The Effect of pH on Methyl Mercury Production and Decomposition in Lake Sediments

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Mercury methylation was measured in surficial sediments taken from unacidified and experimentally acidified lakes in the Experimental Lakes Area, northwestern Ontario. A reduction in the pH of sediments lowered the rate of ^{203}Hg methylation. Methylation was undetectable at pH < 5.0. This decrease in mercury methylation was probably related to a shortage of available inorganic mercury when the pH of the sediment porewater was reduced. Below pH 6.0, inorganic mercury concentrations in porewater, measured with ^{203}Hg, were reduced to less than 20% of that found at unaltered pH. A comparison of methylation and demethylation rates was made at various pH's. The rate of demethylation decreased to a lesser extent than methylation as the pH was lowered. This research indicates that enhanced mercury methylation in the sediment is not responsible for the observed increase in mercury levels in fish from acidified lakes.

On a quantifié la méthylation du mercure de sédiments superficiels recueillis dans les lacs non acidifiés et expérimentalement acidifiés dans la zone de lacs expérimentaux. Une baisse du pH des sédiments a réduit le taux de méthylation du ^{203}Hg ; celle-ci n'était pas décelable à un pH inférieur à 0,5. Cette diminution de la méthylation est probablement liée à une pénurie de mercure inorganique disponible quand le pH des eaux interstitielles du sédiment est réduit. À un pH 6,0, les concentrations (mesurées au ^{203}Hg) de mercure inorganique des eaux interstitielles s'élèvent à moins de 20 % de celles présentes à un pH non modifié. Les taux de méthylation et de déméthylation ont été comparés à divers pH : le dernier a moins diminué que le premier quand le pH était réduit. La présente recherche révèle qu'une méthylation accrue du mercure dans le sédiment n'est pas la cause de l'augmentation observée des niveaux de mercure chez les poissons peuplant des lacs acidifiés.

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One of the early consequences of lake acidification appears to be elevated concentration of mercury in the muscle of fish (Tsai et al. 1975; Jernelov et al. 1976; Suns et al. 1980; R. J. P. Brouzes, Domtar Research Centre, Senneville, Que., pers. comm.). Mercury in fish muscle is mostly methyl mercury produced from inorganic mercury by bacteria active in sediments, in the water column, and in the intestines of fish (Jensen and Jernelov 1969; Furutani and Rudd 1980; Rudd et al. 1980). Chemical methylation of mercury occurs via transalkylation by tin and lead alkyls (Beijer and Jernelov 1979) and is probably not a major contributor to net methyl mercury. After its production, methyl mercury is bioaccumulated by fish from the water and from their food.

There are several possible hypotheses to explain the apparent increase in mercury concentration in fish during the early stages of acidification. For example, more mercury may be entering lakes with acidic deposition because of increased coal combustion (Joensuu 1971; Jernelov and Wallin 1973; Jernelov et al. 1975; NRC 1981). This may elevate rates of mercury methylation which are positively related to inorganic mercury concentration (Rudd et al. 1983). Further, the elevated acidity of atmospheric deposition could cause mobilization of mercury naturally present in watersheds or result in the solubilization of mercury in lake sediments (NRC 1981). Both of these mobilization processes would tend to increase the concentration of soluble divalent mercury, the form methylated by bacteria (DeSimone et al. 1973). In addition, increased lake acidity may cause physiological changes in fish, leading to either an increase in the efficiency of methyl mercury bioaccumulation by fish or a decrease in the rate of mercury depuration. Finally, increased lake acidity may affect the microorganisms, causing them to methylate mercury more rapidly or demethylate more slowly at a reduced pH, even though the mercury concentration may not be changed by acidification. We have tested some of these

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hypotheses by measuring rates of mercury methylation and demethylation in sediments at natural and at reduced pH. We have also studied some of the factors controlling methylation and how these could be affected by changes in pH.

Methods

Study Sites

This research was done at the Experimental Lakes Area (ELA) northwestern Ontario, which lends itself to studies of lake acidification because these lakes are located on granitic rock basins of limited buffering capacity (Brunskill and Schindler 1971). ELA is located in an area that is not significantly impacted by acidic deposition (Schindler and Ruszczynski 1983) and is uncontaminated by mercury. Lake 223 (L223) has been receiving experimental additions of sulphuric acid since 1976. The pH of the surface water has decreased from 6.8 in 1976 to approximately 5.1 in 1982 (Schindler and Turner 1982; Mills 1984). Lake 239 (L239), which has not been acidified, was used as a control lake in this study. Its water chemistry and primary production are similar to that of L223 prior to acidification (D. W. Schindler, Freshwater Institute, Winnipeg, Man., pers. comm.). The porewater pH of epilimnetic sediment is close to 6.2. The recent history of L239 is well documented (Schindler et al. 1980b). Sediment was also collected from L114, L303, and L302N. Lake 114 has been acidified with sulphuric acid since 1979; L302N has been acidified with nitric acid since 1982, and L303 was fertilized with phosphoric acid and sodium nitrate during 1975–76 (Cruikshank 1984).

Sample Collection

Surficial epilimnetic sediments were chosen for this study because they are the only sediments that are exposed to a reduced pH (Kelly and Rudd 1984; Kelly et al. 1984). They were retrieved from 4 m depth in L239 and 6 m depth in L223 using a modified Ekman grab sampler (Burton and Flannagan 1973). Approximately the top 2 cm of this sediment was drawn into a PVC bottle using a hand vacuum pump. One litre of sediment was collected for each experiment. A thermistor (Flett Research Ltd.) was used to measure lake water temperature.

pH Adjustment

In the laboratory, 100 mL of sediment was transferred, under deoxygenated nitrogen (Hungate 1969), into five 125-mL glass reagent bottles fitted with silicone stoppers. An 18-gauge needle attached to a three-way valve (American Hospital Supply) and syringe for addition of HCl and NaOH and a glass combination pH electrode (Fisher Scientific) were inserted through the silicone stopper. The pH electrodes were connected via a six-channel switchbox to a pH meter (Fisher model 630), which enabled us to monitor simultaneously up to six sediment samples. The pH meter was calibrated at pH 4 and 7. Four bottles of sediment were adjusted to four pH values ranging from 4.5 to 7.5 by addition of HCl or NaOH. The pH was adjusted intermittently over approximately a 2-h period until it stabilized. The sediment was stirred during pH adjustment. The pH of the fifth bottle was not adjusted and remained at the natural pH of approximately 6.2–6.5.

Sample Incubation and Extraction

Mercury methylation rates were assayed in duplicate samples taken from each bottle of pH-adjusted sediment using the radioisotopic method of Furutani and Rudd (1980). Because the mercury concentration of the sediment was increased by the addition of radiolabelled and carrier mercuric mercury, this approach yielded a potential methylation rate rather than an actual in situ rate. For this reason our experiments were designed to compare relative methylation rates of manipulated samples with control samples. The main advantage of this approach over other methods for assaying mercury methylation is its high sensitivity which enables methylation to be assayed on fresh sediment samples after only a few hours of incubation. This can be done without stimulation of microbial activity by the addition of artificial substrates. Rates of mercury methylation were linear throughout the incubation period, which indicates that the natural microbial populations were being assayed.

After the sediment pH had been adjusted, duplicate 10-mL samples were drawn into 50-mL glass syringes (American Hospital Supply) that had been flushed with nitrogen. A 5-mL bubble of nitrogen was left in the syringe to facilitate mixing. Two additional 10-mL samples were treated with 1 mL of 4 $mol \cdot L^{-1}$ HCl to stop biological activity and to serve as blanks. Each sample in a syringe received an addition of ^{203}Hg as mercuric chloride (New England Nuclear) to an approximate dosage of 74 $kBq \cdot 2\ \mu g\ Hg^{-1} \cdot g$ dry $sediment^{-1}$. The samples were incubated for 12 or 48 h at in situ temperature (10–23°C). Incubation was terminated by addition of 1 mL of 4 $mol \cdot L^{-1}$ HCl. The contents of each syringe was transferred to a 250-mL glass reagent bottle. Alkylated ^{203}Hg produced during incubation was extracted and assayed as described in Furutani and Rudd (1980), which does not distinguish between monomethyl and dimethyl mercury. This method has a mean percent coefficient of variation of ±10.2% for 14 replicates.

Methylation rates (R) are expressed as nanograms of mercury methylated per gram of dry sediment per hour:

$$R = [(s - b)(d)] \cdot (a)^{-1} \cdot (w)^{-1} \cdot (t)^{-1}$$

where s = sample activity (disintegrations per minute (dpm)), b = activity of the killed blank (dpm), d = dilution factor from the extraction procedure, a = dpm added per nanogram of mercury, w = sample dry weight (grams), and t = incubation time (hours).

Porewater Experiments

The effect of pH on ^{203}Hg and $CH_3{}^{203}Hg$ concentration in the sediment porewater was also determined. Sediment pH was adjusted as previously described. Two samples were taken at each pH and $^{203}HgCl_2$ was added to each of the subsamples in the same concentrations as was used for the measurement of the rate mercury methylation. Radiolabelled methyl mercury ($CH_3{}^{203}Hg$) (562.4 $MBq \cdot mmol^{-1}$; Amersham) was added to a concentration of 1.76×10^4 $dpm \cdot mL^{-1}$ to different duplicate sediment samples. All of the samples were shaken for 1 min and then allowed to stand for 1 h. They were then centrifuged for 45 min at 1200 × g. A known volume of supernatant was filtered through a 0.45-μm filter (Millipore), and a 500-μL subsample of the filtrate was counted in a liquid scintillation counter (Beckman 7000). The mercury that passed through the filter was defined as available porewater mercury (Benes and Havlik 1979). The percent of available ^{203}Hg in the sediment porewater was calculated as follows:

$$\text{Percent of } {}^{203}Hg \text{ or } CH_3{}^{203}Hg \text{ in porewater} = \frac{100pv}{d}$$

where p = porewater activity (dpm per millilitre), v = volume of porewater (millilitres), and d = ^{203}Hg or $CH_3{}^{203}Hg$ as dpm added to each sample.

Acid-Volatile Sulfide (AVS) Experiments

AVS is defined as sulfide-containing compounds, such as some forms of metal-S (e.g. FeS), which are volatilized as H_2S under acidic conditions. If sulfide was released from previously insoluble metal sulfides by acidification of lakes, the formation of insoluble HgS might explain the reduction in mercury concentration and methylation rates that we observed at reduced pH. This possibility was tested by removing the AVS from sediments using a combination of methods developed by Jorgenson (1978) and Howarth and Teal (1979). Sediment was put into 60-mL reaction vessels and the pH was reduced to 2.0 with HCl. The AVS, as H_2S, was removed by bubbling the sediments with deoxygenated nitrogen and trapped in a solution of alkaline zinc acetate as zinc sulfide (Howard and Teal 1979). After the sediment had bubbled for 2 h, the trapped sulfide was quantified by iodimetry (Skoog and West 1969). The amount of AVS removed was calculated as follows:

$$AVS = 0.5(E - A)\cdot C$$

where AVS = micromoles of acid-volatile sulfide, E = millilitres of $S_2O_3{}^{2-}$ required to neutralize a known amount of iodine in a blank trap (no sulfide), A = millilitres of $S_2O_3{}^{2-}$ required to neutralize a known amount of iodine in a sample trap, and C = microequivalents per litre of the $S_2O_3{}^{2-}$.

To determine what effect the acid treatment of AVS had on porewater mercury concentration at natural and adjusted pH, the acid-treated sediment was raised to its original lake pH (approximately 6.3) by the addition of NaOH. Porewater mercury concentrations at natural and altered pH in treated sediments were then compared with porewater mercury concentrations in acid-treated sediments. In addition, FeS was added to another subsample of the acid-treated sediments at the original AVS concentration (Table 1) and the pH of this sediment was also adjusted. Measurement of porewater ^{203}Hg concentration in the three types of treatment of sediments was carried out as previously described.

Demethylation

The rate of microbial breakdown of methyl mercury, or demethylation, was determined by the method of P. S. Ramlal, J. W. M. Rudd, and R. E. Hecky (in prep.). Briefly, ^{14}C-methylmercuric iodide was added to a set of sediment samples acidified to different pH values. The isotope was added as CH_3HgI at a dose of 0.2 μg Hg·g dry sediment^{-1}. The samples were shaken for 1 min after isotope addition and incubated for 12 h at in situ temperatures. After incubation, the samples were acidified and the gaseous endproducts of demethylation were stripped from the samples by bubbling with oxygen (50 mL·min^{-1}) through Vycor tubes packed with copper oxide. The Vycor tubes were heated in a tube furnace to 450°C. This ensured that $^{14}CH_4$ was combusted to $^{14}CO_2$. The gases were collected in a carbon dioxide trap consisting of 10 mL of PCS, 2 mL of methanol, and 2 mL of CO_2mMet (Amersham) in a liquid scintillation vial. Samples were stripped for 1 h, an additional 5 mL of PCS was added to the vial, and the samples were counted to 2% error in a liquid scintillation counter. Both the trapping and stripping efficiency of this method was found to be 100%. In some instances, to facilitate comparison of methylation and demethylation, rates are presented as percentages of the demethylation or methylation rates at natural pH.

TABLE 1. Concentrations of total mercury, total sulfur, and acid-volatile sulfide (AVS) removed from sediments taken from L239 (unacidified) and L223 (experimentally acidified).

Lake	Date (1982)	Treatment	Total S (mg·g^{-1})	AVS removed (μg·g^{-1})	Total Hg (μg·g^{-1})
L239	Sept. 13	None	2.3	0.0	0.35
		pH 2	1.4	4.2	0.36
	Aug. 17	pH 4	—	0.02	—
		pH 2	—	0.17	—
L223	Aug. 17	pH 4	—	4.19	—
		pH 2	—	10.17	—
	Sept. 20	None	2.1	0.0	0.36
		pH 2	2.1	55.8	0.35

Other Methods

Organic carbon and total nitrogen analyses were done using methods developed by Stainton et al. (1977). Dry weight of the sediment was determined by drying sediment to a constant weight at 60°C. Total sulfur analyses were made using a Fisher Total Sulfur Analyzer model 470. The total mercury concentration of the sediments was measured using atomic absorption (Armstrong and Uthe 1971). Reagent grade FeS (Baker) was examined by X-ray diffraction (Phillips) to ensure that it had not been converted to pyrite during storage.

Results

Surficial sediments from L239 and L223 were similar with respect to carbon, nitrogen, and mercury content. The average values (±SEM) of organic carbon and total nitrogen on five replicates from L239 were 42 ± 4.3 mg C·g^{-1} and 3.5 ± 0.3 mg N·g^{-1}. The corresponding values of five replicates from L223 were 56 ± 1.2 mg C·g^{-1} and 5.9 ± 0.3 mg N·g^{-1}. The average concentrations of total natural mercury in L239 and L223 sediments were low (0.35 ± 0.05 μg Hg·g^{-1} (n = 4) and 0.33 ± 0.04 μg Hg·g^{-1} (n = 4) respectively). The sediments differed by an order of magnitude in AVS content, although the total sulfur content was similar (Table 1). The water content of the sediment was 88% for L239 and 90% for L223.

Studies done in 1980 (Fig. 1, 2) using pH-adjusted sediment from both lakes indicated that rates of biological methyl mercury formation decreased with decreasing sediment porewater pH. The response of mercury methylation to change in pH for L239 sediment, which had not been previously exposed to reduced pH, was similar to that of L223. The rate of methylation at unadjusted pH also varied considerably between sampling dates. There was a seasonal trend, with the highest rates occurring in June and July followed by lower methylation rates during August of each year.

The concentration of porewater $CH_3{}^{203}Hg$ was measured as a function of pH using L114 sediment. It was found that 1% of the added radiolabelled methyl mercury remained in the sediment porewater at natural pH (6.1) and that the percentage of dissolved methyl mercury did not vary within a pH range of 4.3–6.8.

On several occasions, the concentration of porewater inor-

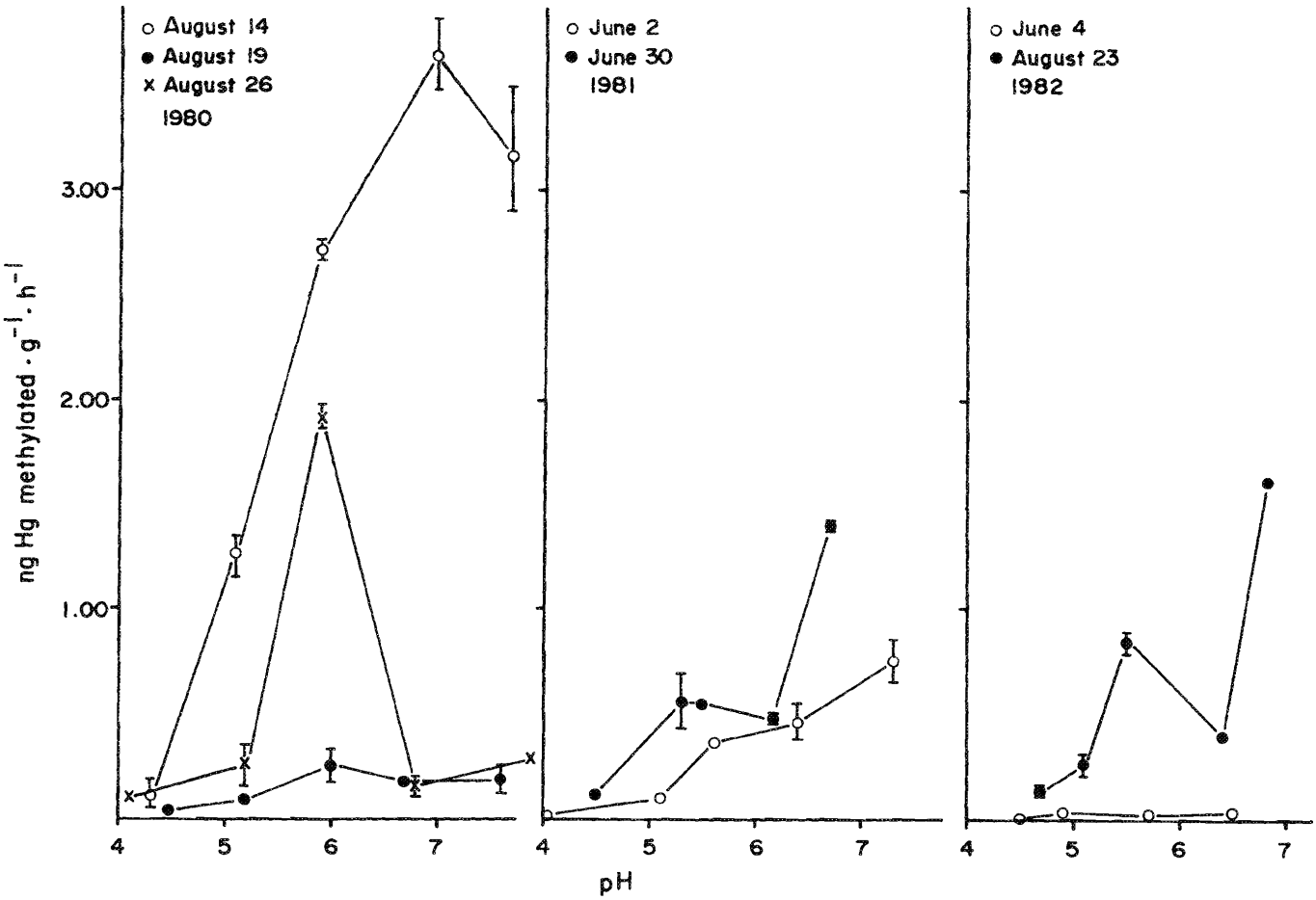

FIG. 1. Rates of $^{203}CH_3Hg$ formation in L239 sediment under different pH conditions during 1980–82. Vertical lines give the range of duplicate samples when variation is greater than the dimensions of the symbol.

ganic ^{203}Hg was measured as a function of pH (Fig. 3). In general, in sediments from both L239 and L223, there was less ^{203}Hg in the porewater at reduced pH levels than there was at pH > 5.5. The concentration ranged from < 0.01% at pH < 6.0 to 1.2% at pH 7.0 in L239 (differences between replicates were 0.0–0.1%). In L223, concentration of ^{203}Hg in porewater varied from 0.6% at pH 4.0 to 4.4% at pH 7.0 (differences between replicates were 0.0–0.4%).

To determine if the observed decrease in the concentration of porewater ^{203}Hg at reduced pH might be related to an increase in the concentration of available sulfide at reduced pH, we investigated the effect of AVS removal on porewater mercury levels. AVS was removed in progressively larger quantities as the pH of the sediment porewater was reduced to pH 4 and 2 (Table 1). When the AVS was not removed (Table 2), pH reduction affected the porewater mercury concentrations, as had been repeatedly observed in other sediment samples (Fig. 3; Table 2); there was an increase in porewater ^{203}Hg above the natural pH and a decrease in concentration below the natural pH (Fig. 3; Table 2). In samples adjusted to lower pH (i.e. < 5.0), removal of AVS increased the porewater mercury concentration and addition of FeS decreased it again (Table 2).

Demethylation and methylation activities were compared in several other lakes (Table 3). In general, reduction in pH had a similar effect on the methylating activity as it did in L239 and L223. Demethylating activity was also reduced (Table 3) as the pH was lowered, but to a lesser extent than methylation.

Discussion

Our results showed that in several ELA lakes that were either unacidified or experimentally acidified, mercury was methylated more slowly at lower pH than it was at the natural pH (Fig. 1, 2; Table 3). Similar results have been obtained in laboratory studies (Baker et al. 1983; Furutani et al. 1984). Baker et al. (1983), using nutrient-amended sediment and long incubation periods (2 wk), found that methyl mercury was only produced at the natural pH. Using L114 sediments maintained in the laboratory, Furutani et al. (1984) found that the rate of mercury methylation was reduced at lower pH and that the mercury methylation rate did not recover to natural levels over a 9-d period. This suggests that the short-term (hours) incubation, used in our study to minimize changes in the natural microbial community, gives an accurate indication of longer term effects of pH change on rates of mercury methylation.

In contrast, Miller and Akagi (1979) found that methyl mercury production in sediment was not influenced over a pH range of 5–7. They also found that the amount of methyl mercury in overlying water increased as the pH decreased. From this they inferred that the lower pH affected the distribution of

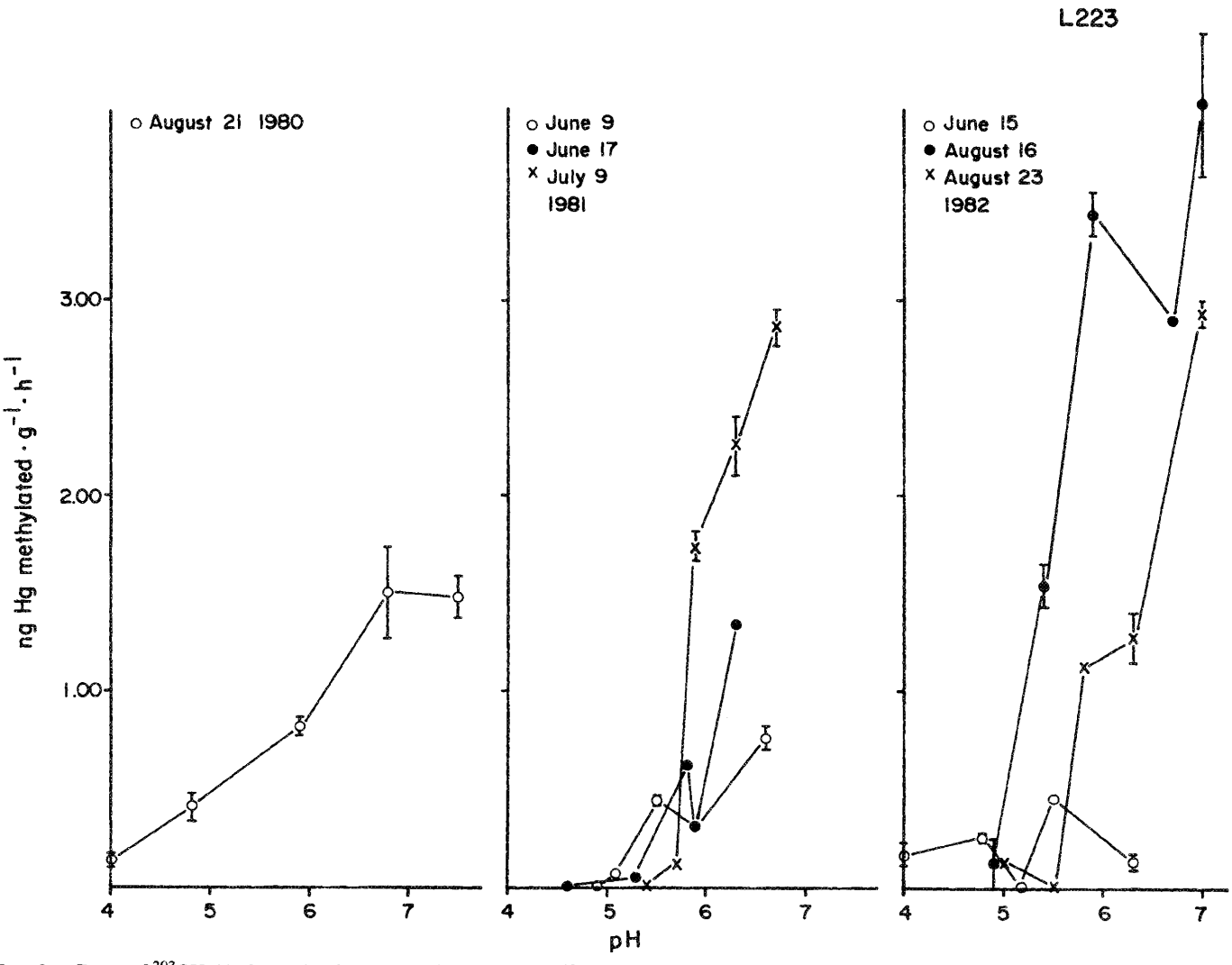

FIG. 2. Rates of $^{203}CH_3Hg$ formation in L223 sediment under different pH conditions during 1980–82. Vertical lines give the range of duplicate samples when variation is greater than the dimensions of the symbol.

TABLE 2. Fraction of total ^{203}Hg in sediment porewater before and after treatment of pH 2 to remove acid-volatile sulfide.

Lake	pH	No treatment	pH 2, no FeS	pH 2, FeS added back
L239	7.0–7.5	0.63	0.11	0.03
	6.3–6.6	0.26	0.04	0.06
	5.5–5.7	0.00	0.07	0.10
	4.9–5.3	0.02	0.11	0.03
	4.4–4.8	0.01	0.55	0.07
L223	6.8–7.2	4.21	0.08	0.13
	6.4–6.7	0.81	0.13	1.16
	5.9–6.3	0.04	0.05	0.06
	5.3–5.8	0.08	0.14	0.06
	4.4–4.8	0.48	1.35	0.05

mercury between sediment and water, leaving the methylation process relatively unaffected. However, we observed that the concentration of radiolabelled methyl mercury did not vary in sediment porewater within a pH range of 4.3–6.8 and that methylation rates were reduced at lower pH. Perhaps these different observations can be explained by differences in the characteristics of the sediments that determine the binding of

TABLE 3. Comparison of mercury methylation and demethylation in sediments collected from several ELA lakes (as measured by % of untreated (u) sediments).

Lake	Depth (m)	Date (1984)	pH	Methylation (% of untreated)	Demethylation (% of untreated)
L303	2	May 21	5.9(u)	100	100
			4.9	0	38
		June 13	6.0	154	136
			5.6(u)	100	100
			5.0	66	85
L114	2	May 23	5.9(u)	100	100
			5.6	90	93
			4.9	34	72
		June 9	7.1	235	58
			6.3(u)	100	100
			5.7	31	98
			5.1	1	82
L302N	13	June 6	6.6	431	104
			6.2(u)	100	100
			5.6	42	71
			5.2	42	38

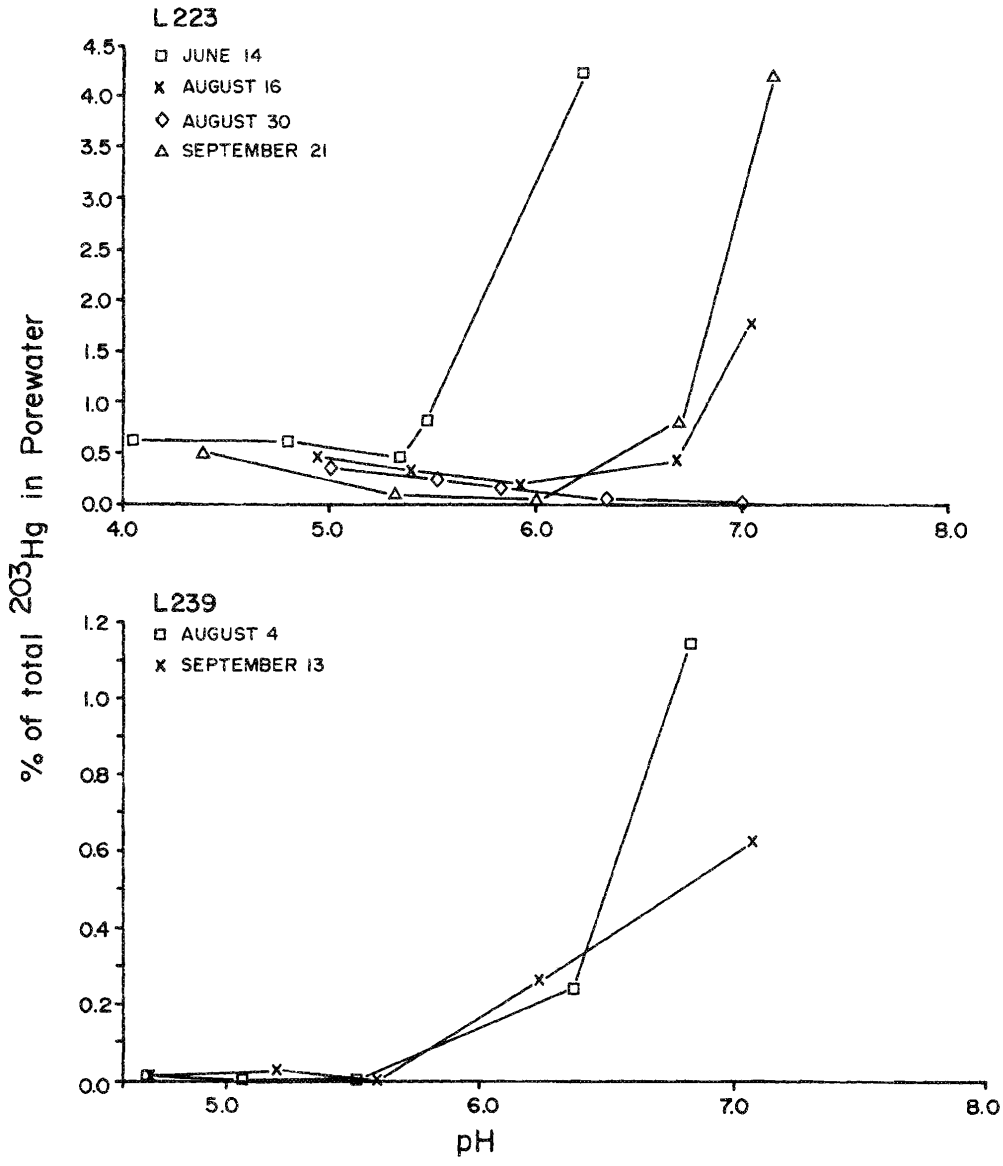

FIG. 3. Fraction of total ^{203}Hg in porewater of pH-adjusted sediment from L239 and L223. All samples were done in duplicate.

inorganic and methyl mercury to particles, or by the trophic state of the lakes. The lakes used in our study were oligotrophic and were not contaminated by mercury.

Our observation that mercury methylation in epilimnetic sediments from unacidified L239 and acidified L223 responded in a similar manner to imposed changes in pH is probably explained by the fact that the pH of most of the sediment porewater of L223 is unaffected by acidification of the lake water (Table 2; Kelly et al. 1984; Kelly and Rudd 1984). The pH of the porewater, which is 5.2 at 1 mm depth below the sediment–water interface, is unchanged from preacidification levels at a depth of 1 cm because of the consumption of H^+ by bacterial activity. At the sediment–water interface, where the porewater pH is reduced, there is evidence that this pH reduction did not decrease microbial activity and so this could not be an explanation for the reduced methylating activity at low pH. Furutani et al. (1984) incubated ELA sediments in the laboratory and found that the reduction in mercury methylation occurred without reduction in microbial activity (CO_2 + CH_4 production). Futhermore, Kelly et al. (1984) have shown that the rate of microbial activity, as estimated by the rate of organic carbon decomposition (CO_2 + CH_4 production), was unaltered by acidification of L223 to pH 5.0.

Another possible explanation for the reduced methylation rates is a decrease in the concentration of available inorganic mercury in porewater at lower pH. The concentration of porewater mercury is a rate-determining factor because it is necessary for mercury to be in solution before it can be biologically methylated (DeSimone et al. 1973). We found that as the pH of the sediment was decreased, the concentration of porewater, or available, ^{203}Hg also decreased. At natural pH the ^{203}Hg in the porewater was always less than 5% of that added. At lower pH the proportion of added ^{203}Hg was further reduced (Fig. 3; Table 2). These data suggest that the observed drop in methylation at lower pH could be related to a decrease in the amount of available inorganic mercury at low pH.

Our observations are in agreement with Hakanson (1974) who showed in laboratory studies that 97% of the mercury

added was adsorbed and deposited with particles at pH 5, whereas only 32.3% was deposited with particles at pH 9. From this he concluded that there is an increased probability of sedimentation of mercury in acid waters. Schindler et al. (1980a), using large in situ enclosures sealed to the sediment of L223, also found that the concentration of ^{203}Hg did not increase in the water column as the pH was decreased.

In comparison with untreated sediments, acid treatment decreased the porewater ^{203}Hg concentration at the higher pH values (>6.3, Table 2). This may have been because the acid treatment "cleaned" the sediment particles, making more sites available for the binding of inorganic mercury. In addition, at pH < 5.5, the acid treatment and removal of AVS reversed the usual pH-related trend of porewater ^{203}Hg concentration (Fig. 3). Instead of a decrease in the porewater mercury concentration at reduced pH, the ^{203}Hg concentration at reduced pH increased. The usual trend was restored by adding FeS back to the acid-treated sediments (Table 2). This experiment suggested that dissolution of metal sulfides in untreated sediments at reduced pH may play a role in lowering porewater mercury concentration at reduced pH by precipitating the Hg^{2+} as HgS. This would decrease the methylation rate, since only dissolved inorganic mercury is available for methylation. While this mechanism seems possible in acidified lakes, since we saw the release of AVS from sediments at pH 4 (Table 1), the reduction in porewater mercury concentration at reduced pH could also be related to changes in the surface binding properties of organic and inorganic particles.

In addition to the effect of decreased porewater mercury concentrations on methylation rates, an increase in the relative rate of demethylation would lead to an overall reduction in the net methyl mercury produced. This was found to be the case (Table 3). Although both methylation and demethylation were inhibited at low pH, methylation was reduced to a greater extent. It is possible that both circumstances, i.e. less porewater mercury and a relative increase in demethylation, occur simultaneously contributing to low net methyl mercury production at reduced pH.

Our data from several ELA lakes suggest that the observed increases in mercury concentration in fish during the early stages of lake acidification cannot be explained by enhanced mercury methylation in the sediment. There are other possible explanations for the observed elevated mercury levels in fish. Two likely explanations are that the rate of net methylation may be enhanced at other sites in the lakes such as the water column (L. Xun, unpub. data), or the mercury concentration in acidified lakes may be increased by higher rates of deposition of mercury and greater loading from the soils.

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References

ARMSTRONG, F. A. J., AND J. F. UTHE. 1971. Semi-automated determination of mercury in animal tissue. At. Absorpt. Newsl. 10: 101–103.

BAKER, M. D., W. E. INNISS, C. I. MAYFIELD, P. T. S. WONG, AND Y. K. CHAU. 1983. Effect of pH on the methylation of mercury and arsenic by sediment microorganisms. Environ. Tech. Lett. 4: 89–100.

BEIJER, K., AND A. JERNELOV. 1979. Methylation of mercury in aquatic environments, p. 203–210. *In* J. O. Nriagu [ed.] The biogeochemistry of mercury in the environment. Elsevier/North-Holland Biomedical Press, Amsterdam. 696 p.

BENES, P., AND B. HAVLIK. 1979. Speciation of mercury in natural waters, p. 175–202. *In* J. O. Nriagu [ed.] The biogeochemistry of mercury in the environment. Elsevier/North-Holland Biomedical Press, Amsterdam. 696 p.

BRUNSKILL, G. J., AND D. W. SCHINDLER. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 139–155.

BURTON, W., AND J. F. FLANNAGAN. 1973. An improved Ekman-type grab. J. Fish. Res. Board Can. 30: 287–290.

CRUIKSHANK, D. R. 1984. Whole lake chemical additions in the Experimental Lakes Area, 1969–1983. Can. Data Rep. Fish. Aquat. Sci. 449: iv + 23 p.

DESIMONE, R. E., M. W. PENLEY, L. CHARBONNEAU, S. G. SMITH, J. M. WOOD, H. A. O. HILL, J. M. PRATT, S. RIDSALE, AND R. J. P. WILLIAMS. 1973. The kinetics and mechanism of cobalamin-dependent methyl and ethyl transfer to mercuric ion. Biochim. Biophys. Acta 304: 851–863.

FURUTANI, A., AND J. W. M. RUDD. 1980. Measurement of mercury methylation in lakewater and sediment samples. Appl. Environ. Microbiol. 40: 770–776.

FURUTANI, A., J. W. M. RUDD, AND C. A. KELLY. 1984. A method for measuring the response of sediment microbial communities to environmental perturbations. Can. J. Microbiol. 30: 1408–1414.

HAKANSON, L. 1974. Mercury in some Swedish lake sediments. Ambio 3: 37–43.

HOWARTH, R. W., AND J. M. TEAL. 1979. Sulphate reduction in a New England salt marsh. Limnol. Oceangr. 24: 999–1013.

HUNGATE, R. E. 1969. A roll tube method for cultivation of strict anaerobes, 3B: 117–132. *In* J. R. Norris and D. W. Ribbons [ed.] Methods in microbiology. Academic Press, New York, NY.

JENSEN, J., AND A. JERNELOV. 1969. Biological methylation of mercury in aquatic organisms. Nature (Lond.) 220: 753–754.

JERNELOV, A., C. HANSSON, AND L. LINSE. 1976. Mercury in fish in Varmland. An investigation of the effect of pH and total phosphorous on the measured variation. I. V. L. Report B282. Swedish Institute for Water and Air Pollution Research, Stockholm. 7 p.

JERNELOV, A., L. LANDNER, AND T. LARSSON. 1975. Swedish perspectives on mercury pollution. J. Water Pollut. Control Fed. 47: 810–822.

JERNELOV, A., AND T. WALLIN. 1973. Air-borne mercury fallout on snow around five Swedish chlor-alkali plants. Atmos. Environ. 7: 209–214.

JOENSUU, O. I. 1971. Fossil fuels as a source of mercury pollution. Science (Wash., DC) 172: 1027–1028.

JORGENSON, B. B. 1978. A comparison for the quantification of bacterical sulphate reduction in coastal marine sediments. I. Measurement with radiotracer techniques. Geomicrobiol. J. 1: 11–27.

KELLY, C. A. AND J. W. M. RUDD. 1984. Epilimnetic sulphate reduction and its role in lake acidification. Biogeochemistry 1: 63–77.

KELLY, C. A., J. W. M. RUDD, A. FURUTANI, AND D. W. SCHINDLER. 1984. Effect of lake acidification on rates of organic decomposition in sediments. Limnol. Oceanogr. 29: 687–694.

MILLER, D. R., AND H. AKAGI. 1979. pH affects mercury distribution, not methylation. Ecotoxicol. Environ. Safety 3: 36–38.

MILLS, K. H. 1984. Fish population responses to experimental acidification of a small Ontario lake, p.117–131. *In* G. R. Hendrey [ed.] Acid precipitation series. Vol. 6. Butterworth Publishers, Boston, MA.

NATIONAL RESEARCH COUNCIL (NRC). 1981. Atmosphere–biosphere interactions: toward a better understanding of the ecological consequences of fossil fuel combustion. Committee for the Atmosphere and Biosphere, National Academy Press, Washington, DC. 263 p.

RUDD, J. W. M., A. FURUTANI, AND M. A. TURNER. 1980. Mercury methylation by fish intestinal contents. Appl. Environ. Microbiol. 40: 777–782.

RUDD, J. W. M., M. A. TURNER, A.FURUTANI, A. L. SWICK, AND B. E. TOWNSEND. 1983. The English–Wabigoon River system: I. A synthesis of recent research with a view towards mercury amelioration. Can. J. Fish. Aquat. Sci. 40: 2206–2217.

SCHINDLER, D. W., R. H. HESSLEIN, R. WAGEMANN, AND W. S. BROECKER. 1980a. Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake. Can. J. Fish. Aquat. Sci. 37: 373–377.

SCHINDLER, D. W., R. W. NEWBURY, K. G. BEATY, J. PROKOPOWICH, T. RUSZCZYNSKI, AND J. A. DALTON. 1980b. Effects of windstorm and forest

fire on chemical losses from forested watersheds and on the quality of receiving streams. Can. J. Fish. Aquat. Sci. 37: 328–334.

SCHINDLER, D. W., AND T. RUSZCZYNSKI. 1983. A test of limnological data from the Experimental Lakes Area, northwestern Ontario, for evidence of acidification. Can. Tech. Rep. Fish. Aquat. Sci. 1147: iv + 17 p.

SCHINDLER, D. W., AND M. A. TURNER. 1982. Biological, chemical and physicial responses of lakes to experimental acidification. Water Air Soil Pollut. 18: 259–271.

SKOOG, D. A., AND D. M. WEST. 1969. Fundamentals of analytical chemistry. 2nd ed. Holt, Rinehart and Winston, Inc., New York, NY. 835 p.

STAINTON, M. P., M. J. CAPEL, AND F. A. J. ARMSTRONG. 1977. The chemical analysis of freshwater. 2nd ed. Fish. Mar. Serv. Misc. Spec. Publ. 25: 180 p.

SUNS, K., C. CURRY, AND D. RUSSEL. 1980. The effects of water quality and morphometric parameters on mercury uptake by yearling yellow perch. Ontario Ministry of the Environment Tech. Rep. LTS 80-1: ii + 16 p.

TSAI, S. C., G. MALLORY BOUSH, AND F. MATSUMURA. 1975. Importance of water pH in accumulation of inorganic mercury in fish. Bull. Environ. Contam. Toxicol. 13: 188–193.