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The influence of clay minerals, oxides, and humic matter on the methylation and demethylation of mercury by micro-organisms in freshwater sediments

Togwell A Jackson
National Hydrology Research Institute, 11 Innovation Boulevard, Saskatoon, Saskatchewan, Canada, S7N 3H5

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Laboratory experiments and analysis of field samples showed that clay minerals, Fe and Mn oxides, and humic matter (colloids) have complex and often dramatic effects on the microbial methylation and demethylation of Hg, and on other microbial activities, in lake sediments. Depending on the nature, abundance, and surface chemistry of the colloids, the source of the sediment, the nature of the microbes, and synergistic/antagonistic effects of environmental variables, the colloids either strongly inhibited or stimulated the Hg transformations, had little or no effect, or alternated in their effects. Most of the results suggest specific effects on particular kinds of microbes, and are not attributable to general inhibition or stimulation of microbial growth or to effects due to the binding of Hg by the colloids. The colloids probably alter the species composition of the microbial community and affect the course of ecological succession, upsetting the dynamic balance between methylation and demethylation and causing alternate increases and decreases in methyl mercury (CH_3Hg^+) levels along with changes in other indicators of microbial activity [CO_2 and CH_4 production and oxidation-reduction potential (Eh)].

The role of clays was critically dependent on surface coatings. Clays often interfered with methylation (while in some cases strongly promoting subsequent demethylation); but iron oxide (FeOOH) often promoted methylation, and FeOOH coatings on clay tended to counterbalance the negative influence of the clay. Removal of oxide coatings depressed both methylation and demethylation. Manganese oxide (MnOOH) coatings sometimes promoted methylation, but larger amounts of MnOOH (unlike FeOOH) strongly suppressed methylation. On addition of organic nutrients, oxide coatings enhanced methylation and impeded demethylation; without nutrient enrichment, the reverse tended to occur. Humic matter in solution tended to stimulate methylation; but humic coatings on clay impeded methylation and fostered demethylation. Thus, the effects of natural colloids on Hg speciation are vitally important but variable, inconsistent, and not altogether predictable.

Keywords: Mercury, methyl mercury, clay minerals, iron oxide, manganese oxide, humic materials, methylation, demethylation, colloids

INTRODUCTION

Mercury (Hg) in natural waters and sediments is mostly in the form of inorganic species.[1–10] However, its harmful biological effects are due chiefly to the much less abundant organometallic compound methyl mercury (CH_3Hg^+), which is synthesized from inorganic bivalent Hg by free-living micro-organisms.[11–19] CH_3Hg^+ is highly toxic and is readily accumulated by aquatic organisms, often rendering fish — especially those at the upper end of the food chain — unsafe for human consumption.[12,14,20]

The bulk of the inorganic Hg in aquatic environments is bound to fine-grained bottom sediments and suspended particles.[4,14,15,21] In the sediments various kinds of micro-organisms gradually transform the Hg into CH_3Hg^+ by methylation of 'available', or biochemically reactive, free Hg(II) species released from this pool of slightly soluble Hg, while other micro-organisms counteract this process by demethylation of CH_3Hg^+.[13,16–19,22] Thus, the net rate of CH_3Hg^+ production under a given set of conditions represents a balance between these two opposing processes. Abiotic methylation and demethylation reactions may also contribute to the overall result.[18,19,23,24]

Environmental conditions control (1) the growth, activities and species composition of the Hg-transforming microbial populations as well as (2) the speciation and solubility, and therefore the availability, of the inorganic Hg.[18] Consequently, the net rate of CH_3Hg^+ production varies as a function of a complex assortment of site-dependent and seasonally fluctuating environmental factors such as pH, Eh, temperature, salinity, and concentrations of nutrients, free oxygen, sulphides, and toxic pollutants.[5–10,13–15,18,25–37] In general, CH_3Hg^+ production tends to be optimized by a plentiful supply of organic nutrient substrates combined with a paucity of both sulphides and free oxygen, provided that other conditions such as temperature and pH are also favourable.[2,9,13,15,34] However, since methylation and demethylation are both mediated by many different microbial species which differ greatly in their ecological requirements, these processes occur under a wide range of environmental conditions.[9,13,16,18,22,26,34] A change in the physicochemical environment (for instance, an increase in the abundance of nutrients or toxic pollutants) may cause a corresponding rise or fall in the net rate of CH_3Hg^+ production owing to changes in total heterotrophic microbial activity or Hg availability or both; but it may also result in a series of alternating increases and decreases in CH_3Hg^+ production as the species composition of the sedimentary microflora shifts in response to the varying conditions in accordance with the principle of ecological succession.[7,8,34,35] Thus, the effects of environmental factors on CH_3Hg^+ formation and breakdown may be extremely complex and variable, and not altogether predictable.

The purpose of the present study was to investigate the effects of clay minerals, iron and manganese oxides, and humic substances on the methylation and demethylation of Hg by micro-organisms in freshwater sediments. It would be surprising if these ubiquitous, abundant, and biogeochemically active, naturally occurring colloids did not strongly influence the microbial transformations of Hg — not only by adsorbing or complexing the Hg (thereby possibly affecting its availability) but also by inhibiting or stimulating vital functions of microbes and altering the state of balance between interacting members of the microbial community (for instance, by determining the outcome of competition between different species). Clay minerals, oxides and humic matter comprise much of the solid matrix in which benthic micro-organisms live, and they are known to play important and diverse roles in microbial ecology in general.[38–61] Nevertheless, little is known about their influence on the microbial methylation and demethylation of Hg, although the scanty existing literature on the subject suggests the occurrence of significant and complex effects which merit further study.[5,6,9,31,34,35]

A few selected results of the present study have been published elsewhere.[9,34,35] A more comprehensive treatment is given here, and additional papers are in preparation.

MATERIALS AND METHODS

Controlled laboratory experiments were performed to test the effects of clay minerals, iron and manganese oxides, and humic matter on the methylation and demethylation of Hg by microbial communities in sediments from different Canadian lakes. In addition, sediment samples from various lacustrine environments were analysed chemically and mineralogically. The research consisted of several separate, though related, studies carried out at different times from 1978 to 1988. Accounts of the methods employed for the fieldwork, laboratory analyses, and experiments, as well as descriptions of the field sites and sample materials, have been published previously.[5–9,34,35] Only a brief summary of them will be given here.

Field sites and sediment samples

Fine-grained bottom sediments (approximately the top 10–15 cm) were collected with an Ekman dredge from (1) East Mynarski Lake, an unpolluted lake in northern Manitoba; (2) Pasqua Lake, a riverine lake of the Hg-polluted Qu'Appelle River system in southern Saskatchewan; (3) two riverine lakes (Clay Lake and Ball Lake) and an intervening stretch of river belonging to the severely Hg-polluted Wabigoon River system in Northwestern Ontario; and (4) four heavy-metal polluted lakes (Schist, Phantom, West Nesootao, and Hamell) located in northern Manitoba and northern Saskatchewan near Flin Flon, Manitoba. Pasqua Lake is situated in a semi-arid prairie region, but all the other lakes are in the Boreal forest zone.

The samples were stored at 4°C in plastic bags from which air had been excluded. All samples were subsequently analysed, but only sediments from east Mynarski Lake, the east basin of Clay Lake, and one of the eastern basis of Pasqua Lake were used for experimental purposes. Pasqua Lake is extremely eutrophic, and the other two lakes are rather productive as well. Therefore, sediments from all three bodies of water were highly suitable as sources of microbes.

Analytical data on the sediment samples are given elsewhere.[5–9,34]

Clays, oxides, and humic matter

The following clays were used in the experiments: (1) 'colloidal' kaolinite from Georgia, USA (Fisher Scientific); (2) montmorillonite (bentonite) (at least 99.75% pure, with minute traces of feldspar, biotite and selenite) from Wyoming or South Dakota, USA (Fisher Scientific); and (3) varved glaciolacustrine silty clay from northern Manitoba, comprising a mixture of illite, chlorite, kaolinite, expandable clay, quartz, potash feldspar, plagioclase feldspar, dolomite, and calcite.[9] To remove the coarser particles, the varved clay was either passed through a 100-mesh (150-μm) screen or was dispersed in water, ultrasonified, and left standing for 1 h, after which the fine-gained fraction remaining in suspension was decanted, dialysed (to get rid of desorbed ions) and gently dried.

Except in a few preliminary experiments, the preparation of these clays for experimental use involved pretreatment with 0.5 mol dm^{-3} $CaCl_2$ followed by dialysis against deionized water to remove adsorbed ions and create a uniform ionic environment on the mineral surface. Subsamples of the clays were also pretreated with citrate/dithionite (C/D) to strip off oxide coatings,[62] and the C/D extracts were analysed for Fe and Mn by atomic absorption spectrophotometry (AA). C/D extraction of the varved clay was preceded by digestion with hot 30% H_2O_2 to decompose organic matter. After C/D treatment, the kaolinite and montmorillonite were bathed in $CaCl_2$ solution. An alternate method of stripping oxides from the varved clay was to pickle the clay in 1 mol dm^{-1} HCl (pH 1) for 16 h; the acid also dissolved the carbonate minerals and may have altered the surface chemistry of the clay minerals and other silicates to some extent.[63] All of these chemical treatments were followed by dialysis to remove excess reagents and dissolved products.

In addition to clays, the following Fe and Mn oxides were used in the experiments: (1) goethite (FeOOH) from Biwabik, Minnesota, USA, and manganite (MnOOH) from the Roberti Mine, Cuyuna Range, Minnesota (Ward's Natural Science Establishment, Inc.); (2) freshly precipitated synthetic oxides; and (3) oxides artifically deposited as adsorbed coatings on kaolinite and montmorillonite crystals. The goethite and manganite were pulverized in a tungsten carbide disc mill (Spex 'shatterbox'). The synthetic oxide precipitates were formed by neutralizing $Fe(NO_3)_3.9H_2O/HNO_3$ solution (pH ~1) with NaOH (1 mol dm^{-3}), by reacting $MnSO_4$ solution with $KMnO_4$ (1 mol dm^{-3}) and NaOH, and by reacting $KMnO_4$ with HCl using procedures described elsewhere.[64–66] They were then dialysed and freeze-dried. Fe oxide coatings were deposited on clay minerals by soaking the clays in $Fe(NO_3)_3.9H_2O$ solution (~0.009 mol dm^{-3}) to saturate the adsorption sites with Fe(III) and then rinsing them with water and drying them. Mn oxide coatings were formed by immersing the clays in $MnSO_4$ solution, rinsing them, oxidizing the adsorbed Mn(II) to Mn(IV) with $KMnO_4$, and then rinsing the clays again and drying them. In this report all oxides will simply be designated as FeOOH and MnOOH for convenience, although the synthetic Mn oxides may actually have been in the form of MnO_2.

Humic matter was extracted from the <150 μm (100-mesh) fraction of the A-horizon of a forest soil in northern Manitoba. The soil was suspended in N_2-purged 0.1 mol dm^{-3} NaOH and ultrasonified, and the mixture was centrifuged. The supernatant was then dialysed in Spectrapor-3 dialysis bags with a molecular weight cut-off of ~3500, using a Pope dialyser. To remove non-humic organic substances such as proteinaceous matter and carbohydrates, the crude extract was hydrolysed with boiling 6 mol dm^{-3} HCl for 19 h, dialysed, adjusted to a slightly alkaline pH with NaOH to make sure that all of the humic matter was in solution, and dialysed again to a final pH of 7.4. Visible spectrophotometry yielded an E_4/E_6 ratio[67] of 3.2, indicating that the humic matter was chiefly or solely composed of relatively high-molecular-weight humic (as opposed to fulvic) acids. Known volumes of the purified humic solution, which had a humic acid content of ~900 mg (dry weight) dm^{-3}, were used in the experimental work.

Furthermore, coatings of humic matter were deposited artificially on kaolinite and montmorillonite crystals. The humic matter was extracted with N_2-purged 0.1 mol dm^{-3} NaOH from a sample of mud collected from an unnamed, unpolluted lake in Northwestern Ontario, and the solution was dialysed. The clays were immersed in the humic solution, after which the slurry was centrifuged and the supernatant discarded. The clay was then rinsed twice with water and dried.

Experiments

The methylation and demethylation experiments were performed in Erlenmeyer flasks with stoppers fitted with valves for sampling head gas. The flasks, stoppers, and solid experimental materials, except (in most cases) the sediments used as sources of microorganisms, were autoclaved beforehand. In certain auxiliary experiments on *abiotic* Hg transformations, the sediments (if any) were autoclaved too. With the exception of the humic acid solution, which was autoclaved, all solutions were sterilized by filtration through autoclaved 0.45-μm membrane filters. Replicate portions of homogenized sediment were weighed into the flasks and suspended in $HgCl_2$ solution (in the methylation experiments) *or* CH_3Hg^+ acetate solution (in the demethylation experiments) with and without known quantities of the substance to be tested (clay, oxide, or humic matter). Other ingredients, which were used in some experiments but not in others, included organic nutrient substrates (plant material from the field area) and $CaCO_3$, which was used for pH buffering. The composition of the slurry in each flask was as follows

(1) *East Mynarski Lake and Pasqua Lake studies:* 10 g (wet weight) of sediment + 20 cm^3 of 10 μmol dm^{-3} $HgCl_2$ *or* 100 nmol dm^{-3} CH_3Hg^+ acetate ± 0.1000 g (dry weight) of pulverized sphagnum moss or bulrush ± 1 g (dry weight) of $CaCO_3$ ± 0.01–5 g (dry weight) of clay or oxide *or* ~9 mg (dry weight) of humic acid (added as an aqueous solution).

(2) *Clay Lake study:* 20 g (wet weight) of sediment + 100 cm^3 of 5,000 μmol dm^{-3} $HgCl_2$ + 5 g (wet weight) of decaying wood chips (paper mill wastes dredged from Wainwright Reservoir upstream from Clay Lake) ± 5 or 10 g (dry weight) of clay or oxide.

All slurries were purged with N_2 gas, and the air in the head space was replaced with N_2. The flasks were then stoppered and incubated at ~20–26°C in the dark with occasional swirling for the required length, or lengths, of time. Each experiment conformed to one of two basic types of experimental design: either (1) the incubation time was varied from 0 to as many as 14 days but the quantity of test substance per flask was kept constant, and 'experimental' systems were compared with 'control' systems, *or* (2) the incubation time was the same for all flasks (about 7 days) and the quantity of test substance was varied (or, in rare cases, kept constant). Starting conditions were assessed by means of controls and experimental systems which were analysed at the outset of the term of incubation. The distinction between experimental systems and controls was based either on (a) the presence or absence of a test substance *or* (b) whether or not the test substance had been altered by removal or addition of surface coatings.

After incubation, head-space gas was analysed for carbon dioxide (CO_2) and methane (CH_4) by gas chromatography (GC), and the pH and oxidation–reduction potential (Eh) of each slurry were measured with a pH-meter. The CH_3Hg^+ content of the slurry was determined by (1) extraction of a weighed portion of slurry with $CuSO_4$ (0.1 mol dm^{-3}), NaBr (3 mol dm^{-3}) mixed with H_2SO_4 (4 mol dm^{-3}), and toluene; (2) extraction of the CH_3Hg^+ from the toluene into $Na_2S_2O_3$ solution (0.005 mol dm^{-3}) diluted 1:1 (v/v) with 95% ethanol; (3) purification of the solution by successive rinsings with benzene; (4) treatment of the purified solution with KI (3 mol dm^{-3}) and benzene, resulting in uptake of the CH_3Hg^+ by the benzene; and (5) analysis of the benzene solution with a Tracor Micro-Tek (MT) 220 or Varian 3300 GC unit employing a column of 10% Sp-1000 on 80/100 'Supelcoport' (Supelco Inc.), with N_2 as the carrier gas, and a tritium or nickel-63 electron capture detector.[68] Results of CH_3Hg^+ analyses were checked by confirmation tests employing Ag_2SO_4.[69] The detection limit for CH_3Hg^+ was 0.1–0.25 ng g^{-1} (wet weight), and the abundance of

CH_3Hg^+ was generally expressed as total nanograms per flask. The relative rate of methylation or demethylation was represented by the increase or decrease, respectively, in the CH_3Hg^+ level during incubation.

In a separate set of experiments, the adsorption of Hg^{2+} and CH_3Hg^+ by the clays and oxides was investigated to ascertain whether differences in adsorption kinetics were relevant to the results of the methylation and demethylation experiments. Different quantities of each material were suspended in equal volumes of $HgCl_2$ or CH_3Hg^+ acetate solution, the volumes and concentrations being the same as in the methylation and demethylation experiments, and the proportions of dissolved and suspended components generally being the same as well. The mixtures were left standing in stoppered flasks, with occasional swirling, for a fixed length of time (3–24 h); they were then centrifuged, and the supernatants were analysed for total Hg or CH_3Hg^+.

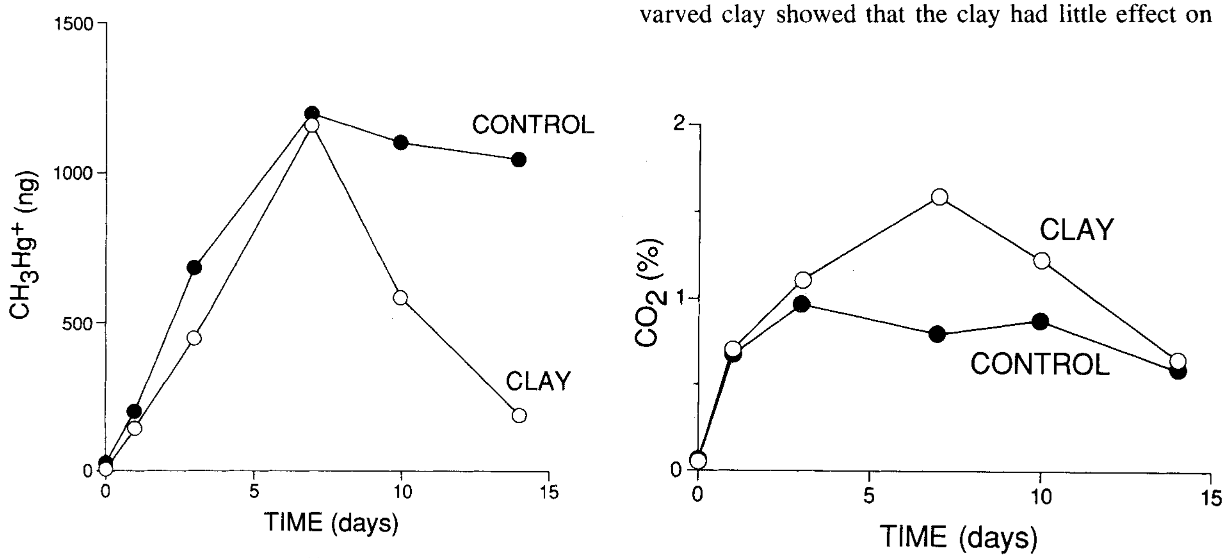
The complexing of Hg^{2+} by humic matter was also studied. Two sets of replicate 20-cm^3 portions of 10 μmol dm^{-3} $HgCl_2$ solution, one with and one without dissolved, hydrolysed humic acid (450 mg dm^{-3}), were dialysed against deionized water in Pope dialysers for different lengths of time using Spectrapor dialysis tubing with a molecular weight cut-off of 3500. As the humic acid was non-dialysable, its ability to bind Hg was assessed by analysing the solutions in the dialysis bags for total Hg and comparing the Hg levels in the two sets of solutions.

In the experiments on Hg^{2+} binding by mineral colloids and humic matter, the total dissolved Hg levels were determined by digestion with hot $HNO_3/H_2SO_4/KMnO_4/H_2O_2$ or (in the Clay Lake study only) $H_2SO_4/KMnO_4/K_2S_2O_8$ followed by reduction of Hg^{2+} to Hg^0 with $SnSO_4$ and estimation of the Hg^0 by flameless AA.[70]

RESULTS

East Mynarski Lake (EML) study

Incubation of control slurries consisting solely of nutrient-enriched, $CaCO_3$-buffered sediment from East Mynarski Lake (EML) suspended in $HgCl_2$ solution resulted in a sharp, continuous increase in CH_3Hg^+ concentration with time over the first seven days, giving way abruptly to a much more gradual progressive decline spanning days 7–14 (Fig. 1A). This two-phase pattern of variation indicates a shift from net production to net decomposition of CH_3Hg^+, implying that demethylating microbes replaced methylators as the dominant Hg-transforming species in the microbial community. Comparisons of the control slurries with experimental slurries containing varved clay showed that the clay had little effect on



(A) (B)

Figure 1 Effects of varved calcareous clay on the production of (A) CH_3Hg^+ and (B) CO_2 by nutrient-enriched, buffered EML sediment suspended in $HgCl_2$ solution. 'Control' slurries contained no added clay. CH_3Hg^+ and CO_2 levels were plotted against incubation time.

Hg methylation but that it had a profound influence on subsequent demethylation (Fig. 1A). Thus, the clay caused a slight, though consistent, decrease in the net rate of CH_3Hg^+ production but brought about a dramatic increase in the net rate of CH_3Hg^+ breakdown, resulting in a drastic drop in CH_3Hg^+ concentration.

CO_2 levels, which served as indicators of microbial activity, showed comparable trends. In both the controls and the experimental systems, CO_2 levels increased initially with time and then declined (Fig. 1B), presumably indicating CO_2 production by heterotrophic microbes followed by CO_2 utilization by certain microbes whose activities predominated after day 7. As with CH_3Hg^+, the rate of decrease was accelerated considerably by the clay, and in the presence of clay (but not in the control systems) the peak in CO_2 concentration coincided exactly with the peak in CH_3Hg^+ concentration. Unlike CH_3Hg^+, however, CO_2 was more abundant in the systems containing added clay than in the controls throughout the term of incubation because the clay enhanced CO_2 production during the first seven days. Despite its mildly inhibitory effect on Hg methylation, the clay apparently had a stimulatory effect — initially, at least — on general microbial activity, on the assumption that most of the CO_2 was produced by microbial respiration and fermentation. Some of the CO_2 may have been generated by dissolution of the carbonates owing to excretion of acids by microbes; but even CO_2 originating in this way would provide an indirect, non-

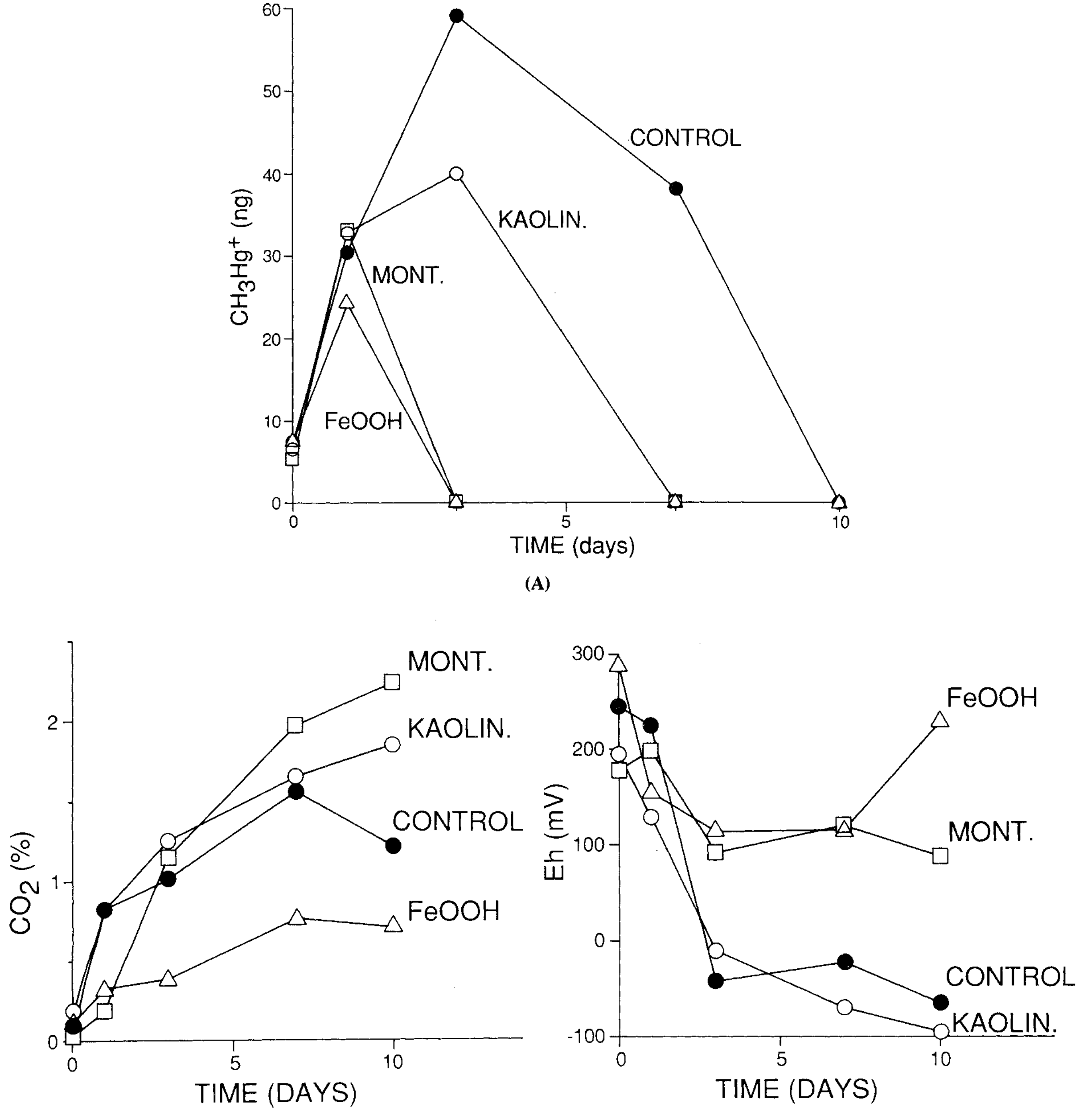
Table 1 The pH values [means ($\bar{x}$) and ranges] of the slurries in the methylation and demethylation experiments whose results are illustrated in the figures

		pH	
Figure	Sample set	$\bar{x}$	Range
1	Control	7.05	6.99–7.19
	Clay	7.22	7.10–7.35
2	Control	6.51	6.09–6.70
	Kaolinite	6.25	6.00–6.48
	Montmorillonite	6.60	6.38–6.72
	FeOOH	6.63	6.25–7.40
4A, 4C, 4D	Control	7.17	6.94–7.36
	Clay	7.17	7.07–7.37
	H_2O_2-treated clay	7.27	7.10–7.54
	C/D-treated clay	7.08	6.91–7.23
4B	Control	7.25	7.01–7.40
	Clay	7.18	6.91–7.36
	H_2O_2-treated clay	7.31	7.15–7.50
	C/D-treated clay	7.15	7.01–7.31
5	Clay	7.22	7.10–7.35
	Acid-treated clay	7.00	6.91–7.10
8, 12E	Control	6.98	6.65–7.70
	Kaolinite	7.14	6.90–7.84
	C/D-treated kaolinite	7.24	7.10–7.35
9, 12A 12B, 12C, 12D	Control	7.02	6.87–7.15
	Montmorillonite	6.79	6.63–6.93
	C/D-treated montmorillonite	6.97	6.73–7.73
10	Control	6.96	6.71–7.22
	FeOOH	7.07	6.79–7.40
	MnOOH	7.02	6.77–7.34
14	Control	6.96	6.71–7.22
	Humic acid	7.19	7.12–7.29
16	Control	5.3	5.2–5.5
	FeOOH	6.8	6.2–7.5
	MnOOH	5.2	4.9–5.4
	Kaolinite	5.8	5.8–5.9
	Montmorillonite	7.6	7.4–7.9
20A	Control	7.34	7.29–7.39
	Kaolinite	7.29	7.26–7.31
	Fe–kaolinite	7.29	7.27–7.32
	Mn–kaolinite	7.31	7.20–7.39
	Humic–kaolinite	7.30	7.22–7.37
20B, 20C	Control	7.04	7.02–7.06
	Kaolinite	7.14	7.08–7.18
	Fe–kaolinite	7.09	7.03–7.14
	Mn–kaolinite	7.13	7.05–7.22
	Humic–kaolinite	7.12	7.04–7.16
21A, 21B	Control	7.12[a]	—
	Kaolinite	7.19	7.10–7.23
	Fe–kaolinite	7.37	7.25–7.50
	Mn–kaolinite	7.34	7.28–7.45
	Humic–kaolinite	7.28	7.23–7.35
	C/D-treated kaolinite	7.26	7.04–7.48
21C	Control	6.44[a]	—
	Kaolinite	7.14	7.09–7.18
	Fe–kaolinite	7.17	7.06–7.23
	Mn–kaolinite	7.24	7.19–7.28
	Humic–kaolinite	7.10	6.75–7.28

[a] Single value.

specific measure of microbial activity. Variations in CH_3Hg^+ and CO_2 levels were independent of pH: the pH values of the experimental and control slurries alike deviated very little from 7 throughout the experiment (Table 1).

A similar experiment[34] in which the $CaCO_3$ was omitted gave essentially the same results except that in the methylation phase the rate of CH_3Hg^+ production was higher in the slurries containing varved clay than in the controls — apparently because pH buffering by the natural carbonate minerals in the clay made the sedimentary environment somewhat more



(A)

(B)

(C)

Figure 2 Effects of kaolinite, montmorillonite, and synthetic FeOOH on (A) CH_3Hg^+ and (B) CO_2 production, and on (C) Eh, in nutrient-enriched, buffered EML sediment suspended in $HgCl_2$ solution. 'Control' slurries contained no added clay or oxide.

favourable for methylating microbes. [In the previously described experiment employing artificially buffered slurries, this effect was cancelled by the addition of $CaCO_3$ to all of the slurries, including the control ones, and the results revealed that the non-carbonate minerals in the clay had a mildly negative influence on methylation (Fig. 1A).] In the demethylation phase the clay, again, induced a sharp drop in CH_3Hg^+ levels with respect to control values.

Further experimentation employing nutrient-enriched, buffered EML sediment suspended in $HgCl_2$ solution but substituting the individual mineral species kaolinite, montmorillonite, and synthetic FeOOH for the heterogeneous varved clay, yielded results that were strikingly similar to those obtained with the varved clay (Fig. 2A). In both the controls and the experimental systems, CH_3Hg^+ levels rose rapidly, attaining a maximum within only one–three days, and then declined steeply and disappeared altogether. The minerals had little or no effect on methylation rate (the only effects, if any, being a slight, and possibly insignificant, enhancement of methylation by the clays, and a slight inhibition by FeOOH, at day 1); on the other hand, the minerals greatly hastened subsequent demethylation — seemingly by making the demethylation phase begin sooner rather than increasing the rate of demethylation. The tendency of the minerals to promote the breakdown of CH_3Hg^+ increased in the order kaolinite < montmorillonite ≤ FeOOH.

CO_2 levels (Fig. 2B) increased with time and continued to increase throughout the term of incubation or declined slightly after day 7. Kaolinite and montmorillonite enhanced the rate of CO_2 production, whilst FeOOH depressed it. A close link between the biochemical pathways of CO_2 and CH_3Hg^+ is not apparent in this case (Figs 1A and 1B).

Table 2 The citrate/dithionite (C/D)-extractable iron and manganese content of the kaolinite, montmorillonite, and varved clay used in the experiments
All numbers represent single analyses.

Sample material	Fe (mg g^{-1})	Mn (mg g^{-1})
Kaolinite	0.242	0.0006
Montmorillonite	2.17	0.0878
Varved clay	5.43	0.208
	3.58	0.168

The variations in Eh (Fig. 2C) were more closely related to the variations in CH_3Hg^+ levels. Eh decreased with time until day 3 (presumably owing to microbial activity) and then stabilized to a greater or lesser degree. The Eh values generally varied in the order kaolinite < montmorillonite ≤ FeOOH, suggesting a relationship with the role of the minerals in demethylation: the greater the mineral's relative effectiveness in promoting demethylation, the higher the Eh of the slurry tended to be. In the presence of FeOOH and montmorillonite, Eh values were consistently positive and, by day 3, considerably higher than in the controls, whereas in the presence of kaolinite the Eh quickly dropped to negative values. A relevant fact which will be discussed below is that the citrate/dithionite (C/D)-extractable Fe oxide content of the montmorillonite exceeds that of the kaolinite by an order of magnitude (Table 2), so that the FeOOH content of the minerals increases in the order kaolinite < montmorillonite < FeOOH.

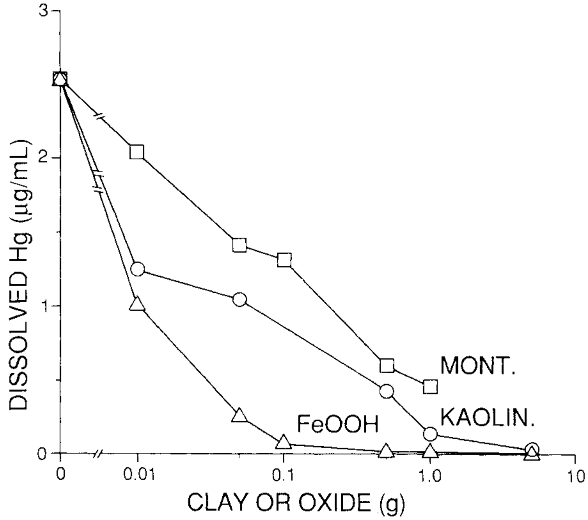


Figure 3 Kinetics of $HgCl_2$ adsorption by kaolinite, montmorillonite, and FeOOH, expressed as quantity of Hg remaining in solution after 24 h plotted against quantity of clay or oxide.

The minerals readily adsorbed Hg^{2+} from solution, approaching or attaining 100% removal of Hg at higher mineral concentrations; but they differed significantly in rate of uptake, which increased in the order montmorillonite < kaolinite < FeOOH (somewhat surprisingly, as montmorillonite is generally a more efficient adsorbent than kaolinite) (Fig. 3). However, the binding of Hg by the minerals appeared to have no relevance to the influence of these minerals on

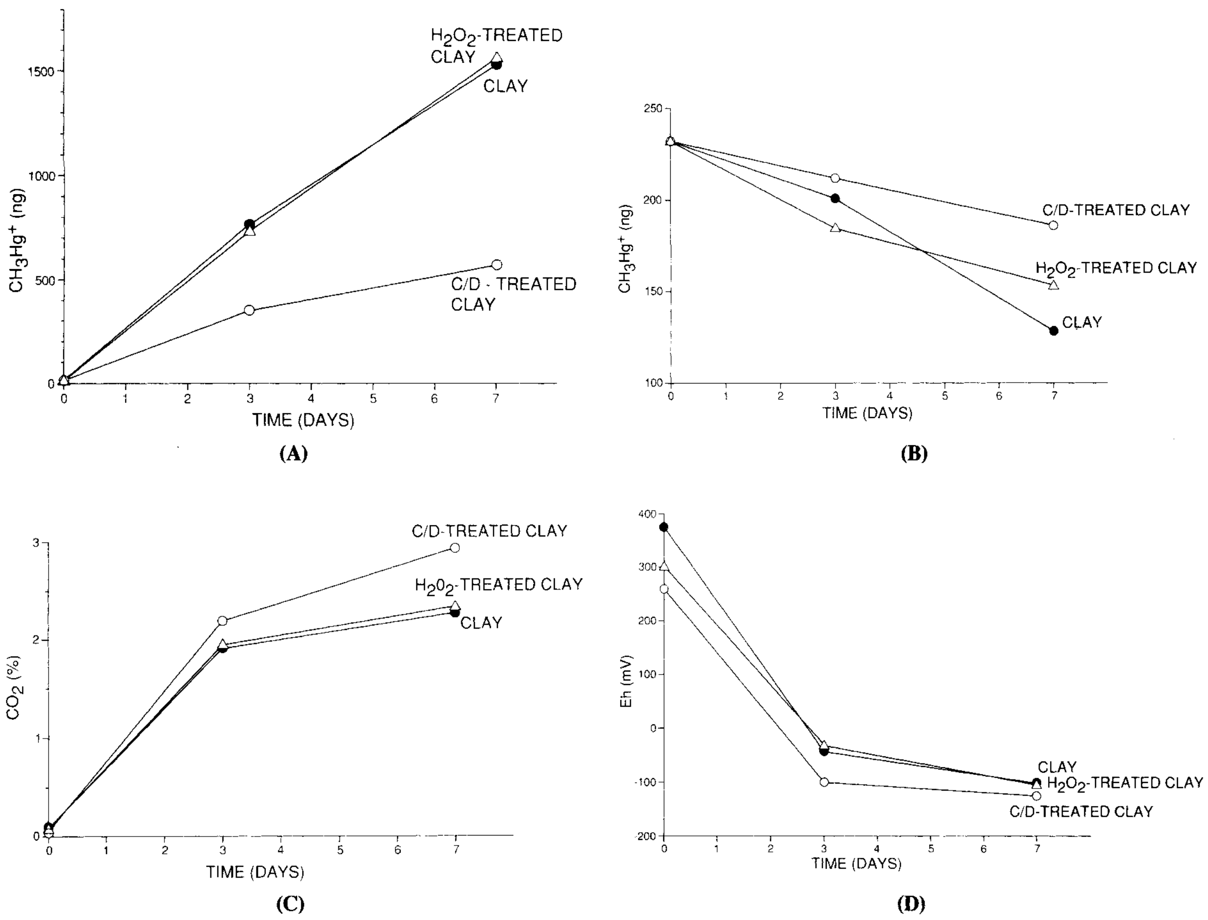


Figure 4 Comparison of effects of H_2O_2- and C/D-treated and untreated varved calcareous clay on (A) CH_3Hg^+ production, (B) CH_3Hg^+ decomposition, (C) CO_2 production, and (D) Eh in nutrient-enriched, buffered EML sediment suspended in $HgCl_2$ (A, C, D) or CH_3Hg^+ acetate (B) solution.

microbial transformations of Hg, with the possible exception of the trivial variations in CH_3Hg^+ production observed at day 1 (Fig. 2A). On the other hand, there was a definite inverse correlation between the rates of Hg adsorption and CO_2 production (Figs 2B and 3), evidently indicating that the efficient removal of dissolved substances from the water tends to reduce the rates of certain metabolic activities of microbes.

Digestion of varved clay with H_2O_2 to decompose organic coatings did not affect any of the observed biological functions of the clay appreciably, but removal of oxide coatings by C/D extraction caused a marked drop in the rates of both methylation and demethylation (Figs 4A and 4B). In contrast, C/D extraction promoted CO_2 production (Fig. 4C), implying stimulation, not suppression, of general microbial activity; it also lowered the Eh (Fig. 4D), reflecting either the intensification of microbial activity or prevention of reactions in which the oxides functioned as oxidizing agents, or both. Leaching of clay with HCl had the same general effect as C/D treatment (Fig. 5): The rate of CH_3Hg^+ production was dramatically reduced, and subsequent demethylation was totally suppressed; yet CO_2 production was increased,[34] and Eh levels were lowered.[71]

The curves representing adsorption of Hg^{2+} by untreated clay and C/D-extracted clay virtually coincided (Fig. 6A), militating against the possibility that the effects of oxide removal on Hg speciation were due to a change in the clay's ability to bind Hg^{2+}. On the other hand, acid-leached clay scavenged dissolved Hg^{2+} more rapidly than untreated clay, although both

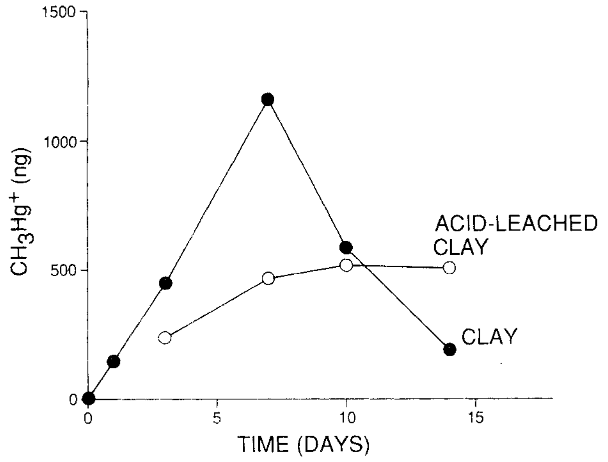


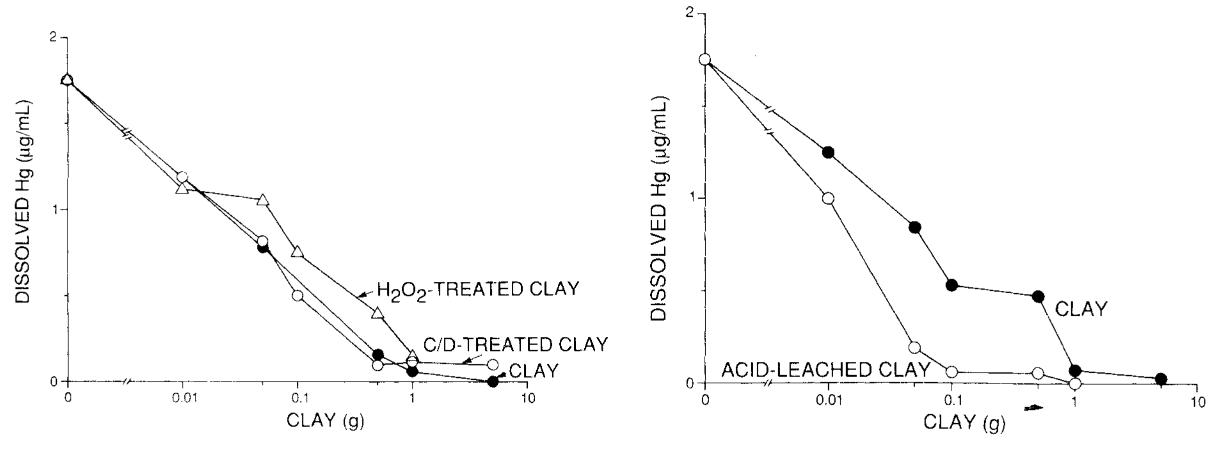
Figure 5 Comparison of effects of HCl-treated and untreated varved calcareous clay on CH_3Hg^+ production by nutrient-enriched buffered EML sediment suspended in $HgCl_2$ solution.

clays adsorbed virtually 100% of the Hg at the highest clay concentrations (Fig. 6B). Possibly the differences in adsorption kinetics contributed to the observed effect on Hg speciation (stronger binding by acid-leached clay rendering Hg less available), but this is questionable because (1) there was little or no difference in Hg uptake rate at the high clay concentration (5 g/flask) used in the methylation experiment, and (2) C/D leaching had basically the same effect as acid leaching on Hg speciation but did not alter the adsorption kinetics, as demonstrated above (Fig. 6A).

In none of these experiments can the effects of oxide removal be attributed to changes in the pH, as the pH values of the slurries were invariably close to 7 (Table 1).

Results of a methylation experiment in which EML sediment suspended in $HgCl_2$ solution was incubated for seven days with varying amounts of varved clay, but without supplementary nutrients and $CaCO_3$, are shown in Fig. 7A. With increasing clay content, the pH rose progressively and smoothly owing to the buffering effect of carbonate minerals in the clay, and finally levelled off at a value of 7.7. The CH_3Hg^+ and CO_2 concentrations and Eh, however, did not vary as simple functions of clay content. Instead, they gave complex zigzag patterns of variation with only a hint of overall trends. Yet the patterns appear to be non-random and are clearly related to one another. The CH_3Hg^+ data gave two large peaks superimposed on a smooth baseline, which declines very gently toward higher clay concentrations, and each of these peaks coincides with a peak in the CO_2 curve and a dip in the Eh curve; one of the CH_3Hg^+ peaks also coincides with a small dip in the pH curve. At the highest clay concentrations there was a third CO_2 maximum but no corresponding CH_3Hg^+ maximum. These results reveal that at certain particularly favourable clay concentrations there were bursts of microbial activity characterized by enhanced CO_2 and CH_3Hg^+ production and a drop in Eh.

Repetition of this experiment with the addition of an organic nutrient supplement to each of the slurries yielded results that were strikingly similar in some respects but different in others (Fig. 7B). As before, the pH increased steadily with increasing clay concen-



(A) **(B)**

Figure 6 Comparison of kinetics of $HgCl_2$ adsorption by (A) H_2O_2-treated, C/D-treated, and untreated varved calcareous clay and (B) HCl-treated and untreated clay expressed as Hg remaining in solution after 24 h plotted against quantity of clay.

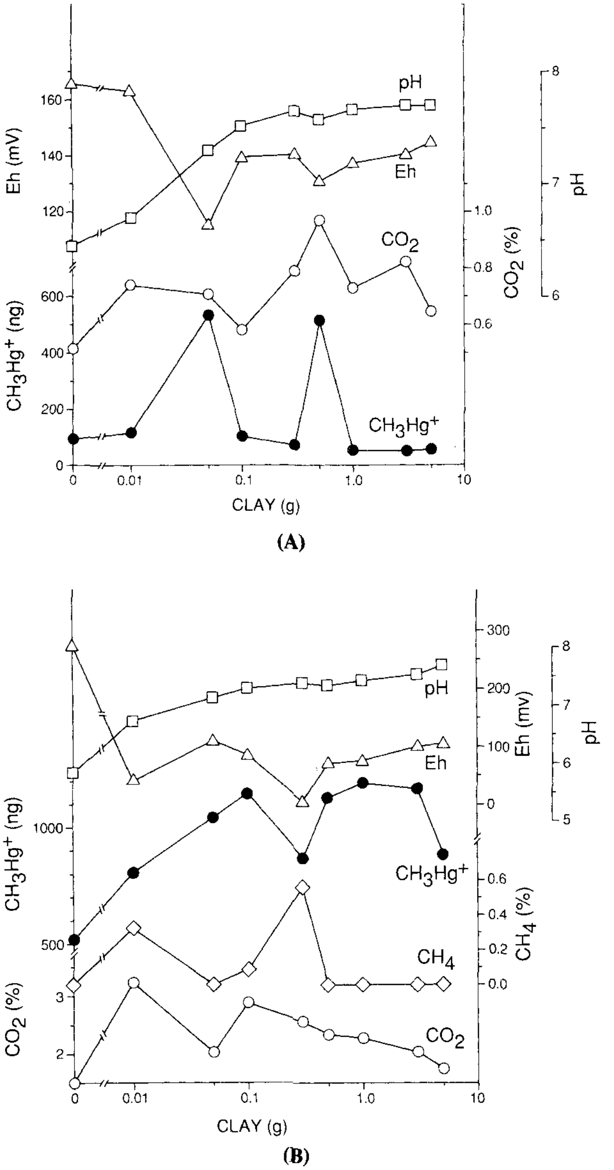


Figure 7 Variations in CH_3Hg^+, CO_2, and CH_4 production, Eh, and pH as functions of quantity of varved calcareous clay added to (A) unaltered and (B) nutrient-enriched EML sediment, representing results obtained after incubation for seven days. (*Note*: Without nutrient supplement, no CH_4 was generated.)

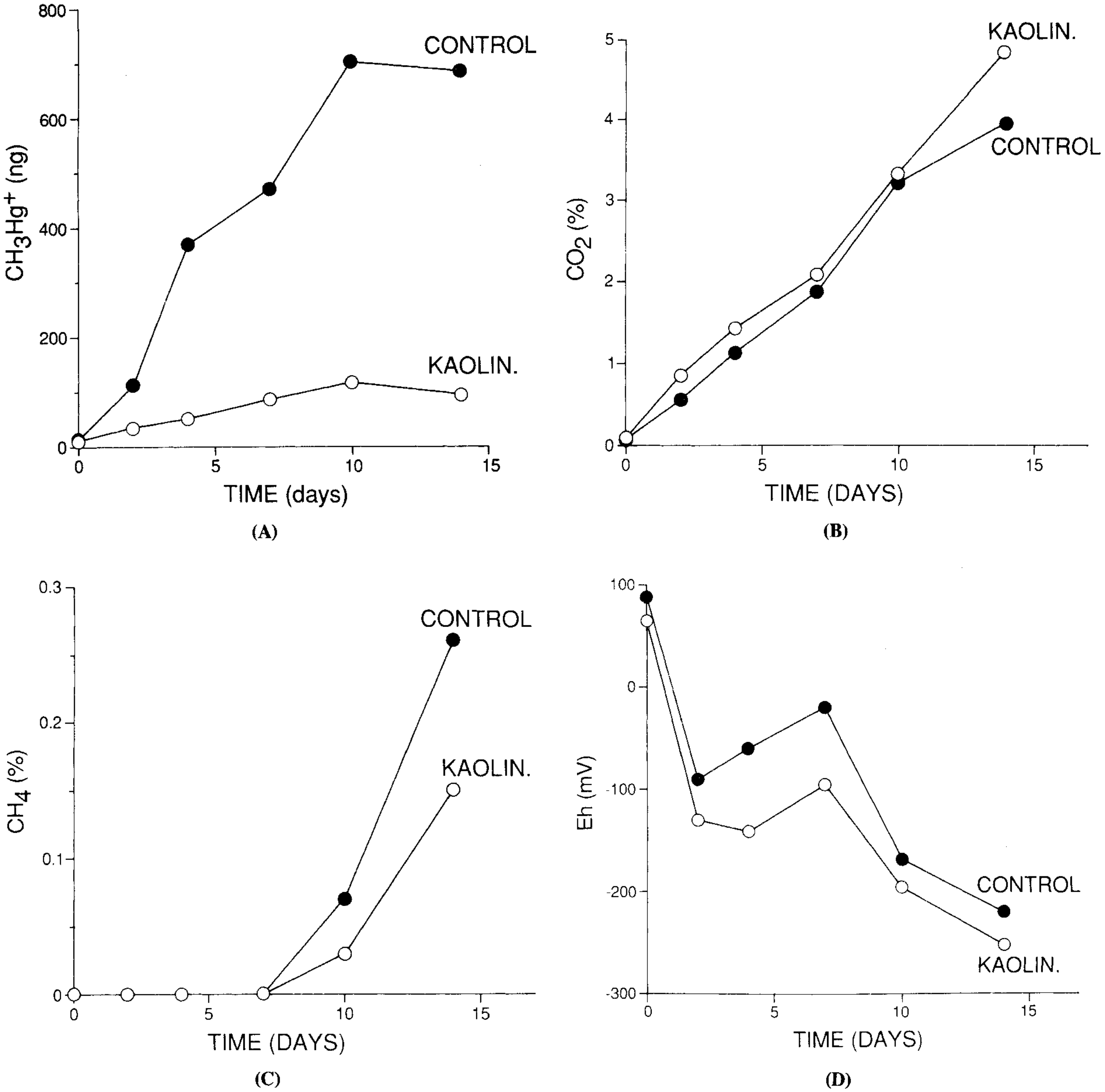
tration, whilst all other variables alternately rose and fell; and again the Eh dipped twice, giving virtually the same pattern of variation as in the other experiment except that the two minima occurred at somewhat lower clay concentrations. However, one major difference was that CH_4 was generated only in the experiment employing nutrient-enriched systems. Understandably, CH_4 production was inversely related to Eh, with the result that there were two CH_4 peaks coinciding with the two Eh minima. In addition, CH_3Hg^+ showed a different pattern of variation which was shaped by environmental and biological factors quite different from those in the other experiment. At first, CH_3Hg^+ levels rose steadily with increasing clay concentration, evidently in response to the rising pH.[34] Then there was a sharp dip in the CH_3Hg^+ curve coinciding with a CH_4 peak and an Eh minimum (even though Eh minima were associated with CH_3Hg^+ *maxima* in the experiment without nutrient enrichment). This indicates an upsurge of specialized microbial activity involving creation of reducing conditions together with CH_4 production and demethylation of Hg. At the highest clay concentration there was a second drop in CH_3Hg^+ level which was not unequivocally related to a change in any of the other variables.

Pasqua Lake (PL) study

Results of experiments employing sediment from Pasqua Lake (PL) were in certain respects qualitatively different from the results obtained with EML sediment, probably owing to differences in the species composition of the microflora. Nevertheless, they reflected the same general principles.

Incubation of replicate samples of nutrient-enriched, $CaCO_3$-buffered PL sediment suspended in $HgCl_2$ solution over a 14-day period with and without added clay or oxide led to a marked rise in CH_3Hg^+ and CO_2 content (Figs 8A, 8B, 9A, 9B, 10A and 10B). The CH_3Hg^+ and CO_2 concentrations increased steadily or rose and then started to level off after several days, but in these experiments there was no sign of a shift from net production to net decomposition of CH_3Hg^+, with one possible exception (Fig. 8A). Both kaolinite and montmorillonite strongly inhibited the net rate of CH_3Hg^+ production, kaolinite to a much greater degree than montmorillonite (Figs 8A and 9A). In contrast, both clay minerals enhanced the rate of CO_2 production, indicating intensification of general heterotrophic microbial activity (Figs 8B and 9B). Montmorillonite, however, caused a considerably larger increase in CO_2 level than did kaolinite.

In these experiments, CH_4 was generated after day 7. Both clay minerals depressed the rate of CH_4 production (Figs 8C and 9C), but the inhibitory effect of montmorillonite was decidedly stronger than that of kaolinite. CH_4 biosynthesis being a strictly anaerobic



(A) (B)

(C) (D)

Figure 8 Effects of kaolinite on (A) CH_3Hg^+, (B) CO_2, and (C) CH_4 production, and (D) Eh in nutrient-enriched, buffered PL sediment suspended in $HgCl_2$ solution. 'Control' slurries contained no added clay.

process, this difference is understandable in the light of the Eh data (Figs 8D and 9D): kaolinite consistently reduced the Eh with respect to the control values, whereas montmorillonite — probably owing to the higher concentration of oxides on its crystal faces (Table 2) — consistently raised it. In experimental and control systems alike, the Eh, reflecting environmental changes brought about by microbes, declined initially but rose again to form a peak and then resumed its decline.

FeOOH (goethite) had a complex and inconsistent effect on methylation. At first FeOOH inhibited CH_3Hg^+ production somewhat, but after day 7 there was a turnabout, and the FeOOH promoted CH_3Hg^+ production appreciably from day 10 onward (Fig. 10A). The curve representing variation in CH_3Hg^+

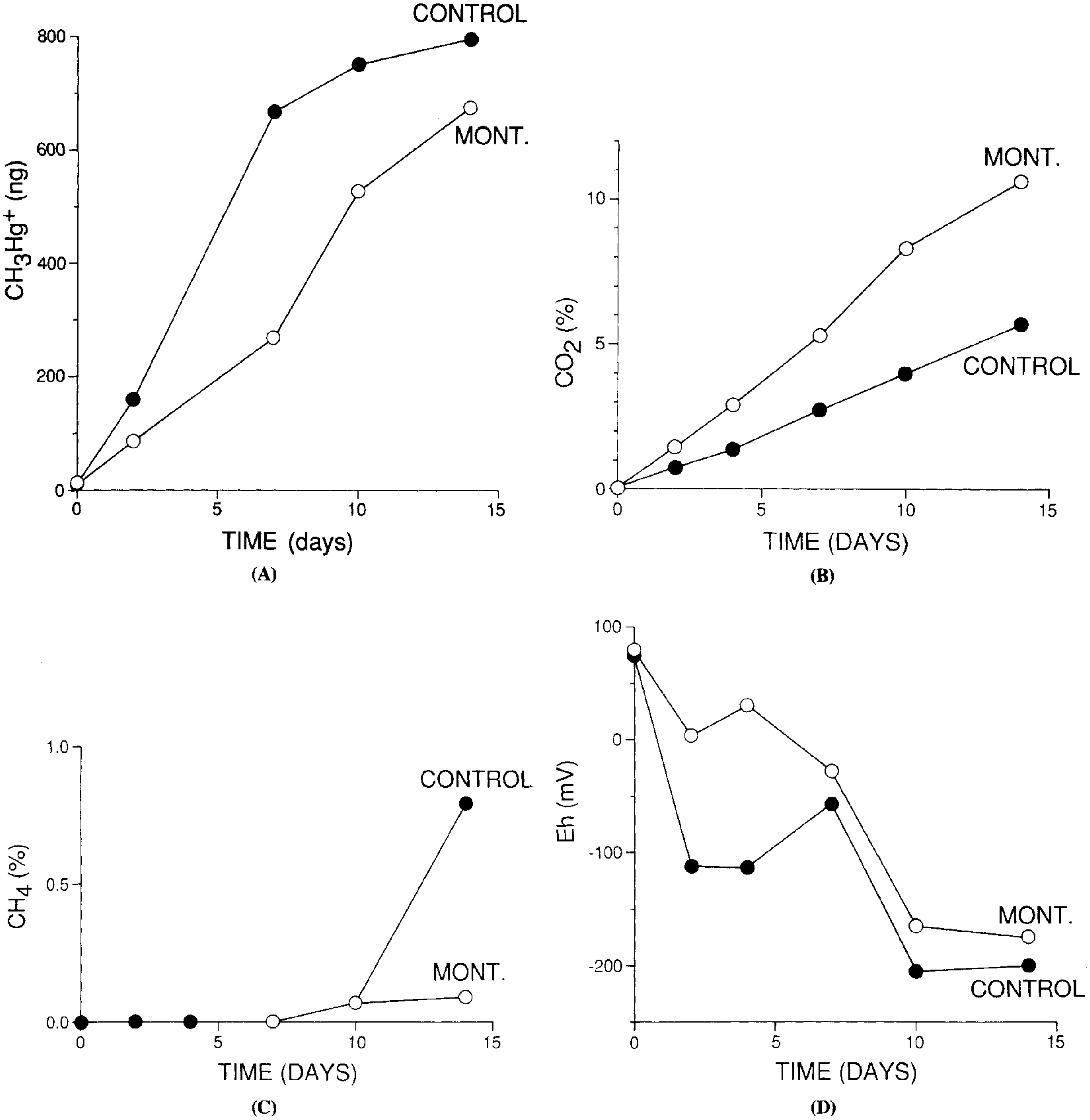


Figure 9 Effects of montmorillonite on (A) CH_3Hg^+, (B) CO_2, and (C) CH_4 production, and (D) Eh in nutrient-enriched, buffered PL sediment suspended in $HgCl_2$ solution. 'Control' slurries contained no added clay.

concentration as a function of time in the presence of FeOOH was S-shaped, resembling microbial growth curves which rise exponentially and then level off; but the control curve was practically linear with an incipient convex-upward shape. This difference in the shapes of the two curves accounts for the reversal of their relative positions, and indicates that the change was due entirely to a shift in the character of the microflora in the slurries containing FeOOH: the controls maintained a constant trend, and the experimental systems varied with respect to it. Alteration of the nature or activities of the microflora was also indicated by the fact that the change from inhibition to enhancement of CH_3Hg^+ production was accompanied by a change in

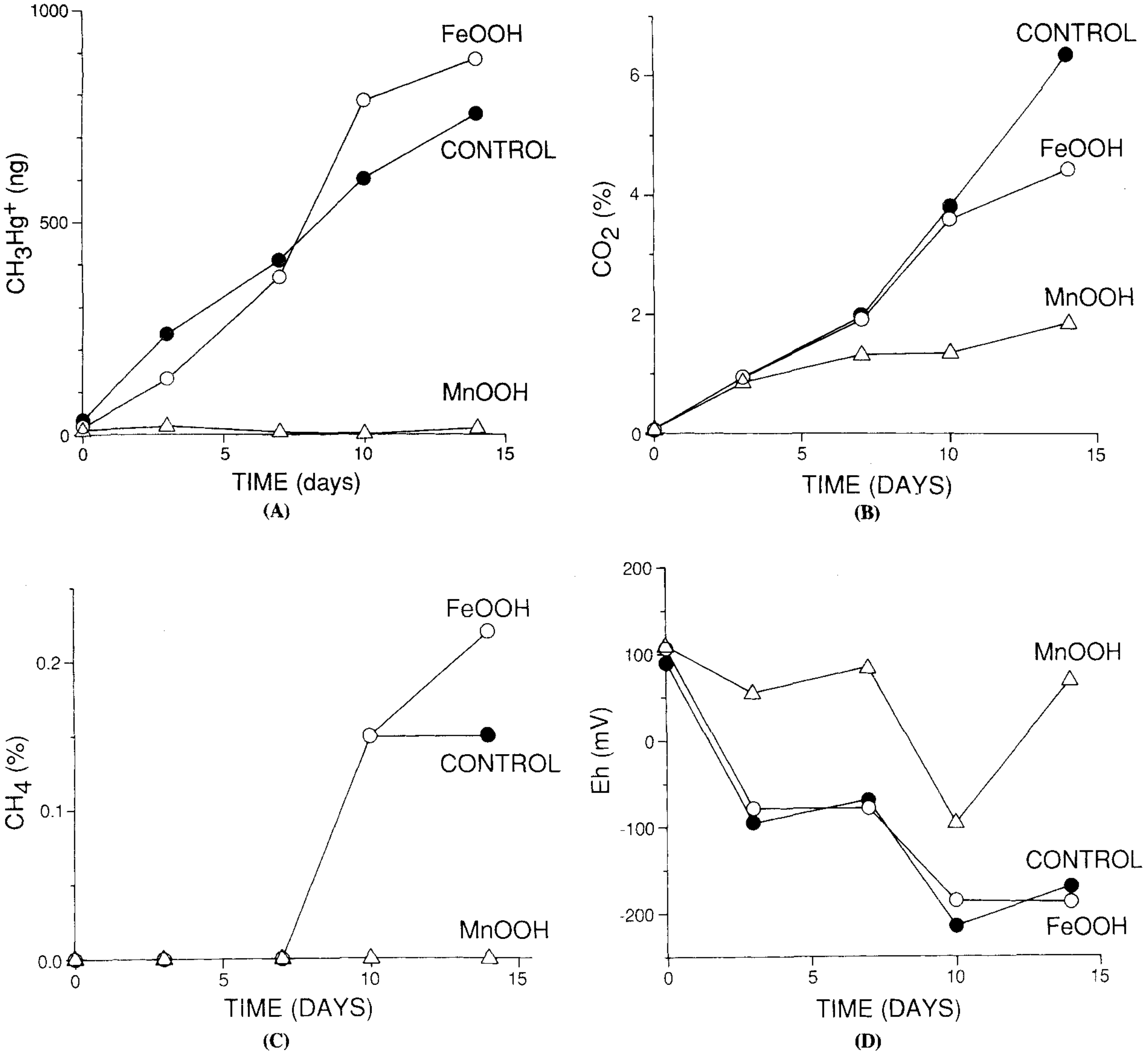


Figure 10 Effects of FeOOH (goethite) and MnOOH (manganite) on (A) CH_3Hg^+, (B) CO_2, and (C) CH_4 production, and (D) Eh in nutrient-enriched, buffered PL sediment suspended in $HgCl_2$ solution. 'Control' slurries contained no added clay.

the CO_2 and CH_4 production rates (Figs 10B and 10C). Initially, the rates of CO_2 and CH_4 production were the same in the experimental and control systems; but then, as the FeOOH began to increase the rate of methylation, it simultaneously lowered the rate of CO_2 output while accelerating CH_4 output. On the other hand, the Eh values of the experimental and control systems did not differ significantly (Fig. 10D). The Eh curves zigzagged, but their overall tendency was to decrease with time, indicating continuous microbial activity. The close parallelism between the two independent curves proves that the zigzags represent real events, not random fluctuations due to experimental error.

MnOOH (manganite) had radically different biogeochemical effects from FeOOH. MnOOH almost totally suppressed methylation (Fig. 10A), inhibited CO_2 production to a much greater extent than FeOOH did (Fig. 10B), completely blocked CH_4 production (Fig. 10C), and caused a large increase in the Eh values (Fig. 10D). These findings suggest general inhibition of microbial growth by MnOOH. The Eh and CH_4

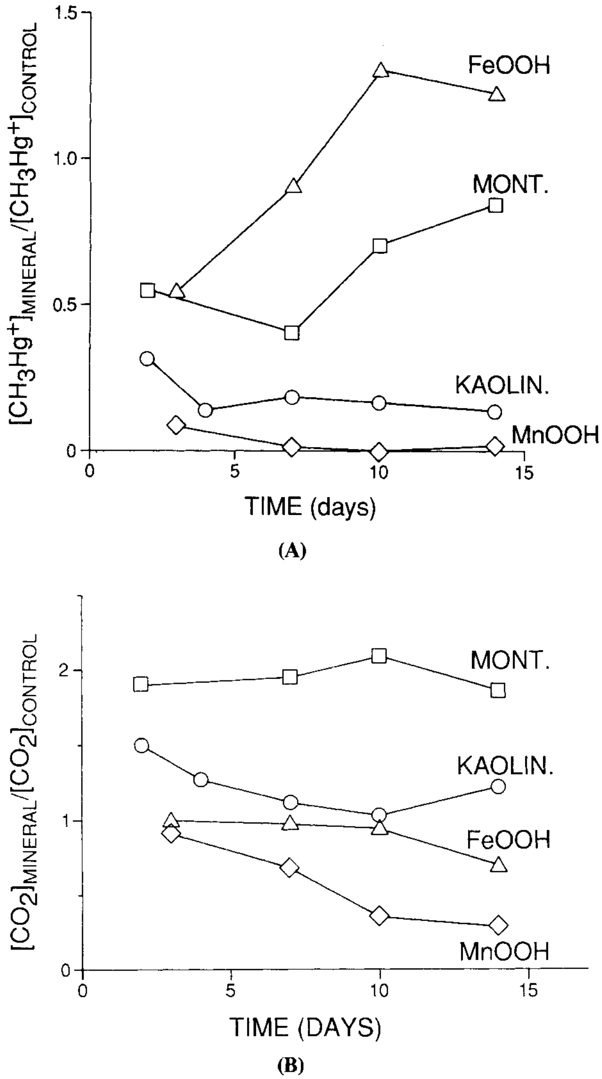


Figure 11 Comparison of effects of kaolinite, montmorillonite, FeOOH (goethite), and MnOOH (manganite) on production of (A) CH_3Hg^+ and (B) CO_2 (see Figs 8–10). CH_3Hg^+ and CO_2 levels in slurries with added clay and oxide were normalized with respect to control values and plotted against incubation time.

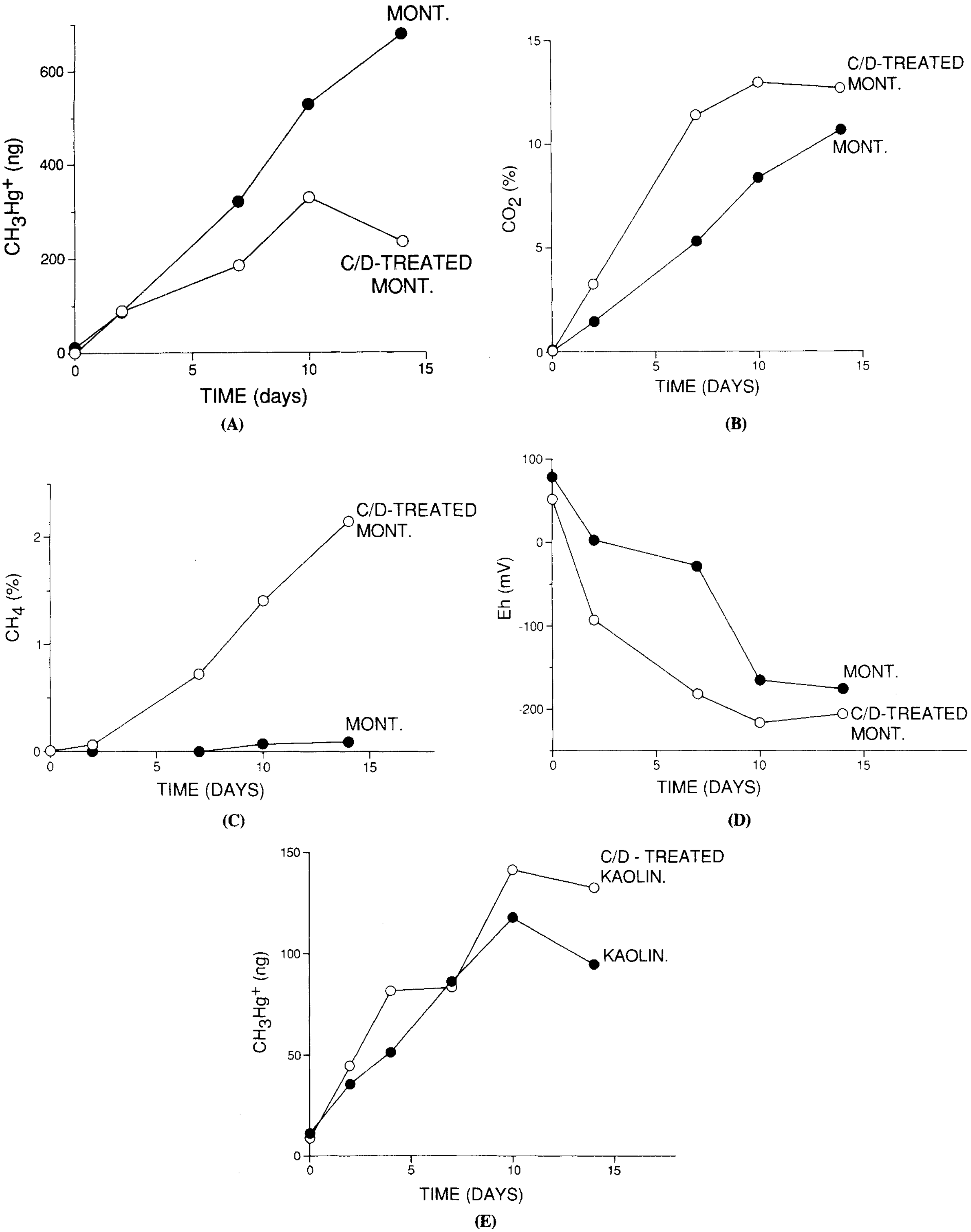
data are also consistent with the possibility that MnOOH acted as an oxidizing agent.

The effects of the clay minerals and oxides on methylation and CO_2 production are summarized and compared in Figs 11A and 11B, in which the CH_3Hg^+ and CO_2 concentrations in the experimental systems normalized with respect to the CH_3Hg^+ and CO_2 concentrations in the control systems are plotted against incubation time. Fig. 11A demonstrates clearly that the influence of the added mineral on CH_3Hg^+ production became increasingly favourable in the order MnOOH < kaolinite < montmorillonite < FeOOH (Fig. 11A). As the abundance of FeOOH associated with the clay increases in the same order (Table 2), the results reveal that, all other factors being equal, FeOOH films on clay crystals may tend to promote microbial methylation of Hg. Fig. 11B, however, shows that both FeOOH and MnOOH interfered with CO_2 production, whereas the two clay minerals fostered it. The effect of the added mineral on CO_2 production became increasingly favourable in the order MnOOH < FeOOH < kaolinite < montmorillonite.

These findings were confirmed by the results of a methylation experiment comparing the effects of montmorillonite with and without its natural coating of oxides. Removal of oxides by C/D extraction lowered the rate of CH_3Hg^+ production (Fig. 12A) but increased the rates of CO_2 and CH_4 production (Figs 12B and 12C), besides reducing the Eh (Fig. 12D). Similar results had been obtained using varved clay and EML sediment (Figs 4A, 4C and 4D). In contrast, removal of oxides from kaolinite, the clay with the smallest oxide content (Table 2), generally *increased* the rate of CH_3Hg^+ production (Fig. 12E), even though its effects on CO_2 and CH_4 production and Eh were the same as with montmorillonite and varved clay.[71] The anomalous results obtained with kaolinite were later corroborated by means of another experiment employing fresh PL sediments.[71]

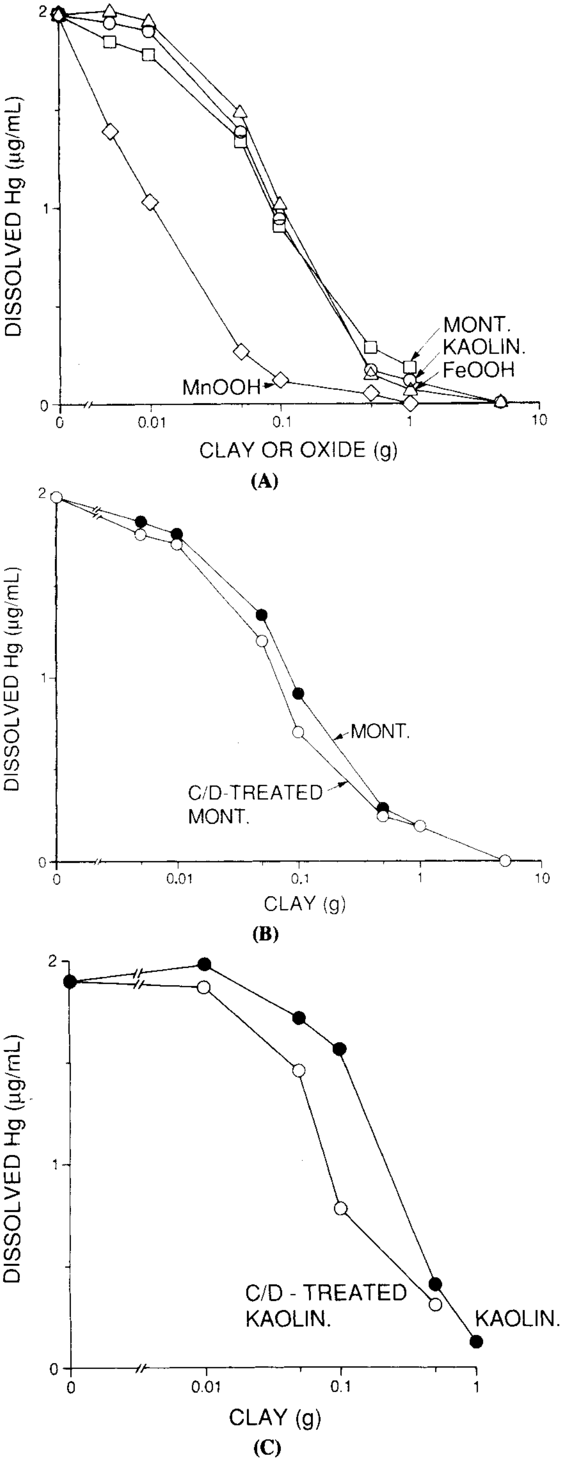
The curves illustrating the kinetics of Hg^{2+} adsorption by kaolinite, montmorillonite, and FeOOH practically coincided (Fig. 13A), ruling out any possibility that differences in adsorption efficiency or binding strength can account for the widely differing effects of these minerals on Hg methylation. MnOOH, however, had a much stronger affinity for Hg^{2+} than did the other minerals, adsorbing it more rapidly and attaining 100% uptake at a lower concentration of adsorbent (Fig. 13A). Therefore, it is conceivable that strong binding of Hg^{2+} by MnOOH played a part in the mineral's suppression of Hg methylation. C/D-extracted montmorillonite and kaolinite adsorbed Hg^{2+} somewhat more efficiently than untreated clay (Figs 13B and 13C); but inasmuch as the leached minerals had opposite effects on Hg methylation, it is doubtful whether adsorption kinetics had anything to do with these effects.

Humic acid, like FeOOH, tended to enhance CH_3Hg^+ production but was not entirely consistent,



(A) (B) (C) (D) (E)

Figure 12 Comparison of effects of C/D-treated and untreated montmorillonite on (A) CH_3Hg^+, (B) CO_2, and (C) CH_4 production and (D) Eh, and (E) effects of production, in nutrient-enriched, buffered PL sediment suspended in $HgCl_2$ solution.



(A)

(B)

(C)

Figure 13 Comparison of the kinetics of $HgCl_2$ adsorption by (A) kaolinite, montmorillonite, FeOOH (goethite), and MnOOH (manganite), (B) C/D-treated and untreated montmorillonite, and (C) C/D-treated and untreated kaolinite. Quantity of Hg remaining in solution after 24 h was plotted against quantity of clay or oxide.

as revealed by the results of a methylation experiment in which nutrient-enriched, $CaCO_3$-buffered PL sediment suspended in $HgCl_2$ solution with and without dissolved humic acid was incubated for varying periods of time up to 14 days (Fig. 14A). At first, the humic acid had no effect on methylation, but then it temporarily stimulated CH_3Hg^+ production. After this burst of activity, the CH_3Hg^+ levels in the experimental systems declined to the levels seen in the controls. Precisely during the interval in which it was promoting methylation, the humic acid engendered a small rise in CO_2 production, but when the CH_3Hg^+ level subsequently dropped, the rate of CO_2 production also fell (Fig. 14B). However, the humic acid induced a dramatic upsurge in CH_4 production at the same time that CH_3Hg^+ and CO_2 were declining (Fig. 14C). These synchronized changes in the rates of CH_3Hg^+, CO_2, and CH_4 biosynthesis are clearly due to temporal variations in the nature and activities of the microflora. Furthermore, the humic acid brought about a consistent lowering of the Eh (Fig. 14D), presumably by functioning as a reducing agent or by stimulating microbial activity, or both.

It is noteworthy that the humic acid either fostered Hg methylation or at least did not interfere with it in spite of having a strong tendency to complex Hg^{2+}. After 30 h of continuous dialysis, a solution of $HgCl_2$ mixed with dissolved humic acid retained about 77% of its original Hg content, whereas an aqueous control solution containing $HgCl_2$ but no humic acid lost all but about 3% of its Hg (Fig. 15).

In none of the methylation experiments treated in this section can any of the results be explained by variations in pH. All of the slurries were buffered with $CaCO_3$, and the pH values never deviated far from 7 (Table 1).

Wabigoon River system

Figure 16 shows the results of a methylation experiment in which replicate portions of nutrient-enriched, unbuffered Clay Lake (CL) sediment suspended in $HgCl_2$ solution were incubated for seven days with and without different quantities of kaolinite, montmorillonite, and synthetic FeOOH and MnOOH. The data demonstrate spectacular preferential stimulation of CH_3Hg^+ production by FeOOH, contrasted with relatively minor enhancement of CH_3Hg^+ production by the two clay minerals and absolute suppression of it by MnOOH (Fig. 16A). Thus, in the presence of

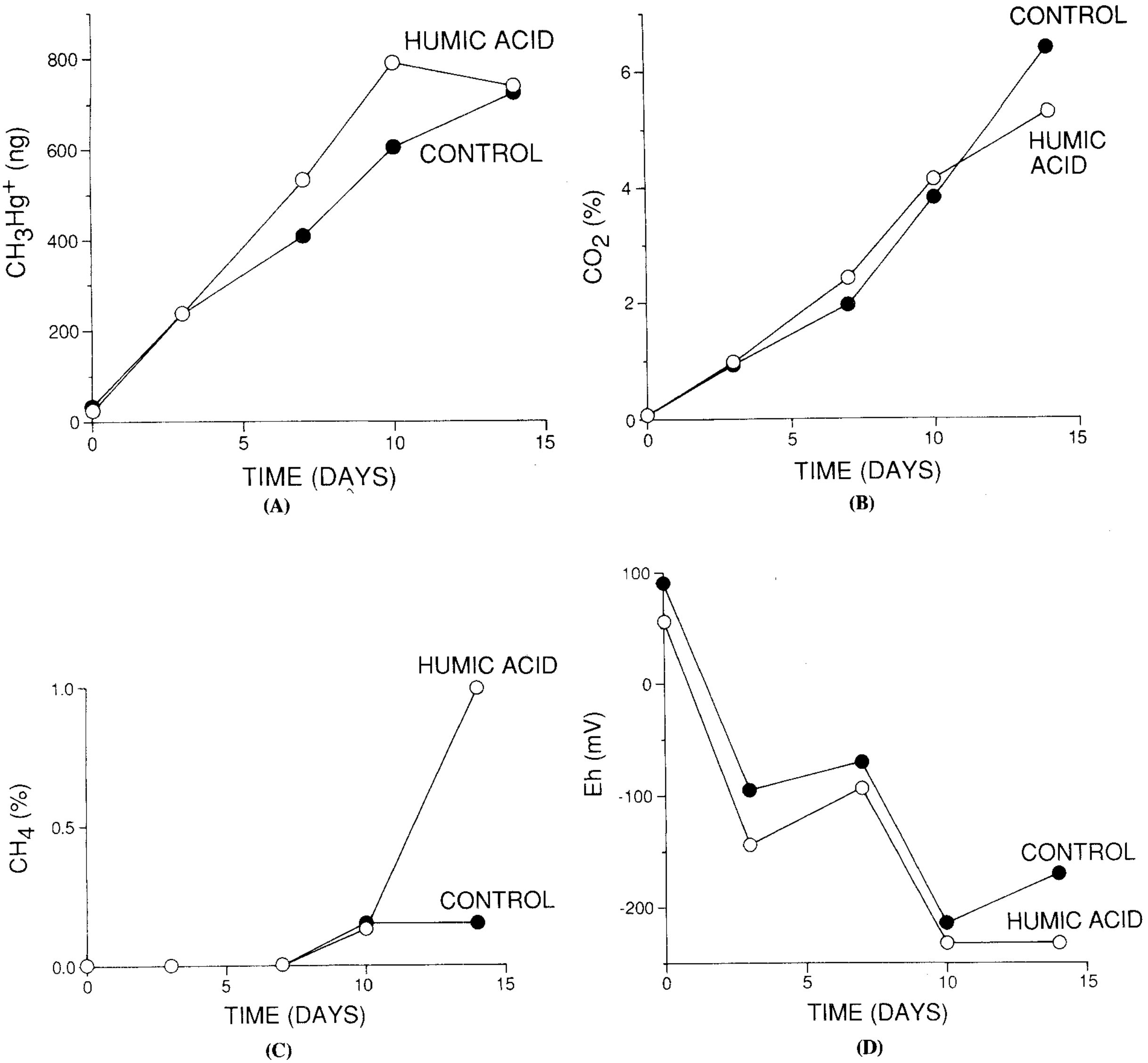


Figure 14 Effects of dissolved, non-dialysable humic acid on (A) CH_3Hg^+, (B) CO_2, and (C) CH_4 production, and (D) Eh, in nutrient-enriched, buffered PL sediment suspended in $HgCl_2$ solution. 'Control' slurries contained no added humic acid.

clays and oxides the CH_3Hg^+ output increased in the order MnOOH < montmorillonite < kaolinite $\ll$ FeOOH. Despite its remarkably favourable effect on methylation, FeOOH lowered the rate of CO_2 production; indeed, CH_3Hg^+ was *inversely* correlated with CO_2, the CO_2 levels increasing in the order FeOOH < kaolinite < montmorillonite < MnOOH (Fig. 16B). CH_4 levels, however, varied independently of CH_3Hg^+ and CO_2, increasing in the order FeOOH < kaolinite < MnOOH $\ll$ montmorillonite (Fig. 16C).

The pH values (Table 1) ranged from 4.9 to 7.9, as the slurries had not been buffered. Nevertheless, the variations in CH_3Hg^+ production were unrelated to the fluctuations in pH. For instance, 10 g of FeOOH gave nearly the same pH as 10 g of montmorillonite, although the CH_3Hg^+ levels were radically different; contrariwise, 10 g of kaolinite and 10 g of montmorillonite yielded similar amounts of CH_3Hg^+ but widely divergent pH values.

The variations in the effects of the clays and oxides

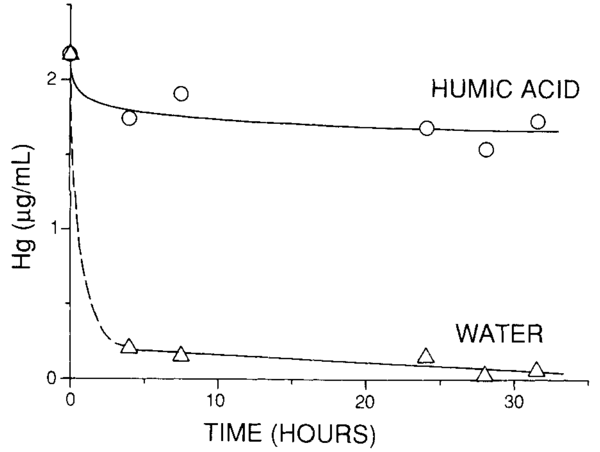


Figure 15 Effects of dissolved, non-dialysable humic acid on the diffusion of dissolved $HgCl_2$ through a dialysis membrane. Quantities of Hg remaining in dialysis bags containing aqueous $HgCl_2$ solutions with and without humic acid were plotted against dialysis time.

on methylation were independent of the ability of the minerals to adsorb Hg^{2+} (Fig. 17). Surprisingly, FeOOH, despite its anomalously favourable effect on CH_3Hg^+ production, was the most efficient adsorbent. Kaolinite adsorbed only about 2% of the dissolved Hg, whereas the other minerals adsorbed ~75–98%. This large disparity, though consistent with kaolinite's relatively weak adsorptive capabilities, is not in keeping with the results of the other, previously mentioned, adsorption experiments (Figs 3 and 13A). The aberration can probably be explained by a major difference in the proportion of dissolved Hg to adsorbent: in the experiment represented by Fig. 17, the proportion was 50–100 µmol g^{-1}, but in the experiments represented by Figs 3 and 13A it was ≤ 0.04 µmol g^{-1}.

Analytical data for sediment samples collected from Clay Lake, Ball Lake, and an intervening stretch of the Wabigoon River testified to the importance of Fe in regulating CH_3Hg^+ production under field conditions, although the relationship is evidently not a simple one. A plot of CH_3Hg^+ concentration against total Fe concentration revealed that the patterns of variation representing the three sampling regions resemble each other closely (Fig. 18). Although in each case the distribution of points zigzags and shows no overall trend, the patterns are not merely the result of random fluctuations or analytical error, as evidenced by the parallelism between the three independent curves. In particular, note that the major peak in each plot occurs

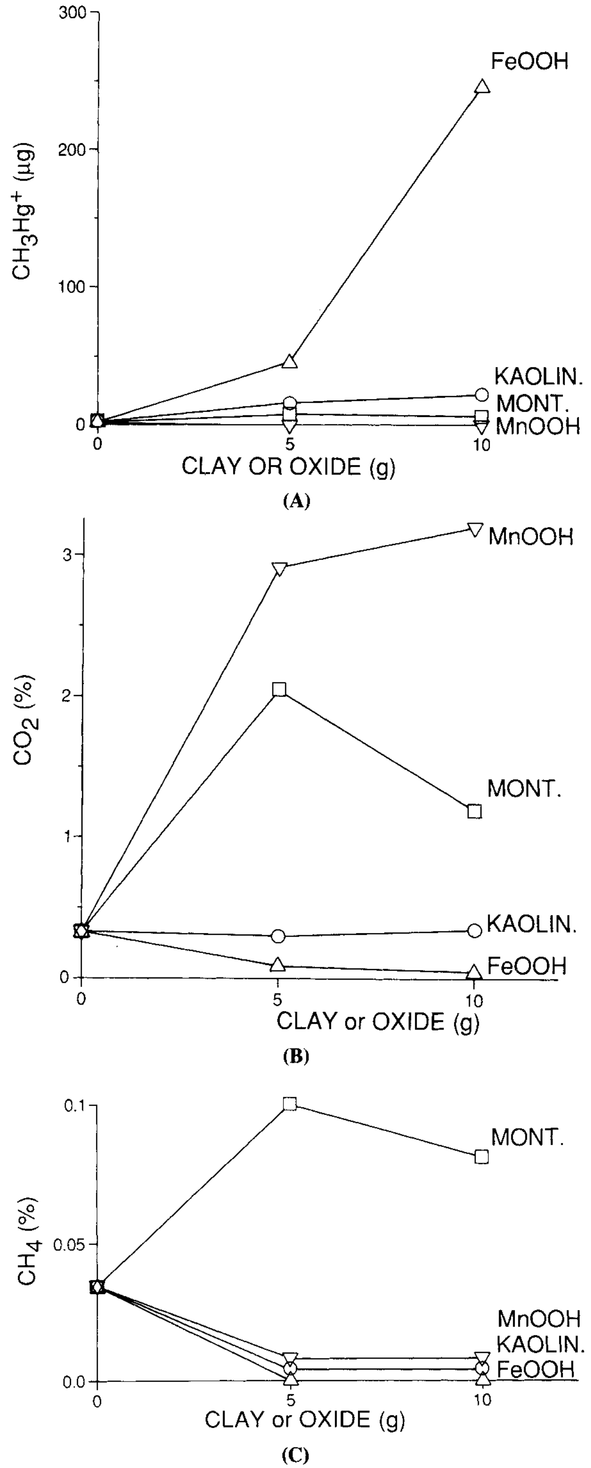


Figure 16 Effects of kaolinite, montmorillonite, and synthetic FeOOH and MnOOH on production of (A) CH_3Hg^+, (B) CO_2, and (C) CH_4 by nutrient-enriched CL sediment suspended in $HgCl_2$ solution. CH_3Hg^+, CO_2, and CH_4 levels attained after incubation for seven days were plotted against quantity of mineral added.

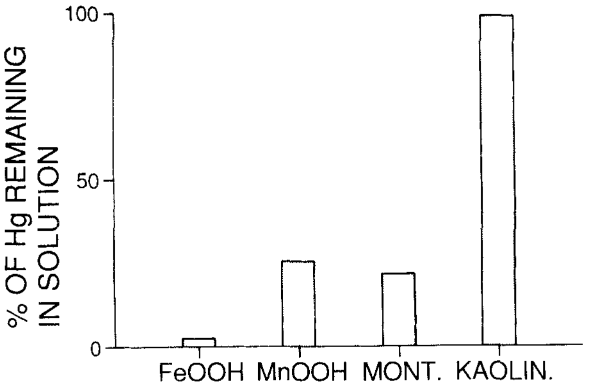


Figure 17 Variations in the efficiency of $HgCl_2$ adsorption by 10-g portions of kaolinite, montmorillonite, and synthetic FeOOH and MnOOH over a 24-h period.

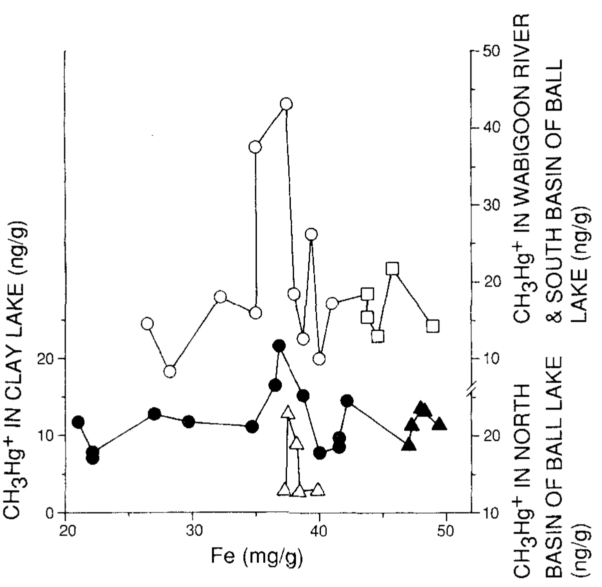


Figure 18 Environmental CH_3Hg^+ concentrations in sediments from Clay Lake, Ball Lake, and the Wabigoon River plotted against the total Fe concentrations in the sediments (analytical data for field samples). ● Clay Lake (east basin); ▲ Clay Lake (west basin); □ Wabigoon River; ○ Ball Lake (south basin); △ Ball Lake (north basin).

within the same narrow range of Fe concentrations (~35–40 mg g^{-1}), with the result that the three peaks almost coincide. There are two other coincident or nearly coincident peaks at higher Fe concentrations. Such striking similarities suggest a single underlying cause. The shapes of the curves recall the zigzag patterns observed in the experimental work (cf. Figs 7A and 7B) and may well be explicable by the same fundamental principle.

The nature of the Fe in the sediments is not well known, but most of it was probably complexed with organic matter. All sediment samples except the ones from the east basin of CL gave a highly significant positive correlation between total Fe and organic carbon (C), whereas only 1.89–8.06% of the Fe was in the Fe oxide fraction (defined operationally as all forms of Fe extracted sequentially with $NH_2OH.HCl$ and C/D).[71] In the east basin of CL there was no significant correlation between Fe and organic C, probably because any association of Fe with natural organic matter was masked by organic paper mill wastes (wood fragments) transported into that end of the lake by the Wabigoon River.[5,6]

The key role of oxide coatings on clay minerals: summary of results for different clays and field sites

According to the experimental data, Fe oxides tend to promote methylation of Hg, whereas Mn oxides tend to suppress it, and the role of clay minerals in methylation is critically dependent on the oxide coatings that commonly occur on their crystal faces. The broad applicability of these generalizations is attested by the fact that experiments involving sedimentary microflora from three widely separated field sites representing very different environments, as well as different natural and synthetic oxides and clays with and without oxide coatings, all gave results which, though differing in detail, led to the same general conclusions. In this section effects of the oxide coatings of varved clay, montmorillonite, and kaolinite on CH_3Hg^+ production by microbes in EML and PL sediments incubated under identical conditions are summarized and compared. Results of the CL experiment are not included because the experimental conditions were different.

The ratio of CH_3Hg^+ produced in the presence of clay to CH_3Hg^+ produced in the presence of the same clay stripped of its oxides by C/D or HCl treatment increased in the order kaolinite < montmorillonite < varved clay (Fig. 19A). Therefore, the greater the quantity of oxide associated with the clay (Table 2), the more favourable the influence of the clay on methylation. C/D-leached and HCl-leached varved clay gave nearly the same results (Fig. 19A).

The ratio of CH_3Hg^+ generated in seven days in the presence of clay to CH_3Hg^+ generated in control slurries without added clay gave a highly significant positive correlation with the C/D-extractable Fe content of the clay (Fig. 19B) and a weaker, though signifi-

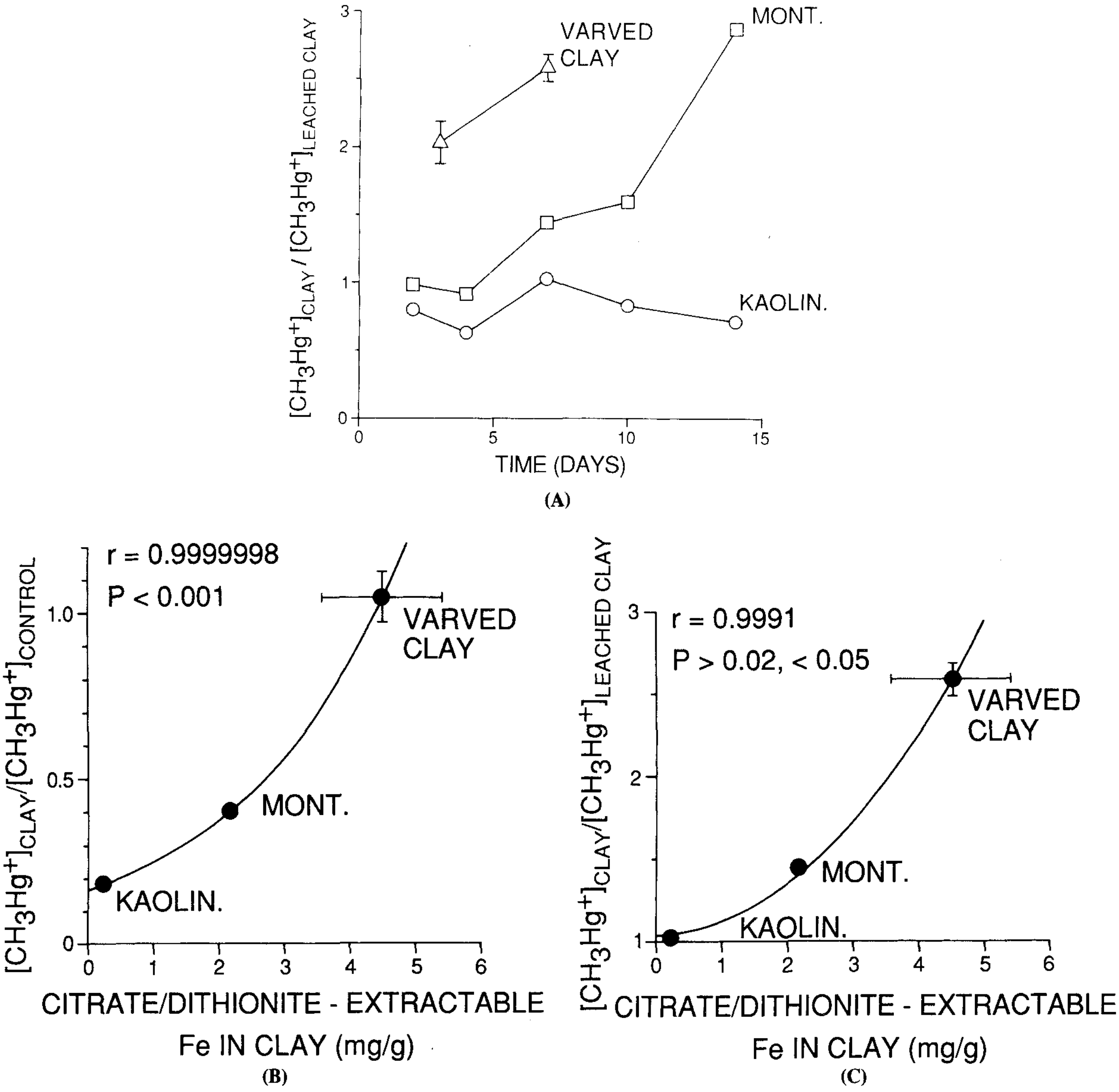


Figure 19 Apparent stimulation of CH_3Hg^+ production in EML and PL sediments by Fe oxide coatings on varved calcareous clay, montmorillonite, and kaolinite. CH_3Hg^+ produced in the presence of untreated clays was normalized with respect to CH_3Hg^+ produced either in the presence of C/D- and HCl-leached clays or in the control systems (i.e. in the absence of added clay) and then plotted against (A) incubation time and (B,C) the C/D-extractable Fe content of the clay. CH_3Hg^+ data shown in B and C are the levels observed at day 7.

cant, correlation with the much less abundant C/D-extractable Mn ($r = 0.9999$; $P \simeq 0.01$). Thus, it would seem that FeOOH coatings on clay surfaces counteract the unfavourable effect of the clay on microbial production of CH_3Hg^+: the greater the quantity of FeOOH, the higher the net rate of CH_3Hg^+ production. The existence of such a strong relationship despite the differences in the clay minerals and sediments employed implies that the oxide concentration was the only important factor responsible for the observed variations in CH_3Hg^+ level. The ratio of CH_3Hg^+ produced in the presence of clay to CH_3Hg^+ produced in the presence of the same clay stripped of its oxide coating also gave a significant positive correlation with C/D-extractable Fe (Fig. 19C), indicating, again, that FeOOH coatings on clay tend to enhance CH_3Hg^+ production. Note that the ratio approaches a value of 1 as the FeOOH concentration approaches zero. C/D-extractable Mn gave an equally significant correlation ($r = 0.9994$; $0.02 < P < 0.05$.

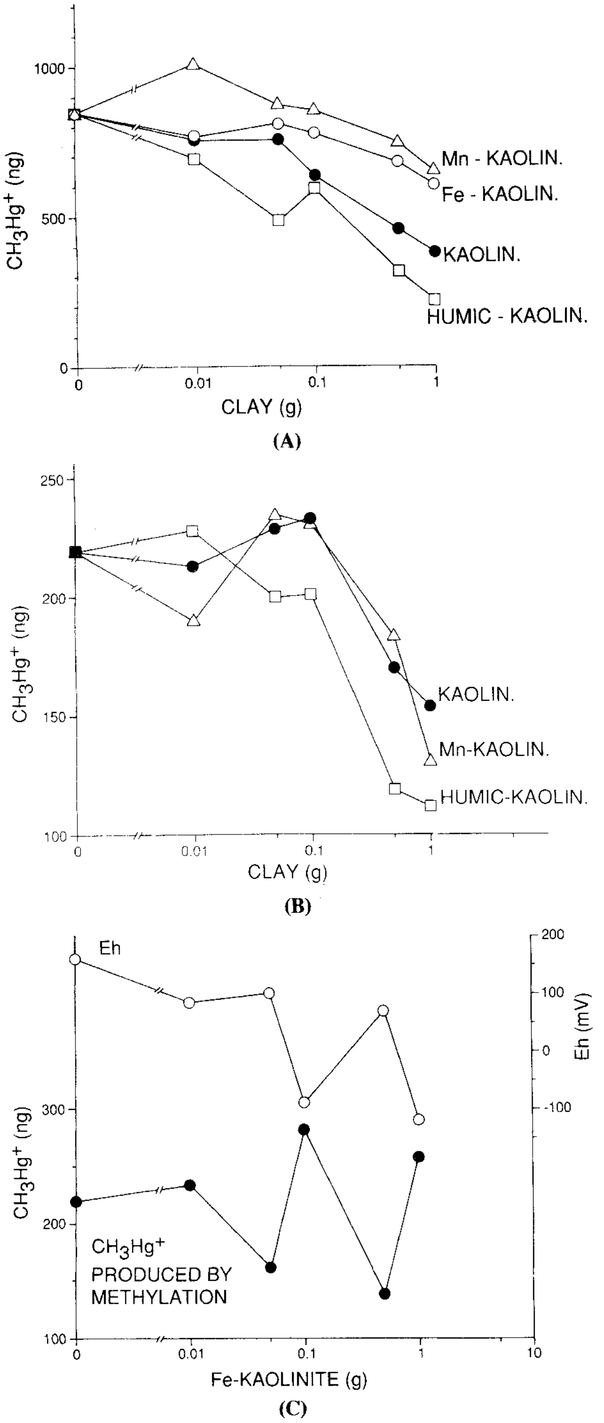


Figure 20 Effects of different quantities of kaolinite with and without artificial coatings of FeOOH, MnOOH, and humic acid on CH_3Hg^+ production and Eh in (A) nutrient-enriched and (B, C) unaltered PL sediments suspended in $HgCl_2$ solution and incubated for seven days.

Interaction between organic nutrients and oxides: their combined effects on methylation and demethylation

A series of methylation and demethylation experiments was performed in which PL sediment was incubated for seven days in the presence of varying amounts of kaolinite with and without artificially deposited FeOOH, MnOOH, and humic acid coatings. C/D-leached kaolinite was also used. In one set of experiments an organic nutrient supplement was mixed with the sediment, and in another (otherwise identical) set of experiments no extra nutrient substrate was added. No $CaCO_3$ buffer was used, but the pH values deviated very little from 7 (Table 1).

The results (Figs 20 and 21) showed that the nutrient substrate profoundly affected the role of oxide coatings in methylation and demethylation reactions, although the role of humic acid coatings was not altered.

In the presence of organic substrate, methylation rates tended to decline with increasing concentration of clay, and all forms of the clay gave similar trends (Fig. 20A). Nevertheless, the clays differed consistently in the extent to which they interfered with CH_3Hg^+ production. The degree of inhibition increased in the following order: Mn–clay < Fe–clay < clay < humic–clay. As expected, a surface coating of FeOOH tended to neutralize the inhibitory effect of the clay; but, surprisingly, an MnOOH coating did as well. Evidently a thin layer of MnOOH on clay can have a positive effect, even though massive amounts of MnOOH may hinder methylation (cf. Figs 10A and 16A). The inhibitory effect of the humic acid coating was also unexpected (cf. Fig. 14A), but it may be due to phenomena such as the masking of oxide films on the clay crystals.

Without nutrient enrichment, methylation again decreased with increasing concentrations of untreated clay, Mn–clay, and humic–clay, except that all three of them gave a maximum or shoulder in the CH_3Hg^+ distribution at 0.05–0.1 g of clay (Fig. 20B). In the presence of Fe–clay, the CH_3Hg^+ levels oscillated wildly and erratically, and showed no overall trend or systematic relationship with the data for the other clays, though they were inversely related to the Eh (Fig. 20C). Of particular interest is the fact that the

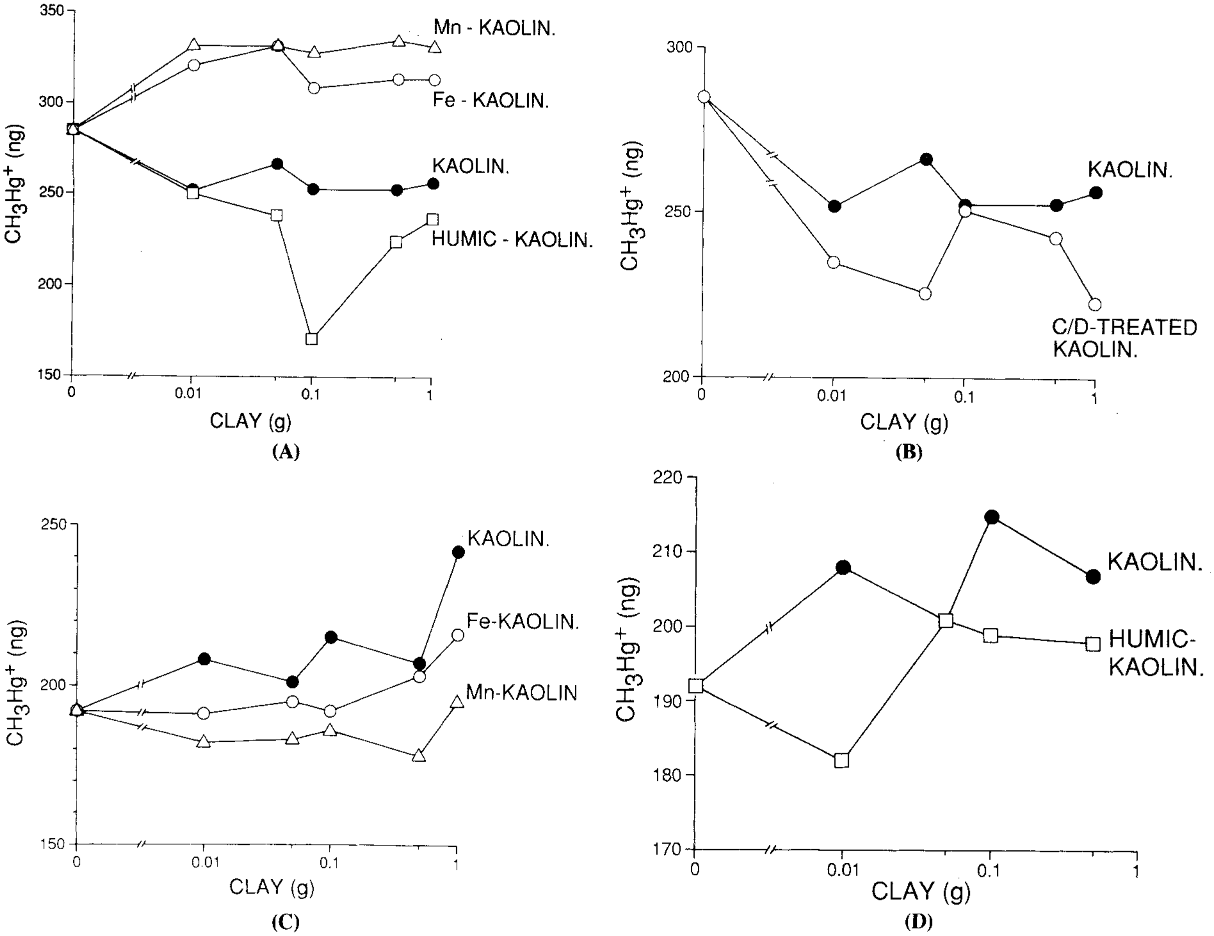


Figure 21 Effects of different quantities of kaolinite with and without artificial coatings of FeOOH, MnOOH, and humic acid on CH_3Hg^+ decomposition in (A, B) nutrient-enriched and (C, D) unaltered PL sediment suspended in CH_3Hg^+ acetate solution and incubated for seven days.

CH_3Hg^+ curves for clay and Mn–clay virtually coincided (Fig. 20B). This means that in the nutrient-poor environment the MnOOH coating did not significantly alter the clay's adverse influence on methylation, whereas in the nutrient-rich environment the MnOOH coating largely counteracted this effect. Yet the role of the humic acid coating was the same in both the nutrient-poor and nutrient-rich systems (Figs 20A and 20B).

In the nutrient-enriched sediment, Fe– and Mn–clays both inhibited demethylation, whereas humic–clay and untreated clay fostered demethylation, the rate of demethylation increasing in the order Mn–clay < Fe–clay < clay < humic–clay (Fig. 21A). By the same token, the enhancement of demethylation by the clay was increased when the clay's natural FeOOH and MnOOH coatings were removed by C/D treatment (Fig. 21B). Indeed, the CH_3Hg^+ data for the C/D-treated and untreated clays are inversely related.

In sediments that received no nutrient supplement the results were entirely different. There, the clays tended to inhibit demethylation, especially at the higher concentrations, but FeOOH, MnOOH and humic acid coatings all boosted the rate of demethylation, partly overcoming the negative effect of clay in its native state (Figs 21C and 21D). The relative rates of demethylation increased in the order clay < Fe–clay ≃ humic–clay < Mn–clay.

These findings may be summarized as follows.

(1) In the environment enriched in organic nutrients, oxide coatings caused relative enhancement of methylation and inhibition of demethylation, whilst humic acid coatings

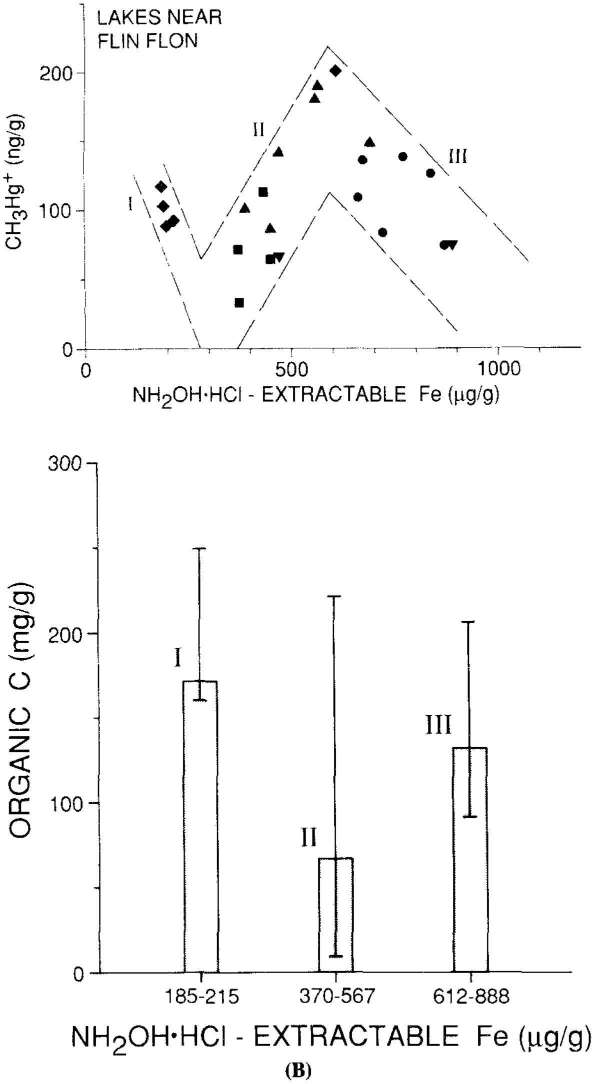


Figure 22 Relationships between the environmental (A) CH_3Hg^+ and (B) organic C concentrations and $NH_2OH \cdot HCl$-extractable Fe ('amorphous' FeOOH) concentrations in sediments from lakes near Flin Flon (analytical data for field samples). ● Schist Lake (northwest Arm); ○ Schist Lake (Inlet Arm); ▲ Phantom Lake; □ West Nesootao Lake; △ Hamell Lake. Fig. 22A is a modified version of a diagram published elsewhere (ref. 7, Fig. 14.7A, p 570).

caused relative inhibition of methylation and enhancement of demethylation.

(2) In the more nutrient-poor environment, MnOOH coatings had no effect on methylation, but both MnOOH and FeOOH coatings enhanced demethylation. Humic acid coatings depressed methylation but enhanced demethylation.

On the basis of these experimental results, one would expect to find that the net rate of CH_3Hg^+ production in nature is subject to synergistic and antagonistic interactions between oxides and organic matter, the CH_3Hg^+ levels in sediments rising and falling with variations in the proportions of these sediment components. The analytical data for sediments from lakes near Flin Flon exhibit just such a pattern of variation (Figs 22A and 22B). With increasing concentration of $NH_2OH \cdot HCl$-extractable Fe ('amorphous' colloidal FeOOH), the CH_3Hg^+ concentration alternately falls, rises, and then falls again, forming a three-limbed zigzag pattern (Fig. 22A) (cf. Fig. 18). The organic C concentrations of the sediments show a corresponding pattern of alternate decrease and increase (Fig. 22B). Thus, in this particular case, variations in the relative amounts of FeOOH and organic matter appear to affect the CH_3Hg^+ levels as follows:

(I) Low FeOOH, high organic C content: CH_3Hg^+ decreases with increasing FeOOH.
(II) Intermediate FeOOH, low organic C content: CH_3Hg^+ increases with increasing FeOOH.
(III) High FeOOH, high organic C content: CH_3Hg^+ decreases with increasing FeOOH.

Abiotic transformations of Hg

Autoclaved sediments from two lakes in northern Manitoba [East Mynarski Lake (EML) and Southern Indian Lake ('Methyl Bay', flooded forest zone)], when incubated with $HgCl_2$ solution for seven days, yielded no detectable CH_3Hg^+, whereas unsterilized sediment yielded copious amounts of it.[9,34] Sterilization of the sediments had totally destroyed their ability to methylate Hg, proving that methylation was strictly a function of micro-organisms.

Sterilization of EML sediment did not completely prevent demethylation, but loss due to abiotic breakdown was small compared with loss due to microbial demethylation. In experiments involving incubation of sterilized and unsterilized sediment suspended in CH_3Hg^+ acetate solution with and without nutrient supplement and $CaCO_3$ buffer, loss through abiotic reactions was only 0–2.41% by day 7 and 0.689–9.06% by day 14; but loss due to microbial demethylation was 61.3–83.4% by day 7 and 82.0–97.0% by day 14.

On the other hand, certain clays were highly effective in promoting non-biological decomposition of

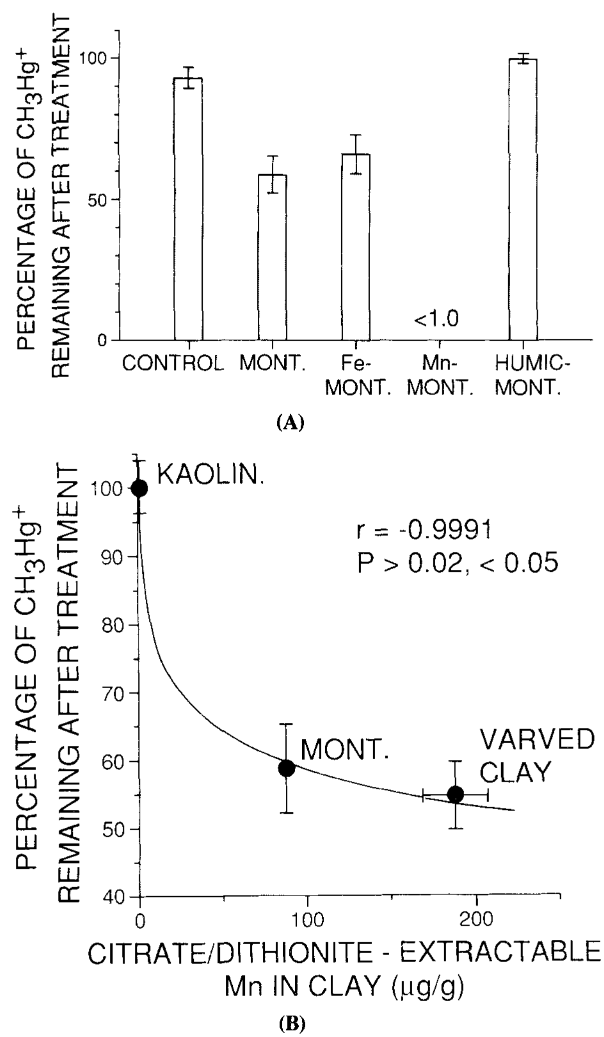


Figure 23 Effects of clays and their oxide coatings on abiotic demethylation of CH_3Hg^+ as represented by percentage loss of dissolved CH_3Hg^+ acetate after incubation with and without minerals for seven days: (A) effects of montmorillonite with and without artificial coatings of FeOOH, MnOOH, and humic acid; (B) apparent effects of C/D-extractable natural MnOOH coatings on kaolinite, montmorillonite and varved calcareous clay.

CH_3Hg^+, apparently owing to reactions catalysed by oxide coatings. In an experiment on abiotic demethylation in which dissolved CH_3Hg^+ was incubated for seven days in the presence and absence of montmorillonite with and without FeOOH, MnOOH, and humic acid coatings, only ~7% of the CH_3Hg^+ originally present in the clay-free control solution disappeared, but ~41% was broken down by montmorillonite, ~34% by Fe–montmorillonite, and 100% by Mn–montmorillonite, whereas none of the CH_3Hg^+ was destroyed by humic–montmorillonite (Fig. 23A). Under the same experimental conditions, kaolinite caused no loss of CH_3Hg^+, although montmorillonite and varved clay caused ~35–50% loss. The quantity of CH_3Hg^+ remaining by the end of the experiment decreased in the order kaolinite > montmorillonite > varved clay and gave a significant inverse correlation with the concentration of natural C/D-extractable MnOOH associated with the clay (Fig. 23B). C/D-extractable FeOOH gave a similar correlation, but it was highly insignificant ($r = -0.948$; $P \gg 0.1$).

These results indicate that MnOOH catalysed nonbiological demethylation of CH_3Hg^+, whereas FeOOH did not. Humic acid provided full protection against abiotic breakdown, probably by coating the oxide surfaces responsible for catalysing demethylation or by otherwise neutralizing their effects.

The results of these experiments are consistent with the possibility that abiotic demethylation is at least partly responsible for the observed suppression of microbial CH_3Hg^+ production in the presence of MnOOH (Figs 10A and 16A). However, this is an exceptional case. In the presence of unsterilized sediment, microbial transformations of Hg almost always predominated over any nonbiological reactions, as, for instance, in the experiments in which (1) Mn-clay *inhibited* demethylation (Fig. 21A), (2) humic-clay *promoted* demethylation (Figs 21A and 21D), and (3) clays become *less* effective in lowering the net rate of CH_3Hg^+ production as their natural MnOOH content *increased* (Fig. 19B; Table 2).

DISCUSSION

Microbial communities in sediments consist of different co-existing species occupying diverse ecological niches and may interact with each other in complex ways ranging from mutualism to fierce competition and antagonism. With a change in the physicochemical environment, either imposed from the outside or caused by the organisms themselves (as when aerobic microbes create anaerobic conditions by exhausting the local oxygen supply), ecological succession may take place. Changes in total microbial activity and population density may also occur. In this manner the microflora may exhibit a sequence of shifts in species composition and population size, and in the nature and intensity of its biochemical activities. Accordingly, as the sedimentary environment varies, Hg methylating and demethylating microbial populations can be expected to undergo successive changes of this kind.

Methylation and demethylation of Hg are natural processes mediated by many different kinds of heterotrophic sedimentary microbes which, judging by the wide range of environmental conditions under which these transformations occur, belong to a great diversity of ecological niches. Consequently, spatial and temporal variations in the physicochemical properties of their habitat (apart from any change in the quantity and availability of the Hg supply) could result in an increase or decrease, or alternating increases and decreases, or perhaps no appreciable net change, in the abundance of CH_3Hg^+ owing to the various effects of these environmental changes on the Hg-transforming microbial species. The findings presented here demonstrate effects of clay minerals, oxides, and humic matter on the production and decomposition of CH_3Hg^+ in sediments. These substances (which, in this discussion, will be collectively termed 'colloids' for convenience) are major, ubiquitous constituents of fine-grained sediments, and, as revealed here, they are not merely inert structural units of the matrix in which sedimentary microbes live; as in soil, they play active biogeochemical and ecological roles. The results show that colloids have diverse, complex, and often dramatic effects on the methylation and demethylation of Hg by microbes, and on other microbial activities that may be directly or indirectly linked to, or may influence, the Hg transformations. Depending on the nature, quantity, and surface chemistry of the colloid, the source of the sediment (and therefore, the nature of the microbial community), and environmental factors such as nutrient levels, such a substance may greatly suppress or promote Hg methylation or demethylation or it may have little or no effect. As shown in this paper, the effects are not altogether consistent and predictable, although it is possible to formulate certain broad generalizations (see below).

Generally, the intervention of colloids in pathways of CH_3Hg^+ formation and breakdown appeared to be due to effects on particular kinds of microbe, and ecological succession was evidently a common occurrence. These conclusions are based on the following observations.

(A) Variations in CH_3Hg^+ level and other indicators of microbial activity with respect to incubation time or concentration of colloid commonly showed a zigzag pattern rather than a smooth, simple trend, suggesting ecological succession and accompanying shifts in the balance between methylators and demethylators (Figs 1, 2A, 5, 7, 8D, 9D, 10D, 14D, 18, 20B, 21A and 22A). Similar patterns shown by independent sets of samples proved that these complex variations were not fortuitous (Figs 1, 2, 7, 8D, 9D, 10D, 14D, 18, 20B and 21A). Particularly striking instances of presumed microbial succession resulting in radical changes in Hg speciation were seen in methylation experiments involving EML sediment, in which clays and oxides had little or no effect on CH_3Hg^+ production but strongly promoted subsequent decomposition of CH_3Hg^+, when methylators apparently became inactive and were displaced from their original position of dominance by demethylators (Figs 1 and 2).

(B) Colloid-induced changes in the dynamics of Hg speciation were commonly accompanied by distinctive changes in other indicators of microbial activity (CO_2 and CH_4 levels and Eh), suggesting that the colloids caused shifts in the character and biochemical activities of the microflora (Figs 1, 4, 7, 8, 9, 10, 12, 14, 16 and 20C). Most of these variations imply that the changes in Hg speciation were due to specific effects on the nature of the microflora. Striking examples of such apparently selective effects included (1) alternate increase and decrease in CO_2 coinciding with increase and decrease in CH_3Hg^+ (Figs 1, 7A and 14); (2) stimulation of CO_2 and CH_4 production despite inhibition of methylation and demethylation (Figs 4 and 12); (3) decreases in Eh accompanying increases in CH_3Hg^+ (Fig. 7A); (4) association of CH_4 production and Eh decrease with demethylation (Fig. 7B); (5) stimulation of CO_2 production and inhibition of CH_4 production accompanying inhibition of methylation (Figs 8 and 9); (6) inhibition of CO_2 production and enhancement of CH_4 production accompanying stimulation of methylation (Fig. 10) *or* inhibition of methylation (Fig. 14). As can be seen, some of these relationships are not consistent (for instance, the association of enhanced CH_4 production with increased methylation *and* demethylation). This probably reflects the fact that methylation and demethylation are mediated by a wide variety of microbial species. Possibly both methylation and demethylation may, in certain circumstances, be mediated by CH_4 producing bacteria.

(C) Sediments from differed field sites (EML and PL), when incubated with $HgCl_2$ under nearly identical conditions, different qualitatively in their Hg speciation kinetics as affected by clay (compare Figs 1 and 2 with Figs 8 and 9). This is probably due to a site-related difference in the species composition of the microbial community. In EML sediment, a methylation phase only slightly affected by clay was followed by a clay-enhanced demethylation phase; but in PL sediment, clay-enhanced demethylation may well have occurred *simultaneously* with methylation instead of following it, resulting in a large net inhibition of CH_3Hg^+ production right from the beginning, but without a large drop in CH_3Hg^+ level afterwards.

(D) Most of the colloid-induced variations in Hg speciation cannot be attributed to general stimulation or stifling of microbial activity as a whole, and they are apparently unrelated to the ability of a colloid to bind and immobilize Hg or to catalyse or impede abiotic breakdown of CH_3Hg^+. The one obvious exception was the suppression of the methylating activity of PL sediment by MnOOH (Fig. 10). This could possibly have been due to non-specific inhibition of microbes (Figs 10B–10D), immobilization of Hg^{2+} by strong adsorption (Fig. 13A), or abiotic decomposition of CH_3Hg^+ by heterogeneous catalysis or oxidation (Figs 23A and 23B) or a combination of these processes. On the other hand, suppression of the methylating activity of CL sediment by MnOOH (Fig. 16A) cannot be due to general suppression of microbial activity (Figs 16B and 16C) nor to immobilization of Hg^{2+} (Fig. 17).

Clay minerals and varved silty clay in their native state (with natural surface coatings intact) often inhibited methylation to a greater or lesser extent (Figs 1A, 7A, 8A, 9A, 11A, 19B, 20A and 20B) and fostered demethylation (Figs 1A, 2A and 21A), although in some cases they fostered methylation (Figs 1A, 2A, 7A and 21A) and inhibited demethylation (Fig. 21C). It is not certain from these results whether inhibition of CH_3Hg^+ production was due to active interference with methylation or acceleration of concurrent demethylation (or both).

Further investigation established that the apparent role of clay in microbial Hg transformations was actually a function of the nature and concentration of associated oxides, which were probably in the form of coatings on the mineral surfaces, as is commonly the case with clay. When the oxides were removed, the behaviour of the clay with respect to Hg changed dramatically. Bare clay surfaces, as compared with oxide-coated surfaces, caused pronounced inhibition of both methylation (Figs 4A, 5 and 12A) and demethylation (Figs 4B and 5) except in one anomalous case in which methylation (Fig. 12E) and demethylation (Fig. 21B) were enhanced by oxide removal.

The experiments repeatedly demonstrated the critically important influence of oxides on the methylation and demethylation of Hg. Fe oxide, whether in the form of goethite, synthetic FeOOH, or unidentified naturally occurring deposits on clay crystals, generally tended to promote methylation, offsetting the inhibitory effect of associated clay minerals (Figs 4A, 5, 10A, 11A, 12A, 16A, 19–C and 20A; Table 2). FeOOH was also capable of mildly inhibiting methylation under certain circumstances (Fig. 10A) and was able to promote demethylation (Figs 2, 4B, 5 and 21C), although inhibition of demethylation was observed as well (Fig. 2A).

Unlike FeOOH, MnOOH strongly suppressed methylation when present in relatively large quantities (5–10 g MnOOH/10–20 g sediment) (Figs 10A and 16A). On the other hand, smaller amounts of MnOOH present as deposits on clay surfaces actually stimulated methylation (Fig. 20A) or else had no appreciable effect (Fig. 20B), and either inhibited demethylation (Fig. 21A) or enhanced it (Fig. 21C), depending on whether the sediment had been enriched in nutrients. A distinctive characteristic of MnOOH, but not FeOOH, was that in the absence of viable microbes it caused rapid non-biological decomposition of CH_3Hg^+, even when present only as a surface film on clay (Figs 23A and 23B).

As pointed out above, the effects of oxides on methylation and demethylation can be attributed to selective effects on microbial activity, with the exception of one case in which suppression of CH_3Hg^+ production in the presence of MnOOH could have been due to non-specific inhibition of microbial growth (as measured crudely by CO_2 production), abiotic breakdown of CH_3Hg^+, or strong fixation of Hg^{2+}, or a combination of these factors (Figs 10, 13A, 23A and 23B).

The mechanisms whereby oxides and clays exerted the observed effects are unknown, but heterogeneous catalysis and adsorption and ion-exchange reactions are obvious possibilities (although it would seem that adsorption of Hg^{2+} was usually not a factor). In addition, the oxides may, in some cases, have acted as oxidizing agents. This is suggested by the fact that oxides, including oxide coatings on clay, usually raised the Eh of the sediment (Figs 2C, 9D and 10D), even if they also stimulated microbial activity (as represented by CO_2 production) (Figs 2B and 9B), which ought to have *lowered* the Eh. By the same token, removal of oxide coatings from clay lowered the Eh (Figs 4D and 12D); but the meaning of this observation is ambiguous, as CO_2 production was increased (Figs 4C and 12B), signifying that the drop in Eh could have been due at least partly to intensified microbial activity. Kaolinite, which had the lowest concentration of associated oxide, did not raise the Eh, and in fact lowered it (Figs 2C and 8D). Interestingly, FeOOH in the form of goethite was the only oxide that had no appreciable effect on the Eh (Fig. 10D), whereas MnOOH in the form of manganite raised the Eh considerably (Fig. 10D) under the same experimental conditions. The marked difference between MnOOH and FeOOH with regard to their effects on Hg speciation may well be related to their different capabilities as oxidizing agents.

It is also noteworthy that the respective roles of FeOOH and MnOOH in the creation and breakdown of CH_3Hg^+ were essentially independent of the source of the sediment and the nature of the clay which served as a carrier for the oxides. This implies that the observed phenomena are of widespread, if not universal, occurrence and that the oxides were the principal factors regulating microbial Hg transformations in the experiments.

An important result of the study was that the effects of oxides and clay on microbial Hg transformations were strongly dependent on environmental variables such as organic nutrient levels (Figs 7, 20, 21 and 22). For instance, FeOOH and MnOOH enhanced demethylation in PL sediment (Fig. 21C), but inhibited demethylation in the same sediment on addition of an organic nutrient supplement (Fig. 21A). Possibly the nutrients, by fostering microbial growth, offset the oxidizing ability of the oxides, favouring an increase in methylation at the expense of demethylation. Whatever the explanation, the general implication is that synergistic and antagonistic interactions between colloids and other environmental factors may profoundly alter the effects of the colloids on Hg transformations.

Like oxides and clays, humic matter had different effects on microbial Hg transformation, the variations being linked to microbial succession. Dissolved humic acid either enhanced Hg methylation or had no effect at all, depending on fluctuations in the nature of the microflora, but there was no inhibition of CH_3Hg^+ production (Fig. 14). Perhaps one reason for this is that humic acid acted as a mild reducing agent, consistently lowering the Eh (Fig. 14D). As a rule, anaerobic conditions tend to favour methylation, provided that sulphide levels are low.[9,34] This points to a common denominator in the behaviour of humic acid and FeOOH: both substances tended to promote methylation and at the same time either had no effect on the Eh or lowered it, in contrast to MnOOH, which promoted demethylation and also raised the Eh. A common denominator in the chemistry of the two substances is that both humic matter and FeOOH contain Fe: the humic matter was not analysed for Fe, but humic matter, as is well documented in the literature, has a strong tendency to complex Fe(III) and is generally associated with Fe in the form of Fe^{3+} or FeOOH.[54,67] Furthermore, the humic acid could conceivably have enhanced the rate of methylation by transferring CH_3-groups abiotically to inorganic Hg,[23,24] although the fact that the humic matter initially had no effect on methylation and started to exert an influence only when there was a change in the microflora (Fig. 14) militates against this interpretation.

Surprisingly, humic acid, in the form of a coating on clay, inhibited methylation and enhanced demethylation, and the effect was the same regardless of whether the sediment had been enriched with organic nutrients (Figs 20 and 21). The reason for the radical difference in the behaviour of dissolved and clay-bound humic acid is unknown, but it may be related to the masking of the clay's oxide coatings by films of adsorbed humic acid. The insensitivity of the adsorbed humic acid's behaviour to the concentration of organic nutrients (as contrasted with the sensitivity of oxides to this factor) suggests involvement of humic acid's role as a reducing agent: in the presence of humic acid, oxidation may have been kept in check with or without the stimulation of microbial growth by added nutrients.

A striking effect of humic acid coatings on clay was the total suppression of abiotic demethylation which would otherwise have been catalysed by MnOOH coatings on the clay (Fig. 23A). The most likely reason for this is that a film of adsorbed humic matter poisoned the MnOOH surfaces, or that reduction by the humic acid offset oxidation by the MnOOH. In the presence of viable micro-organisms, however, the Hg chemistry was entirely different and was evidently controlled by microbial activity, not by abiotic reactions (Figs 20 and 21).

Finally, to what extent do the experimental results apply to natural environments? A natural environment is vastly more complex than a simple, controlled experimental system; consequently, field observations are apt to be ambiguous, and it is often difficult to establish cause and effect relationships amid the intricate interplay of different phenomena. Nevertheless, analytical data for samples from two widely separated and unconnected field areas yielded patterns of variation which mimic patterns seen in the experimental data to a truly startling degree (Figs 18 and 22). They not only display zigzag patterns suggesting microbial succession but also indicate important effects of Fe on the formation and breakdown of CH_3Hg^+. Moreover, there is evidence for synergistic/antagonistic interactions between organic matter and FeOOH (Figs 22A and 22B). The study of field samples has also yielded evidence that silty clay eroded into a river-lake system from shoreline deposits (the same 'varved clay' used in the experimental work) causes inhibition of CH_3Hg^+ production in the sediments.[9,34] The experiments suggest that this could be due largely to preferential stimulation of demethylation (Fig. 1A), although other factors such as dilution and rapid burial of nutrients and Hg by the detrital sediment could also be involved.[9]

As for effects of MnOOH, data for field samples from two different river–lake systems have revealed relatively high CH_3Hg^+ levels in sediments and water from certain vigorously flushed, well-aerated lake environments in which the bottom sediments contained an abundance of Mn nodules.[5,9] Sediment from at least one of the lakes also showed a relatively high rate of CH_3Hg^+ production. Even if the MnOOH had a negative impact on CH_3Hg^+ production (and this is not known), it was offset by the favourable influence of other factors, such as enhanced availability of inorganic Hg due to rapid decomposition of sulphides and organic complexes at the sediment–water interface under the prevailing conditions of strong current action and aeration.[5,9]

In one of the same two field areas, increased microbial growth caused by the introduction of organic nutrients (decomposing plant remains) into aquatic environments from recently flooded forest- and muskeg-covered land has caused an upsurge in Hg methylation rates.[9,34] Although nutrient enrichment has been established as the primary cause of the problem,[9,34] stimulatory effects of humic matter, which abounds in the soils of the region,[55] could also be a contributing factor. However, on the basis of data from field samples alone, it is probably not possible to differentiate between these two effects.

In conclusion, further work combining controlled experiments with field studies is needed to elucidate the effects of clays, oxides, and humic matter on the methylation and demethylation of Hg in nature, but the combination of experimental results and field observations presented here indicates that these effects are as important as they are complex and variable.

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