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Biomethylation of Mercury(II) Adsorbed on Mineral Colloids Common in Freshwater Sediments

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The effects of freshwater sediment components such as kaolinite, montmorillonite and birnessite (δ-MnO_2) on the biomethylation of mercury(II) in a synthetic growth medium (M-IIY) were assessed. Additions of kaolinite or montmorillonite to media containing mercuric nitrate [$Hg(NO_3)_2$; 12 μg Hg ml^{-1}] had no significant effect on either bacterial growth or the production of methylmercury (CH_3Hg^+). However, whereas the addition of birnessite resulted in only a small (*ca* 4%) increase in bacterial growth, it also produced a significant decrease (*ca* 50%) in the production of CH_3Hg^+. Further, it was demonstrated that, with the exception of kaolinite, adsorption of mercury(II) onto the sediment components before they were added to the M-IIY medium decreased its bioavailability, i.e., the amounts of CH_3Hg^+ produced from the adsorbed mercury(II) were significantly lower than those produced from equivalent concentrations of $Hg(NO_3)_2$ in the absence of the mineral colloids. In the case of montmorillonite, CH_3Hg^+ production was decreased by 21% relative to the control system. Most striking was the case of birnessite, in which no CH_3Hg^+ was detected after a 25 h incubation period and only very small quantities of CH_3Hg^+ (3–7 ng l^{-1}) were present in the medium after 336 h. These data demonstrate that mineral colloids common in freshwater sediments significantly influence the extent of biomethylation of mercury(II) adsorbed on their surfaces. Birnessite, in particular, is a very effective inhibitor of the biomethylation of surface-bound mercury(II). Therefore, it may be possible to reduce the severity of mercury pollution in some aquatic environments by adding a reactive manganese oxide, such as birnessite, to the system and thereby to inhibit the transformation (methylation) of inorganic mercury(II) into the much more toxic CH_3Hg^+ species. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Sediments play a major role in the dynamics of natural water systems; they act as both source and sink for nutrients and pollutants in the freshwater environment. Indeed, the mobility and fate of both nutrients and pollutants in sedimentary environments depend largely on the mineralogy and surface chemistry of the sediments[1,2] as well as on the chemistry of the interstitial water[3,4] and environmental factors.[5] Moreover, colloidal materials in surficial sediments and suspended in the water column act as scavengers of toxic metals, immobilizing these metals and thereby reducing their bioavailability and toxicity.[6]

The mercury (Hg) in aquatic environments exists primarily in the form of inorganic species bound to particulate matter in sediments. Despite this, the mercury found in fish and other aquatic organisms is almost entirely in the form of the organometallic methylmercury (CH_3Hg^+) species.[7] The presence of CH_3Hg^+ in aquatic environments poses a potentially serious environmental hazard, because of both its extreme toxicity and the ease with which it can be bioaccumulated and biomagnified up the food chain.

The occurrence of CH_3Hg^+ in freshwater ecosystems is a result of microbial transformations of inorganic Hg(II) species.[8–10] Consequently, the

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Table 1 Sources and properties of mineral colloids, common in freshwater sediments, and used in the biomethylation studies

Sample	ID	Source	Description	CEC (cmol(+) kg^{-1})	Specific surface (m^2 g^{-1})	Hg(II) adsorbed (mg g^{-1}/24 h)
Kaolinite	KGa-1	CMS[a]	Well-crystallized kaolin	2.0	10	0.24
Montmorillonite	STx-1	CMS[b]	Ca^{2+}-saturated	84.4	599	0.65
Birnessite (δ-MnO_2)	—	Synthetic[c]	Poorly crystaline Mn oxide	167	262	1.05

[a] Clay Minerals Society Source Minerals Repository; Department of Geology, University of Missouri, Columbia, MO, USA. Sample origin: Washington Co., GA. Chemical characteristics obtained from Ref. 21.
[b] Clay Minerals Society Source Minerals Repository; Department of Geology, University of Missouri, Columbia, MO. Sample origin: Gonzales Co., TX. Chemical characteristics obtained from Ref. 21.
[c] Synthesized according to the procedure of McKenzie (Ref. 22); described in Refs 23 and 24.

production of CH_3Hg^+ in aquatic systems is dependent, in part, upon the bioavailability of these inorganic Hg(II) species. Competitive complexation by inorganic and organic ligands as well as by colloids is considered to be one of the principal factors controlling the bioavailability of inorganic Hg(II) in aquatic environments.[6,11] Indeed, it is presumed that methylation requires dissolved concentrations of Hg(II) and that only 'free' Hg^{2+} (i.e., aquo complexes of the type $Hg^{2+}\cdot xH_2O$) is directly available for methylation.[12] Whereas the effects of inorganic and organic ligands on the aqueous speciation, bioavailability and methylation of mercury have received considerable attention,[4,13–19] there have been far fewer efforts to elucidate the impact of mineral colloids on the bioavailability and biomethylation of Hg(II) in aquatic systems.[6,20]

This investigation was initiated to assess the impact of mineral colloids on the microbially mediated methylation of inorganic Hg(II). The specific objective of this study was to investigate the biomethylation of Hg(II) adsorbed on selected mineral colloids common in freshwater sediments (i.e., the clay minerals kaolinite and montmorillonite, and the manganese oxide birnessite) by a mercury-resistant strain of *Pseudomonas fluorescens* common to the Qu'Appelle River basin in Saskatchewan, Canada.

MATERIALS AND METHODS

Bacterial isolates

Surface sediments were collected from four sites at Buffalo Pound Lake in the Qu'Appelle River basin, Saskatchewan, Canada. Samples were collected with an Ekman dredge, transferred into acid-washed, sterile polyethylene bottles, and packed on ice until processed in the laboratory. Bacterial isolates were obtained from each of the individual sediment samples, screened for mercury resistance and identified.[19] Stock cultures were prepared by streaking the isolates onto tryptic soy agar (TSA) slants, which were incubated for 48 h at 25°C and then stored at 4°C. Working cultures of the isolates were prepared by streaking the stock cultures onto fresh TSA–Hg plates (i.e. TSA supplemented with 100 μg Hg ml^{-1}, added as $HgCl_2$) which, after incubation for 48 h at 25°C, were stored at 4°C. Methylation studies were carried out using the mercury-resistant isolate *P. fluorescens* BPL85-48.[19] This isolate was chosen because (1) it was common in the Qu'Appelle River Basin, (2) it exhibited a significant tolerance to Hg(II), and (3) it exhibited excellent growth characteristics. Fresh working cultures of this isolate were prepared every six to eight weeks.

Adsorption of mercury(II) on mineral colloids

The sources and properties of the clay minerals and manganese oxide used in this study are reported in Table 1. Mercury was adsorbed onto the mineral colloids following the procedure outlined in Fig. 1. Briefly: triplicate 1.0 g samples of the mineral (105°C oven-dry weight basis) were suspended in 100 ml of an 80 μM $Hg(NO_3)_2$ solution and the pH of the suspensions was adjusted to 7.0 ± 0.1 with 0.1 M NaOH. The suspensions were maintained at 25 ± 0.2°C in a water bath with continuous shaking for 24 h, filtered by ultrafiltration (0.01 μm pore size), and washed with distilled de-ionized water to ensure that the samples were free of dissolved Hg(II). Mercury in the filtrates was determined by cold vapor flameless atomic absorption spectrophotometry (CV AAS),[25] and the amount of Hg adsorbed by the colloid was calculated from the

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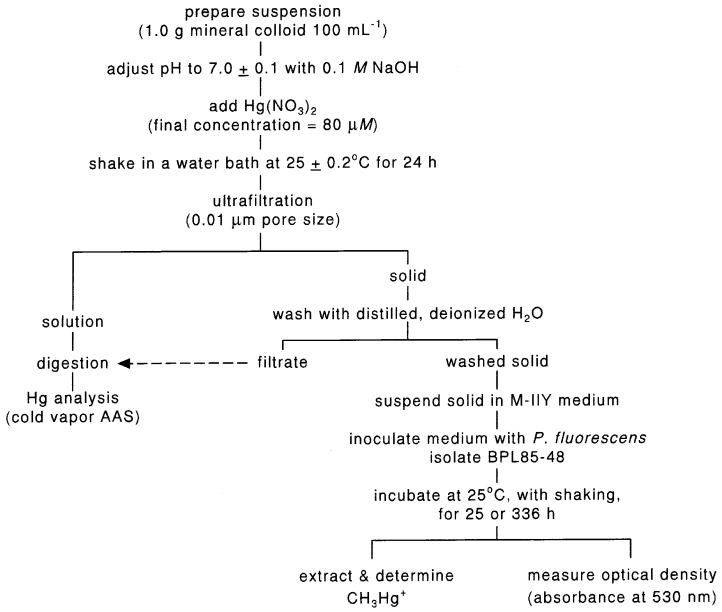

Figure 1 Procedure used to prepare Hg-treated mineral colloid samples.

difference in Hg concentration in the equilibrating solution and filtrates.

Methylation of mercury

Methylation experiments were conducted in a synthetic growth medium using *P. fluorescens* isolate BPL85-48. The synthetic growth medium (M-IIY) was a minimal salts medium amended with 0.1% yeast extract and 0.1% glycerol, and was made chloride-free by substituting appropriate nitrate salts.[19] The biomethylation of Hg(II) was assessed under three sets of experimental conditions (Table 2). In the control system, Hg(II) was added to the M-IIY medium as aqueous $Hg(NO_3)_2$ and methylation took place in the absence of any

Table 2 Treatment combinations used in the methylation studies

		Mineral colloid	
Treatment no.	Hg(II) source[a]	Mineral	conC (g/100 ml)
1.1	$Hg(NO_3)_2$	—	—
1.2	$Hg(NO_3)_2$	—	—
1.3	$Hg(NO_3)_2$	—	—
2.1	Adsorbed Hg[b]	Kaolinite	5.02
2.2	Adsorbed Hg[b]	Montmorillonite	1.85
2.3	Adsorbed Hg[b]	Birnessite	1.15
3.1	$Hg(NO_3)_2$	Kaolinite[c]	5.02
3.2	$Hg(NO_3)_2$	Montmorillonite[c]	1.85
3.3	$Hg(NO_3)_2$	Birnessite[c]	1.15

[a] Total conC = 12 μg Hg ml^{-1}.
[b] Mercury was adsorbed on the mineral colloid before its addition to the M-IIY medium. The total amount of Hg(II) added to the M-IIY medium in adsorbed form was the same (6 μmol), regardless of which mineral was added.
[c] Aqueous $Hg(NO_3)_2$ was added to the M-IIY medium and allowed to equilibrate for *ca* 4 h; the mineral colloid was added to the Hg-amended medium after the equilibration period.

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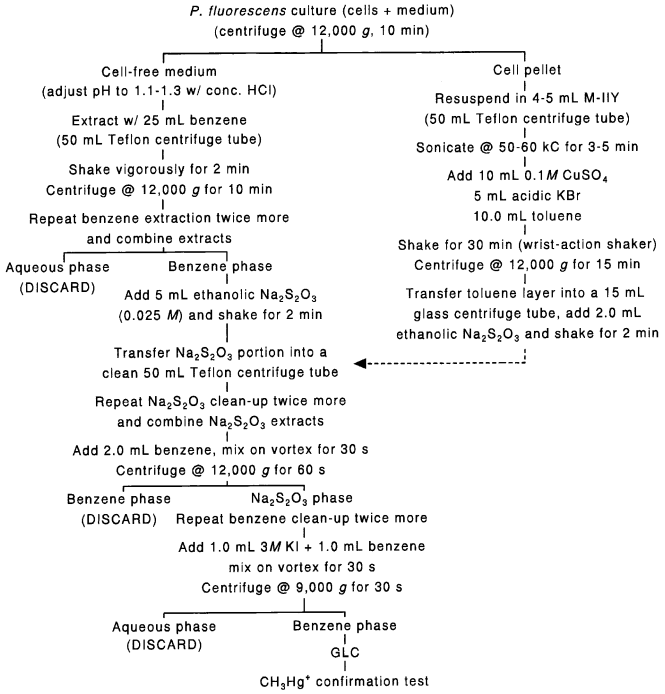

Figure 2 Procedure used to extract methylmercury (CH_3Hg^+) from the bacterial cultures. Modified from the procedure of Longbottom *et al.*[26]

added mineral colloid (treatments 1.1–1.3 in Table 2). In the Hg-colloid system, Hg(II) was added in the adsorbed form with the mineral colloid suspended in the M-IIY medium (Fig. 1; treatments 2.1–2.3 in Table 2). In addition, the influence of the mineral colloids on the biomethylation of aqueous Hg(II) was determined by adding $Hg(NO_3)_2$ to the M-IIY medium prior to the addition of the mineral colloids (treatments 3.1–3.3 in Table 2). Under these conditions, the adsorption of Hg(II) onto the mineral would be in direct competition with the organic and inorganic ligands in the medium and, in this way, the effects of competitive complexation were assessed. In all cases, the total concentration of Hg(II) in the medium was 60 μM (12 μg ml^{-1}).

Methylation studies were carried out as follows: (1) bacterial inoculant was prepared by transferring a loopful of the working culture of *P. fluorescens* BPL85-48 into 50 ml of M-IIY supplemented with additional yeast extract (total concentration = 0.5%) and incubating for 24 h at 25°C with shaking (115 ± 5 rpm); (2) Hg-amended media were prepared by adding 6 μmol Hg(II) to 100 ml of M-IIY medium in 300 ml Erlenmeyer flasks (final concentration of Hg(II) = 60 μM; final pH = 8.00 ± 0.05) [Case 1 — Hg(II) was added as $Hg(NO_3)_2$; Case 2 — Hg(II) was added as an equivalent amount of Hg (6 μmol) adsorbed on the mineral colloids (Table 2); Case 3 — Hg(II) was added as $Hg(NO_3)_2$, allowed to equilibrate for 4 h, and then supplemented with an appropriate amount of the mineral colloid (Table 2)]; (3) the Hg-amended media were inoculated by transferring 1.0 ml of bacterial inoculant to each treatment flask; (4) the flasks were sealed with foam plugs and incubated at 25°C with shaking (115 ± 5 rpm). Growth of the bacterial culture (measured as optical density, i.e. absorbance at 530 nm) and methylmercury production (following solvent extraction and gas-chromatographic analysis) were measured after 25 h.

Determination of methylmercury

Methylmercury in the complete cultures (i.e., cells plus medium) was extracted following the procedure outlined in Fig. 2 and was determined by gas–

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Table 3 Biomethylation of Hg(II) adsorbed on mineral colloids common in freshwater sediments, by *P. fluorescens* isolate BPL85-48 during a 25-h incubation period.

Sample ID	Hg(II) source[a]	Optical Density		CH_3Hg^+ (ng l^{-1})[c]
		Absorbance at 530 nm	RGI[b]	
Blank[d]	—	0.551	1.28 a	—
Control[e]	$Hg(NO_3)_2$	0.430	1.00 d	32.86 ± 0.67 a
KGa-1	Kaolinite	0.423	0.98 d	30.53 ± 1.64 ab
STx-1	Montmorillonite	0.451	1.05 c	25.96 ± 4.17 b
MnO_2	Birnessite	0.478	1.11 b	ND[f] c

[a] Total concentration of Hg(II), added as $Hg(NO_3)_2$ or in adsorbed form, was 6 μmol/100 ml.
[b] Relative growth index = optical density of colloid-amended medium/optical density of the control. Values followed by the same letter are not significantly different ($P \leq 0.05$; least significant difference test, LSD = 0.04).
[c] Values followed by the same letter are not significantly different ($P \leq 0.05$; least significant difference test, LSD = 5.30 ng CH_3Hg^+ l^{-1}).
[d] Isolate grown in the M-IIY medium in the absence of Hg(II).
[e] Isolate grown in M-IIY medium supplemented with $Hg(NO_3)_2$; total Hg(II) concentration = 60 μM.
[f] ND, not detectable.

liquid chromatography. Methylmercury was quantified using an HP-5890 gas chromatograph equipped with a ^{63}Ni electron capture detector and a Pyrex glass column [4 ft (122 cm) × 2 mm, i.d.] packed with Chromosorb W (80/100-mesh) coated with 5% FFAP (free fatty acid phase; Chromatographic Specialists, Inc.). The column was conditioned according to the procedure of Hight and Capar.[27] Under the operating conditions [injector 200°C; column 160°C; detector 300°C; and carrier gas (N_2) flow 65–70 ml min^{-1}], the CH_3Hg^+ peak appeared 2–3 min after sample injection. Methylmercury peaks were confirmed using the procedure described by Jensen.[28]

The detector was calibrated with a series of methylmercuric iodide (CH_3HgI) standards, and the measured peak heights were plotted versus CH_3HgI concentration. Calibration curves, prepared by using linear least-squares regression analysis, were used to calculate the concentration of CH_3Hg^+ (pg/5 μl) in each injected sample. The final concentration of CH_3Hg^+ in each sample was calculated as described previously.[19]

RESULTS AND DISCUSSION

Although it has been firmly established that the vast majority of Hg in freshwater systems is bound by sediments, to be released into the interstitial water and overlying water column only when an appropriate change in environmental conditions occurs,[5,6,29] the rate at which Hg is released from the sediment depends largely on the affinity of the sediment components for Hg[30–32] and the ionic environment of the surrounding interstitial water. Oscarson *et al.*[33] reported that the principal clay minerals in sediments from the rivers, streams and lakes making up the Qu'Appelle River system were smectite, vermiculite, kaolinite and mica. Furthermore, they found that amorphous oxy(hydroxides) of aluminium, iron, manganese and silicon occurred in both the colloidal (presumably as coatings on the clay minerals) and non-colloidal fractions of these sediments. Reimers and Krenkel[34] reported that the affinity of clay minerals for inorganic Hg(II) increases in the order: kaolinite < montmorillonite ≪ illite. Likewise, Rogers *et al.*[31] reported that Hg(II) adsorption by poorly crystallized oxides of aluminium, manganese and iron increases in the order: $Al(OH)_3 \ll Fe(OH)_3 \approx MnO_2$. Despite the implications of these data, there is little information available regarding the biomethylation of sediment-bound Hg.[2,6] Therefore the present study was undertaken to determine how various sediment components affect the biomethylation of inorganic Hg(II) bound to their surfaces.

The data presented in Table 1 demonstrate that, as expected, the adsorption of Hg(II) by the sediment components increased in the order: kaolinite ≪ montmorillonite ≪ birnessite. Not surprisingly, the amount of Hg(II) adsorbed by the sediment components varied greatly and, in general, increased as the cation-exchange capacity (CEC) of the component increased. Indeed, there was a significant positive correlation between the amount of Hg(II) adsorbed and the CEC of the

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sediment components ($r = 0.999$; $P \leq 0.001$). Thus, if specific adsorption mechanisms were not involved, it would be expected that the release and subsequent methylation of the bound Hg(II) would increase in the same order. However, the data presented in Table 3 show that no such relationship was observed. Indeed, just the opposite occurred, indicating that specific adsorption mechanisms were involved in the adsorption of Hg(II) by the sediment components. These results mirror those of Jackson,[2,6] who reported that the effects of clay minerals and metal oxides on Hg(II) transformations in sediment were largely independent of the capacity of the mineral/oxide to adsorb Hg(II). At the same time, our results indicate that the *affinity* of the colloid for Hg(II) may dictate the intensity of microbially mediated Hg(II) transformations in aquatic systems.

The data presented in Table 3 also illustrate that growth of the *P. fluorescens* isolate was suppressed at the concentration of $Hg(NO_3)_2$ (60 μM) used in the methylation experiments, i.e., there was a 28% decrease in the optical density of the 25 h culture. The addition of an equivalent amount of Hg(II) adsorbed on the mineral colloids produced variable effects on both bacterial growth and the production of CH_3Hg^+ (Table 3). Additions of Hg(II) adsorbed on kaolinite had no significant effect on either bacterial growth or CH_3Hg^+ production. This suggests that much of the Hg(II) adsorbed on the kaolinite was readily released into the M-IIY medium, presumably via exchange reactions involving the major cations (K^+, Na^+, Ca^{2+} and Mg^{2+}) in the medium and dissolution of the adsorbed Hg(II) through its complexation with the organic and inorganic ligands in the M-IIY medium.[35] The Hg(II) adsorbed on montmorillonite, however, was apparently less bioavailable, i.e., there was a small increase in bacterial growth (5% relative to the control) accompanied by a rather large (21%) reduction in CH_3Hg^+ production. Although the possibility of specific adsorption of Hg(II) by montmorillonite cannot be ruled out, it is more likely that these results reflect a decrease in the accessibility of Hg(II) bound in the interlayer spaces of the mineral.

The most striking results, however, were obtained with the Hg–birnessite system (Table 3). Unlike the Hg(II) adsorbed on the clay minerals, the Hg(II) adsorbed on birnessite was bound to such an extent that it was apparently retained on the mineral rather than being released into solution. That is, there was a significant increase in bacterial growth relative to the control and no detectable amounts of CH_3Hg^+ were recovered from the 25 h cultures. It was observed, however, that increasing the incubation period to 336 h resulted in small quantities of CH_3Hg^+ (3–7 ng l^{-1}) being produced in the Hg–birnessite system. Likewise, Jackson[2] reported that the production of CH_3Hg^+ in lake sediment was inhibited almost entirely by additions of manganite (MnOOH) to the sediment. Moreover, he reported that the manganite had a much stronger affinity for Hg(II) than either kaolinite or montmorillonite and that this effect may have played a role in suppressing the methylation of Hg(II) in lake sediment. Together, these results indicate that the Hg(II) sorbed by manganese oxides (including birnessite) is bound primarily by specific adsorption mechanisms. For example, McBride[36] suggested that covalent bonding makes an important contribution to the adsorption of trace metals on the surface of manganese oxides. In the case of Hg(II), adsorption to the surface of birnessite occurs through an MnO–Hg bond, a bond that is predominantly (*ca* 65%) covalent.[37] Moreover, assuming that the adsorption reaction is energetically favorable, desorption would be expected to require an activation energy to overcome at least the adsorption energy.[36] As a consequence of this, it is to be expected that the desorption of Hg(II) from manganese oxides would occur at a considerably slower rate than adsorption. Indeed, the results of the methylation studies suggest that desorption of Hg(II) from the birnessite occurred very slowly and was controlled, at least in part, by dissolution of the adsorbed Hg(II) through its complexation with organic and inorganic ligands in the medium. Rogers *et al.*[32] also reported that the desorption of Hg(II) from poorly crystallized oxides of manganese, iron, aluminium and silicon was slow and was controlled by the diffusion of Hg(II) from the oxide surface.

In nature, the adsorption of Hg(II) by sediments occurs in a chemically complex, heterogeneous environment and is greatly affected by the interplay between sediment components and ligands (both inorganic and organic) in the interstitial waters. Thus, to gauge the adsorptive capacity of the sediment components for Hg(II) in the presence of competing ligands, an additional set of experiments was conducted in which $Hg(NO_3)_2$ was added to M-IIY medium prior to the introduction of the sediment components (total Hg concentration = 60 μM). Addition of the mineral colloids to the Hg-amended M-IIY medium had no significant effect on bacterial growth during the 25 h incubation, i.e., in all cases the relative growth index

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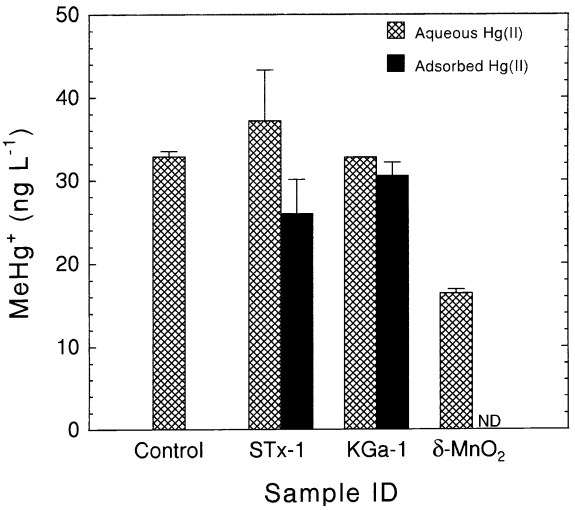

Figure 3 Effect of mineral colloids on the biomethylation of Hg(II) in the M-IIY medium. ▨, Mineral colloid added to M-IIY medium containing aqueous $Hg(NO_3)_2$ ■, Hg(II) added to the M-IIY medium as the Hg–mineral colloid complex. Cultures were incubated at 25°C for 25 h. sample descriptions are given in Table 1; ND, not detectable.

(RGI) for the control and colloid-amended systems differed by less than 4%. The effects of mineral colloids on the methylation of Hg(II) in the M-IIY media are presented in Fig. 3. In general, more CH_3Hg^+ was produced when the mineral colloids were added to media containing aqueous $Hg(NO_3)_2$ than when the Hg(II) was added in the adsorbed form. Clearly, however, this difference was significant only in the case of birnessite. These results suggest that the adsorption of Hg(II) by kaolinite and montmorillonite is controlled simply by ion exchange and that the dissolved ligands in the M-IIY medium are more effective scavengers of Hg(II) than are the clay minerals. Likewise, the data suggest that complexation effects limited the amount of free Hg(II) available to bind with the MnO_2, thus suppressing Hg(II) adsorption and making more Hg(II) available for methylation. Indeed, it is generally acknowledged that metal–ligand stability at surfaces is correlated with metal–ligand stability in solution.[36] Farrell *et al.*[35] calculated that, at pH 7–8, the 1:2 complexes of Hg with alanine, glycine, valine and hydroxide accounted for more than 90% of the total Hg(II) in the M-IIY medium. Though these soluble Hg–ligand complexes were apparently unavailable for surface adsorption, data from the present study indicate that at least some of the Hg(II) in these complexes remained in bioavailable form.

In summary, our data indicate that there is little relationship between the adsorptive capacity of sediment components for Hg(II) and the subsequent methylation of this Hg(II). Indeed, there was no significant correlation ($r = 0.329$, ns) between the quantity of Hg(II) adsorbed by the sediment components and the amount of CH_3Hg^+ produced. The data do suggest, however, that by controlling desorption kinetics the mechanism of adsorption (be it ion exchange or specific adsorption) plays a pivotal role in the methylation of sediment-bound Hg(II). Likewise, competitive complexation reactions involving mineral surfaces and the inorganic and organic ligands in solution exert a considerable influence over both the adsorption and methylation of sediment-bound Hg(II).

Our results provide further insights into the influence of naturally occurring mineral colloids on the bioavailability and methylation of Hg(II) in aqueous systems. In particular, they demonstrate that MnO_2 (birnessite) is a very effective scavenger of Hg(II) — being capable of adsorbing large quantities of Hg(II), even in the presence of competing ligands — and is also an effective inhibitor of the methylation of Hg(II). However, in addition to sequestering Hg(II) in a bio-unavailable form, the possibility that MnO_2-catalyzed abiotic demethylation of CH_3Hg^+ may also have occurred should not be overlooked.[2] Nevertheless, manganese oxides such as birnessite may be considered useful as agents which, by inhibiting Hg methylation, can assist in the remediation and restoration of Hg-polluted aquatic environments.

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