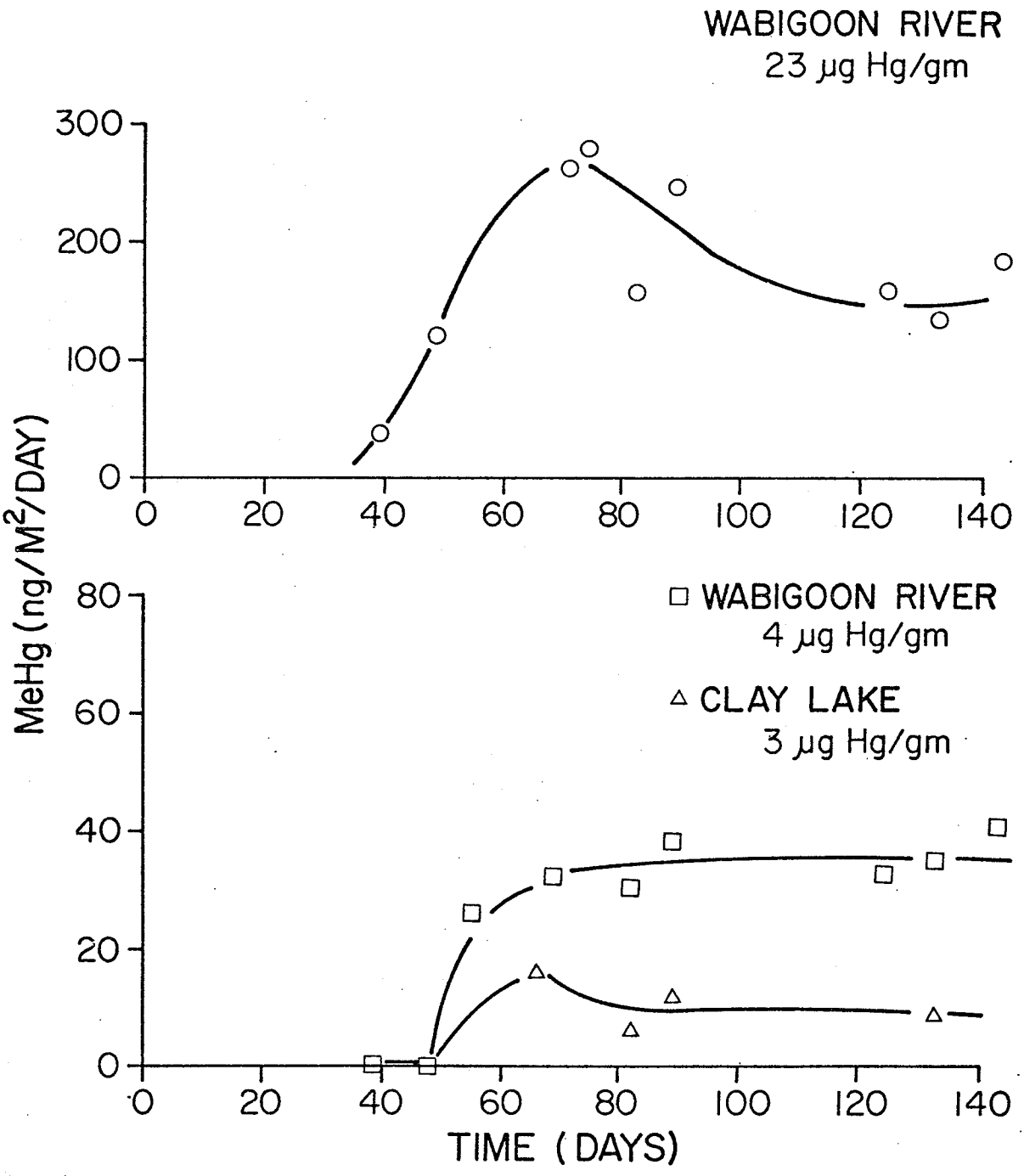

WABIGOON RIVER
23 µg Hg/gm
300
200
100
0
0
20
40
60
80
100
120
140
MeHg (ng/M²/DAY)
□ WABIGOON RIVER
4 µg Hg/gm
△ CLAY LAKE
3 µg Hg/gm
80
60
40
20
0
0
20
40
60
80
100
120
140
TIME (DAYS)

TABLE 2. Percent net methyl mercury release, in microbasin effluent, at 4°C relative to 20°C (using level-off values).

	µg Hg/gm in sediment	%
Clay Lake	3	63
Wabigoon River	4	70
Wabigoon River	22	50

Effect of Direct TSB Addition to L239 Sediments

Lake 239 sediments were enriched with mercury so that methyl mercury release from newly contaminated sediments could be measured. As shown in Fig. 14, net methyl mercury release from L239 sediment enriched to 48 µg Hg/gm (dry wt.) was extremely low (< 40 ng/m^2/day over an 8 month period). However, when L239 sediments were enriched with nutrient (TSB), as well as mercury, during the basin set-up process, net methyl mercury release was greatly enhanced (Fig. 14). Although net methyl mercury production in nutrient enriched L239 basins was not at a stable level, it is clear that without nutrient addition, the release of methyl mercury from L239 mercury contaminated sediments was only about 1% of that from the (4 gm TSB) nutrient enriched L239 basin.

Effect of TSB Addition to Microbasin Inflow Water

The L239 water passing over the surface of mercury contaminated L239 sediments was enriched with TSB (approx. 35 mg TSB/day). Prior to the nutrient addition the L239 basin had released only a minimal amount of methyl mercury but, as shown in Fig. 15, the addition of nutrient resulted in an immediate increase in methyl mercury production. Clay Lake sediments treated similarly demonstrated the same effect (Fig. 15).

Figure 14. Net release of methyl mercury from L239 nutrient (TSB) and/or mercury enriched sediments as a function of time.

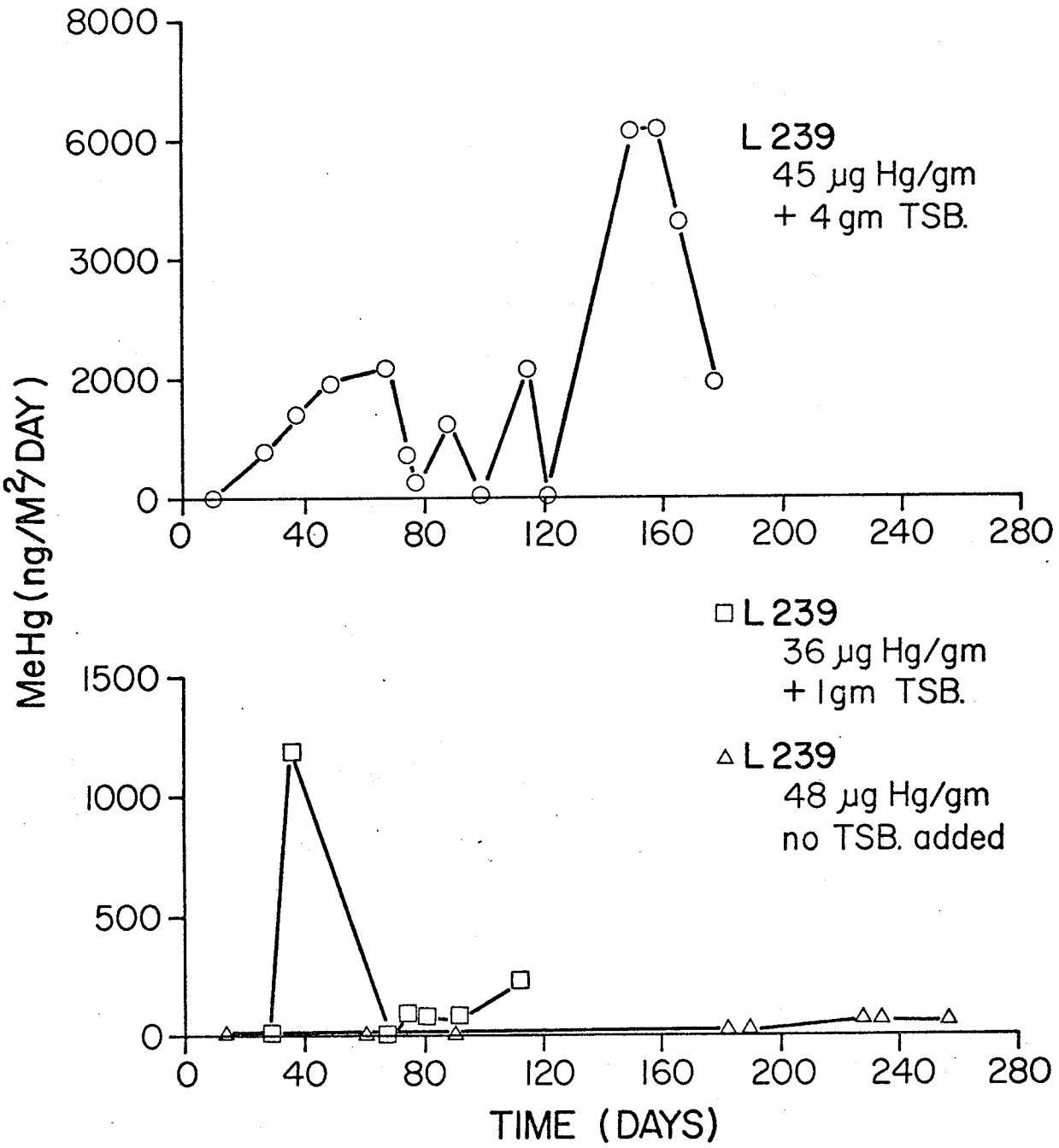
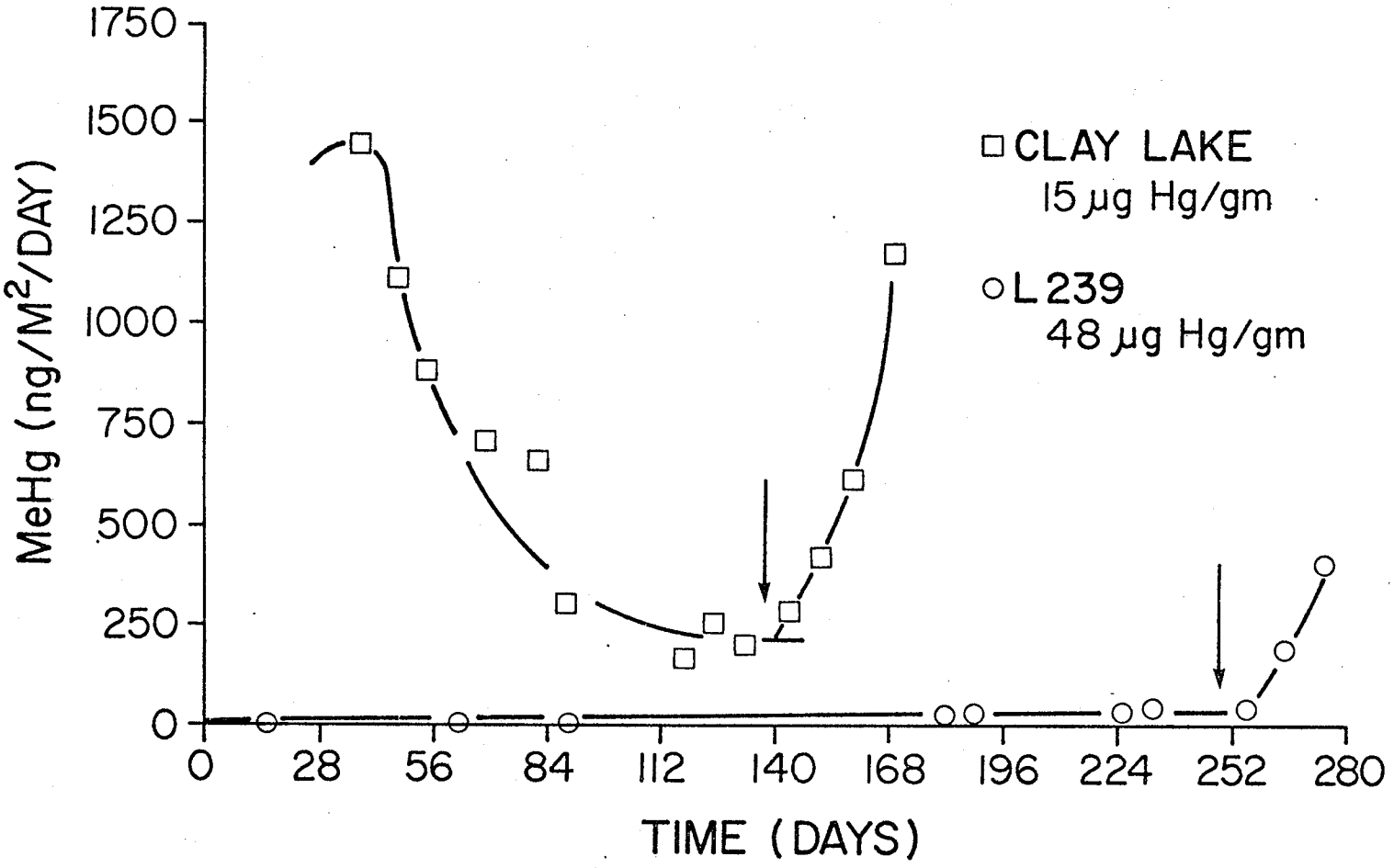

8000
6000
3000
2000
0
0
40
80
120
160
200
240
280
L 239
45 µg Hg/gm
+ 4 gm TSB.
MeHg (ng/M²/DAY)
□ L 239
36 µg Hg/gm
+ 1gm TSB.
△ L 239
48 µg Hg/gm
no TSB. added
1500
1000
500
0
TIME (DAYS)

Figure 15. Net release of methyl mercury from Clay Lake and L239 sediments as a function of time, showing the effect of addition of (approx.) 35 mg TSB/ microbasin/day to inflow water. The arrows indicate when nutrient addition began.


1750
1500
1250
1000
750
500
250
0
MeHg (ng/M²/DAY)
0
28
56
84
112
140
168
196
224
252
280
TIME (DAYS)
□ CLAY LAKE
15 µg Hg/gm
○ L 239
48 µg Hg/gm

Overall Release of Methyl Mercury from English-Wabigoon River System Sediments

The sediment from the Wabigoon River, contained much wood chip and fiber and differed significantly in its makeup from the predominantly clay sediments obtained from Clay Lake. Regardless of sediment origin, however, a relationship was found between total sediment mercury concentration and net methyl mercury release (Figs. 16, 17 and 18). The figures suggest that the methylation process exhibits typical saturation kinetics in that a further increase in the total inorganic mercury concentration in methyl mercury producing sediments (in excess of 15-20 µg Hg/gm, dry wt.) did not result in an increased net production of methyl mercury.

Methyl Mercury Production in Dialysis Cylinders

In a preliminary dialysis experiment, effluent from 2 dialysis cylinders (one with L239 water and one with TSB enriched L239 water) was collected and analyzed for methyl mercury on day 7 and 10 of the experiment. As can be seen in Table 3, a net production of methyl mercury was observed in the TSB enriched dialysis cylinder showing that methyl mercury can be produced in a column of water, as well as in sediments. No methyl mercury was detected in the non-enriched cylinder's effluent. It should be

Figure 16. Log maximum net release of methyl mercury (maximum values taken from Figs. 8 and 9) from Clay Lake and Wabigoon River microbasins, as a function of total sediment mercury concentration.

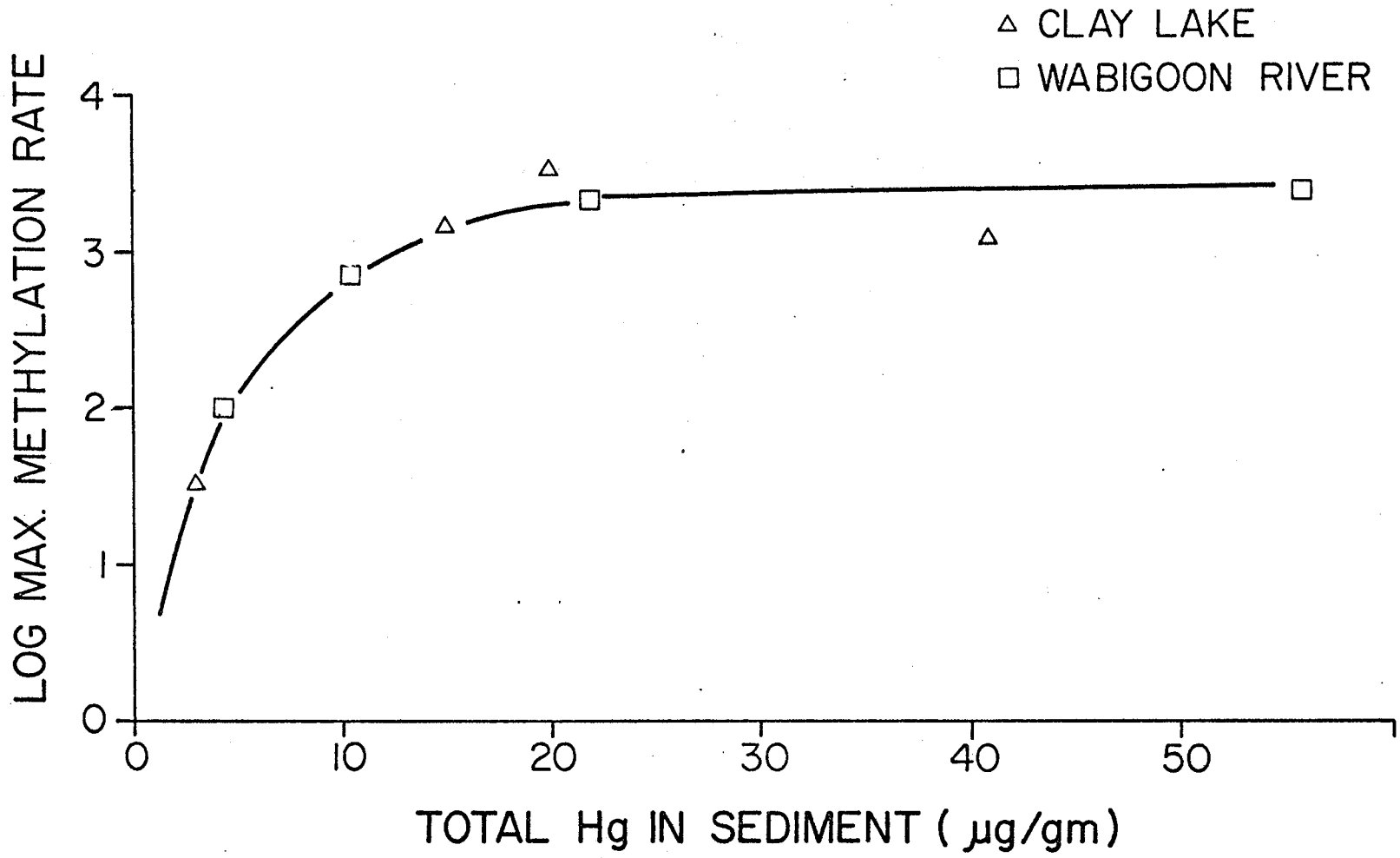

△ CLAY LAKE
□ WABIGOON RIVER
LOG MAX. METHYLATION RATE
0
1
2
3
4
0
10
20
30
40
50
TOTAL Hg IN SEDIMENT (µg/gm)

Figure 17. Log level-off rate of net methyl mercury release (level-off values taken from Figs. 8 and 9) from Clay Lake and Wabigoon River microbasins as a function of total sediment mercury concentration.

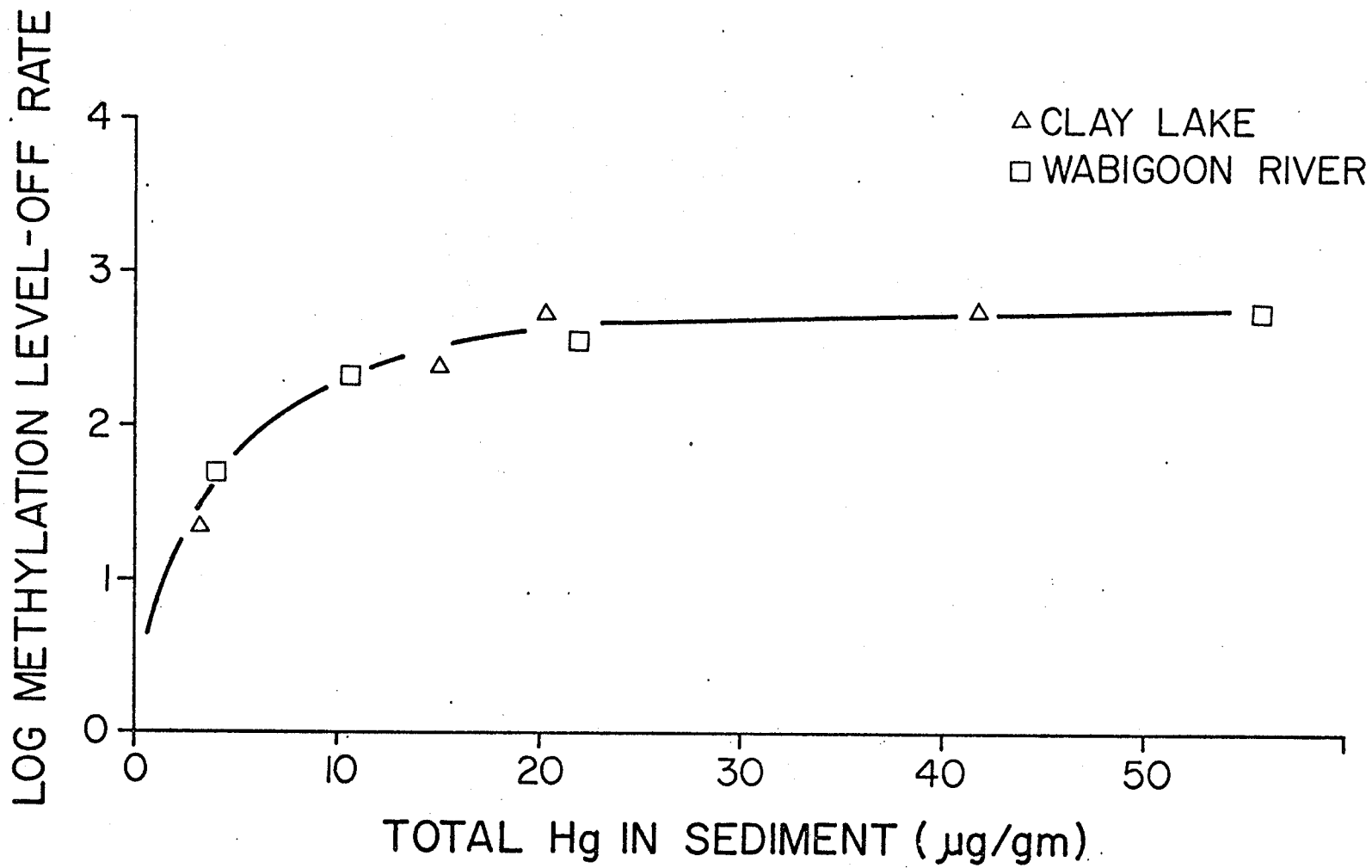

LOG METHYLATION LEVEL-OFF RATE
4
3
2
1
0
0
10
20
30
40
50
TOTAL Hg IN SEDIMENT (µg/gm)
△ CLAY LAKE
□ WABIGOON RIVER

Figure 18. Percent decrease of level-off net methyl mercury release from maximum net methyl mercury release for Clay Lake and Wabigoon River microbasins (from Figs. 8 and 9) as a function of total sediment mercury concentration. The percent decrease of level-off net methyl release from maximum net methyl mercury release was determined for each total mercury concentration by:

$$\frac{\text{maximum release - level-off release}}{\text{maximum release}} \times 100$$

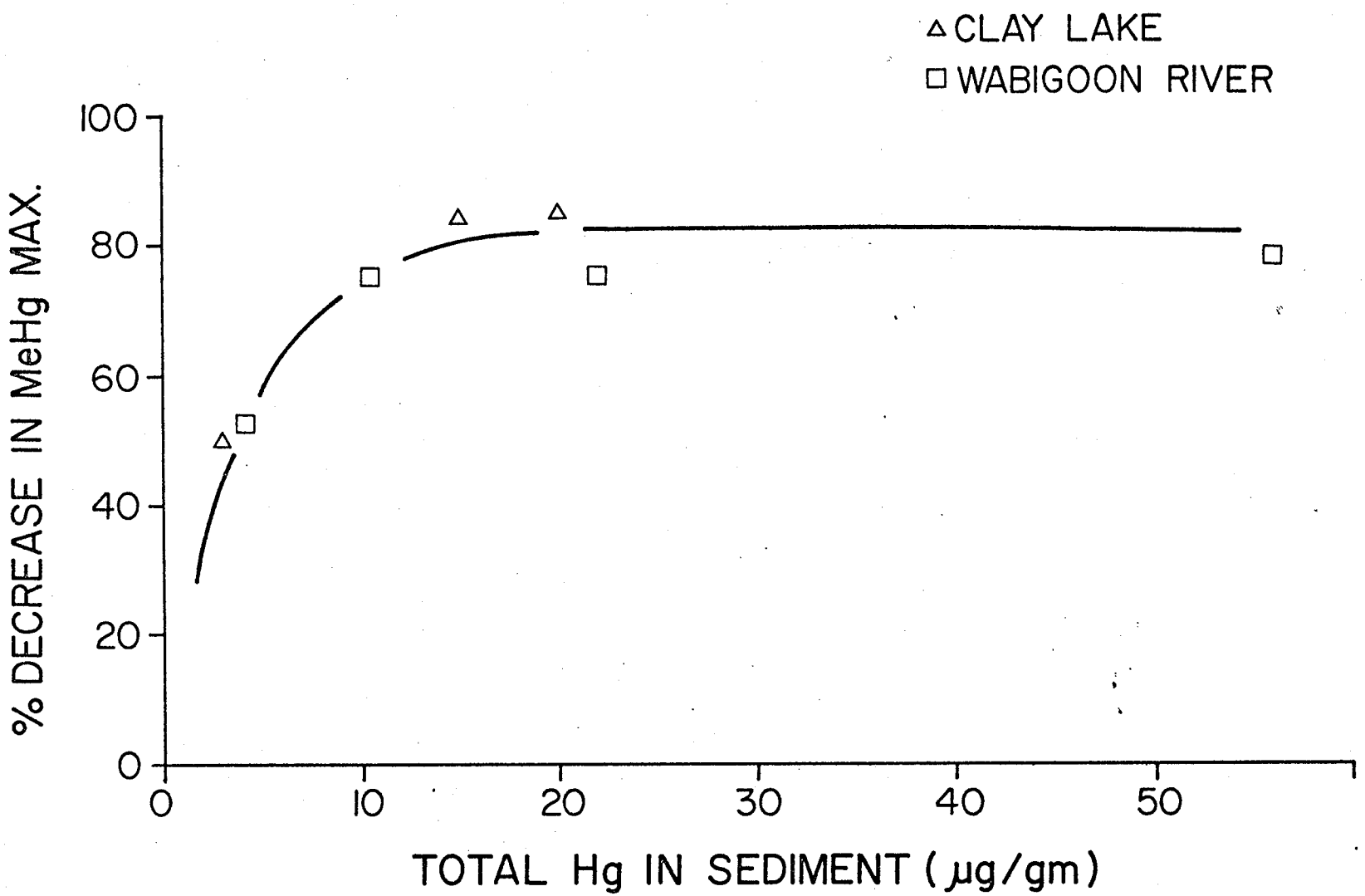

△ CLAY LAKE
□ WABIGOON RIVER
% DECREASE IN MeHg MAX.
100
80
60
40
20
0
0
10
20
30
40
50
TOTAL Hg IN SEDIMENT (µg/gm)

TABLE 3. Net methyl mercury production (ng/day) observed in dialysis cylinder effluent.

	day 7	day 10
dialysis cylinder with L239 water	-	-
dialysis cylinder with L239 water + 35 mg TSB/day	2.5	2.0

noted, however, that the methyl mercury production values obtained for the enriched cylinder are just above the detection limit of the methyl mercury analysis method. It is possible, therefore, that there was a lower rate of methyl mercury production in the non-enriched dialysis cylinder but that it was not detected.

D I S C U S S I O N

DISCUSSION

Microbasin Dynamics

The lag phase characteristically observed during the week after the setting up of a basin can probably be attributed to the effects of handling of the sediment during the collection, transport, and microbasin set-up process. The lag would be particularly pronounced where microbiological communities responsible for methyl mercury production are stratified with respect to chemical and physical features in sediment such as Eh, for example. During the lag period in methyl mercury release a small amount of a compound tentatively identified (from its elution time) as ethyl mercury was often detected in basin effluents. The ethyl mercury was transitory in its occurrence, disappearing as soon as methyl mercury production began, and so it appeared as though a succession of microorganisms was occurring within microbasin sediments.

The presence of demethylating microorganisms in methyl mercury producing sediments has been clearly documented (12, 13, 64). Spangler *et al* (64) showed that after an initial increase in methyl mercury levels over a 1½ month period, the concentration of methyl mercury in their batch flask-sediment cultures dropped drastically.

The methyl mercury decrease corresponded directly to an increase in elemental mercury released from their experimental flasks and they concluded that demethylating organisms were responsible for the methyl mercury loss. Billen et al (12) conducted experiments dealing with an observed methyl mercury mineralizing activity and they concluded that in mercury polluted environments an equilibrium between methylation and mineralization might be reached. The decrease in net methyl mercury production in microbasin experiments (Figs. 8 and 9), and the resulting steady-state methyl mercury levels observed thereafter, may well be a result of such a methylating-demethylating (mineralizing) equilibrium.

Comparison of the Total Sediment Mercury Concentrations Used in Microbasin Experiments with Total Sediment Mercury Concentrations Found in Nature.

Other investigators (9, 52, 54, 58, 59) have shown that an increased mercury concentration in sediments results in a rise in methyl mercury production. The microbasin studies have shown that in the concentration range of up to 15-20 μg Hg/gm there was an increase in net methyl mercury production greater than one would expect with a linear increase in sediment mercury concentration. Above this concentration range the net methyl mercury release was at a stable level. These observations are important because the mercury concentration range examined in micro-

basin experiments was within that observed to occur in nature. Armstrong and Hamilton (23) found that although the mean mercury concentration in Clay Lake sediments examined was 3.1 µg Hg/gm, the range was from 0.1 to 7.8 µg Hg/gm. According to microbasin results, the Clay Lake sediments with a high concentration of mercury (6-8 µg Hg/gm) may be "hot spots" for the release of methyl mercury. That is, a preliminary mass balance estimates that 9% of the total sediment surface area of Clay Lake (which is at approx. 7 µg Hg/gm) may contribute about 40% of the total net production of methyl mercury in the entire lake on a per unit time basis. Isolated regions of high net methyl mercury production may be even more prevalent in the Wabigoon River where mercury content of sediment varies greatly from one location to another (69).

It has been estimated that there is about 10,000 kg of mercury in Clay Lake sediments (23). If the rates of net methyl mercury production in microbasin experiments are at all similar to those occurring under natural conditions, the percent of total mercury released by sediments as methyl mercury on a yearly basis is very small (probably < 0.1%). Other investigators have made a similar estimate (16).

Methylation of Mercury at 4°C

It has been shown that methyl mercury production increases rapidly with a rise in temperature (51, 53, 54). However, when considering methylation in lakes such as those in Canada, where for a large portion of the year water temperature is very low, information concerning methyl mercury production at reduced temperatures is essential. The microbasin studies have shown that net methyl mercury release at 4°C is 50-70% of that from similar sediments at 20°C. Assuming a temperature in Clay Lake of 4°C for 8 months of the year and 20°C for 4 months, for example, as much as 50-60% of the methyl mercury in Clay Lake fish may be present as a result of low temperature methylation.

That methyl mercury production at low temperatures is so high should not be surprising as most investigators believe that microorganisms use the methylation process to detoxify their environment of mercury (40, 44, 73). If organisms find mercury as toxic at 4°C as at 20°C, then regardless of their reduction in metabolic activity at the reduced temperature, the rate at which they methylate mercury may have to remain above a certain critical level. At low temperatures microorganisms may have to utilize a greater percentage of their available energy for methylation to protect themselves from mercury's toxicity. If true, methylation would not, therefore, be

linear with respect to temperature. Additional low temperature methylation data support this conclusion (54).

Methylation of Mercury Upon Nutrient Addition

It is believed that nutrient availability is an important factor when considering the methylation process (51). In microbasins, L239 sediments contaminated with mercury over an extended period of time released very little methyl mercury, but the direct addition of 1 or 4 gm TSB during the basin set-up process (Fig. 14) resulted in large methyl mercury releases after 30 and 12 days of basin operation, respectively. Microbasin experiments also showed that the addition of a much smaller amount of nutrient (35 mg TSB/day) to the inflow water of a L239 microbasin, which had exhibited negligible methyl mercury production for over 240 days, caused a rapid increase in methyl mercury release (Fig. 15).

Other investigators have also shown that methyl mercury production rises as nutrient concentrations increase (51, 52, 53). However, microbasin results indicate that the overall concentration of nutrient in mercury methylating sediments is not so important as is the amount of nutrient available for microbial activity near the sediment-water interface. This suggests that methyl mercury released to the water column is methyl mercury that was produced in the very top layer of the

sediments. Although the methylation process might be active to great depths within sediments, it may well be that the methyl mercury produced more deeply in sediments is demethylated prior to its release from the sediments. This is probably why siltation (15), the covering of methyl mercury producing sediments with clean sediments, can be an effective means of reducing sediment methyl mercury releases.

A Preliminary Attempt to Evaluate Amelioration Through Controlled Eutrophication

The rate of formation of methyl mercury from HgS has been shown to be slower by (approx.) a factor of 10^3 than methyl mercury production from non-sulfide bound inorganic mercury (55). It has been suggested (55) that inorganic mercury in contaminated sediments might be sequestered as the highly insoluble and unreactive sulfide under the proper reducing conditions, thereby reducing the methyl mercury burden upon aquatic organisms. One practical means of attaining the proper reducing conditions might be through controlled eutrophication (T. Jackson, personal communication).

A preliminary testing of this proposal was carried out when TSB was added to the L239 water flowing through 2 microbasins (Fig. 15), one of which had released only an

insignificant amount of methyl mercury over an 8 month period. After the addition of nutrient the microbasins' sediments developed a general black patchiness. As well, the sediments characteristically exhibited a sulfide odor. However, as mentioned previously, the nutrient addition resulted only in an immediate increase in methyl mercury release.

Further microbasin - HgS formation experiments should examine the method and length of addition of less readily oxidizable substrates. Perhaps over an extended period of time such substrates could produce the proper reducing conditions without the accompanying observed surge in microbial methylation.

Water Column Methylation

The literature, with the exception of studies regarding methylation on and in fish, contains no experimental evidence to suggest that methyl mercury is produced at locations within lakes and rivers other than in mercury polluted sediments. Only indirect evidence points to the existence of a non-sediment methylation process. A decline in the methyl mercury levels in Clay Lake fish over the last 5 years correlates well with a decrease in total mercury in Clay Lake water while total mercury in Clay Lake sediment has remained essentially unchanged (F.A.J. Armstrong, personal communication).

Microbasin studies, as well as studies carried out by other investigators (51, 52, 53), have shown that the rate of production of methyl mercury declines with a decrease in total mercury concentration. Clay Lake water has less total mercury than Clay Lake sediment by a factor of 10^4 - 10^5 (50 ng/ℓ vs 3 mg/kg). It is therefore not surprising that methyl mercury production in the water column of Clay Lake, for example, has not been observed as the amount of methyl mercury formed per unit volume Clay Lake water must be very much smaller than that produced per unit Clay Lake sediment on the basis of total mercury concentration alone. As well, sediment methylation is really an "area" phenomenon whereas water column methylation deals with volumes. Clay Lake, for example, has a sediment surface area of 3×10^7 m^2, but it has a volume of 2.4×10^8 m^3 (23). Even if, under natural conditions, water column methylation is lower than sediment methylation by many orders of magnitude, water column methylation may still be extremely significant in a mass balance of total lake and/or river methylation.

For the most part, the first evidence for methyl mercury production in sediments was that increasing levels of methyl mercury in mercury containing sediments was detected through the use of very sensitive analytical techniques (9). The observation that the concentration of methyl mercury in sediments decreased under certain conditions was proof of the existence of demethylation (64).

It therefore seems likely that the potential for water column methylation has been ignored in the past primarily as a result of analytical restraints.

Information concerning the location of methylation within aquatic systems is fundamental to our understanding the methylation process as a whole. In the dialysis cylinder study, the actual amount of methyl mercury produced in the experiment is not important, but instead the significance of the dialysis cylinder experiment is that an initial attempt to detect methyl mercury formation within a simulated water column was successful.

An Overview of the Methyl Mercury Problem in the English-Wabigoon River System

Approximately 10,000 kg of inorganic mercury were released to the English-Wabigoon River system during 1962-1970 from a pulp and paper mill-chlor-alkali complex in the town of Dryden (23). The mercury losses have been curtailed, but the river system is still subjected to large discharges of organic matter from the pulp and paper mill. Parks (69), for example, notes that the average 1975 BOD_5 800 m upstream from the mill was 0.88 mg/ℓ whereas it was 28 mg/ℓ 1100 m downstream from the mill.

The microbasin nutrient addition experiments raise the serious question of what is the result when a readily oxidizable substrate is added to a mercury polluted river

in nature. As can be seen from Table 4, the amount of organic carbon (added as TSB) in microbasin experiments (Fig. 15) is at about the same level as that appearing in the Wabigoon River just downstream from the pulp and paper mill outfall. The microbasin experiments clearly showed that the addition of nutrient, at a level of equivalent to that being discharged to the river system, resulted in immediate increases in the release of methyl mercury from Clay Lake and L239 mercury contaminated sediments.

Although the microbasin nutrient enrichment experiments are only an artificial representation of nature, the results obtained are likely indicative of what can be expected to be observed in a natural system. The effect of temperature upon the methylation process, when considering the entire river-lake system, is likely insignificant. However, elevated localized concentrations of mercury in sediments and increased inputs of organic matter to the Wabigoon River probably result in an elevated methyl mercury burden upon aquatic organisms within the river system. Clearly, the industrial operation in question should immediately concentrate upon reducing its discharge of organic materials to the English-Wabigoon River system.

TABLE 4. Comparison of dissolved organic carbon (DOC) levels used in microbasin (and dialysis cylinder) experiments with that found in the Wabigoon River.

	mg DOC/ℓ
Wabigoon Lake (2 km upstream of Dryden)	5
Wabigoon River (4 km downstream from Dryden)	18
L239 reservoir water*	6
L239 water + TSB addition**	24

* courtesy of ELA chemistry laboratory

**This is a maximum amount, as TSB (assumed to be 50% DOC) was added to nonsterile L239 water in the reservoir. The TSB-L239 reservoir was cleaned, and fresh TSB-L239 water was made up, every 48 hours.

The Microbasin and Dialysis Cylinder Systems as Tools

Most of the work that has been done concerning methyl mercury production has only involved the analysis of lake or river sediments as to their total methyl mercury content. There has been some criticism of this approach in terms of the complex chemical and biological equilibria which are known to exist in the aquatic mercury cycle. To measure the total concentration of methyl mercury in sediment is of little value (40) as the measured concentration may not be indicative of the rate of methyl mercury synthesis in sediments as methyl mercury concentrations are probably at a steady-state level in sediments. In this study, through the use of the microbasin flow-through system, only the net release of methyl mercury from sediment was observed, and this observed net release is likely to be fundamentally related to the methyl mercury from sediment, which is available for uptake by organisms in the aquatic environment. As well, the dialysis cylinder study has cast doubt upon the supposition that all methyl mercury found to be enriched in fish was originally produced in sediments.

The significance of the experimental methods described in the present study is that practical tools are offered in the form of microbasins and dialysis cylinders which can be used in future laboratory (for example, amelioration) and field (in situ) methylation

studies. Microbasins and dialysis cylinders could also be useful in studying the mobilization and biotransformation of other contaminants (i.e. metals, metalloids, pesticides, etc.) in the environment. Obviously further in depth investigations are essential so as to identify the methylating sites in aquatic systems and the importance of each of these sites in the overall aquatic and global mercury cycles.

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