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## Potential bioavailability of mercury in humus-coated clay minerals

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### ABSTRACT

It is well-known that both clay and organic matter in soils play a key role in mercury biogeochemistry, while their combined effect is less studied. In this study, kaolinite, vermiculite, and montmorillonite were coated or not with humus, and spiked with inorganic mercury (IHg) or methylmercury (MeHg). The potential bioavailability of mercury to plants or deposit-feeders was assessed by  $\text{CaCl}_2$  or bovine serum albumin (BSA) extraction. For uncoated clay, IHg or MeHg extraction was generally lower in montmorillonite, due to its greater number of functional groups. Humus coating increased partitioning of IHg (0.5%–13.7%) and MeHg (0.8%–52.9%) in clay, because clay-sorbed humus provided more strong binding sites for mercury. Furthermore, humus coating led to a decrease in IHg (3.0%–59.8% for  $\text{CaCl}_2$  and 2.1%–5.0% for BSA) and MeHg (8.9%–74.6% for  $\text{CaCl}_2$  and 0.5%–8.2% for BSA) extraction, due to strong binding between mercury and clay-sorbed humus. Among various humus-coated clay particles, mercury extraction by  $\text{CaCl}_2$  (mainly through cation exchange) was lowest in humus-coated vermiculite, explained by the strong binding between humus and vermiculite. The inhibitory effect of humus on mercury bioavailability was also evidenced by the negative relationship between mercury extraction by  $\text{CaCl}_2$  and mercury in the organo-complexed fraction. In contrast, extraction of mercury by BSA (principally through complexation) was lowest in humus-coated montmorillonite. This was because BSA itself could be extensively sorbed onto montmorillonite. Results suggested that humus-coated clay could substantially decrease the potential bioavailability of mercury in soils, which should be considered when assessing risk in mercury-contaminated soils.

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### Introduction

It is well known that soil composition can significantly affect mercury (Hg) biogeochemistry in soils (Chen et al., 2012; Dai et al., 2013; Gagnon and Fisher, 1997). Among the soil components, clay can bind large amounts of Hg, partly because of its relatively high surface area (Abollino et al., 2008; Brigatti et al., 2005; Cruz-Guzmán et al., 2006; Lothenbach et al., 1998; Sdiri et al., 2014; Zhong and Wang, 2008a). It has been reported

that clay minerals are important in regulating metal–solid interactions (Kongchum et al., 2011) and play a role in Hg biogeochemistry (Zhong and Wang, 2008b). Meanwhile, soil organic matter has high affinity for Hg (Yu et al., 2006; Zhong and Wang, 2006a), and thus could strongly affect partitioning and bioavailability of Hg in soils.

Montmorillonite and vermiculite are both 2:1 clay minerals with large surface area and high cation exchange capacity (do Nascimento and Masini, 2014). Kaolinite is a 1:1 layer mineral

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with small cation exchange capacity and restricted surface area (Bernaus et al., 2005). Differences in characteristics could possibly lead to large differences in binding capacity and affinity toward Hg in various clay minerals. Furthermore, differences in surface area may also affect sorption of organic matter on clay and could greatly influence Hg binding in soils. Hence, it would be interesting to compare Hg binding and bioavailability in different clay and organic-coated clay minerals. Such research could greatly improve the understanding of Hg biogeochemistry in soils.

In the present study, three of the most common clay minerals, kaolinite, vermiculite and montmorillonite, were coated or not with humus. Partitioning, geochemical fractionation (quantified by a sequential extraction method), and potential bioavailability (assessed by a chemical extraction method) of both inorganic mercury (IHg) and methylmercury (MeHg) were then quantified. The main objective was to explore the effects of various organic-coated clay minerals on sorption and potential bioavailability of Hg in soils.

## 1. Materials and methods

### 1.1. Chemicals and containers

Kaolinite, montmorillonite, humic acid (HA) and fulvic acid (FA) were purchased from Sigma-Aldrich, USA. Vermiculite was purchased from Lingshou DingWang Company, China. All clay particles were sieved through a 75  $\mu\text{m}$  mesh before use. Mercury chloride ( $\text{HgCl}_2$ ) and methylmercury chloride ( $\text{CH}_3\text{ClHg}$ ) were obtained from Sigma-Aldrich (USA) and Brooks Rand (USA), respectively. Calcium chloride ( $\text{CaCl}_2$ ) and bovine serum albumin (BSA), used for Hg extraction, were purchased from Sigma-Aldrich, USA. Trace metal grade nitric acid ( $\text{HNO}_3$ ) and hydrochloric acid (HCl) were purchased from Sinopharm Chemical Reagent (China) and Nanjing Chemical Reagent (China), respectively. Potassium hydroxide (KOH) was from Chinasun Specialty Products (China). Methanol ( $\text{CH}_3\text{OH}$ ) and  $\text{NaBe}_4$ , used for MeHg digestion and determination, were from TEDIA (USA) and Brooks Rand Lab (USA), respectively. All chemicals were of analytical reagent grade or higher. Background Hg levels in all chemicals were tested and found to be extremely low.

Polypropylene centrifuge tubes (Corning, USA) were used in this study, e.g., for organic coating, chemical extraction, sample digestion and dilution. Diluted nitric acid solution (2%) was used to rinse the tubes, and Hg in the rinsing solution was below the detection limit. These tubes were thus considered relatively Hg free. Adsorption of IHg or MeHg on the tubes was also tested and found to be extremely low.

### 1.2. Humus coating and Hg spiking

A total of 6 treatments (each with 3 replicates) were applied in this study: kaolinite; vermiculite; montmorillonite; humus-coated kaolinite; humus-coated vermiculite; and humus-coated montmorillonite. To prepare humus-coated clay particles, kaolinite, vermiculite, or montmorillonite was mixed with humus solutions (mixtures of 10 g/L HA and 10 g/L FA) in a centrifuge tube at a ratio of 0.1 g/1 mL, respectively. The mixtures were incubated in an incubator shaker at 25°C and 300 r/min.

After one month incubation, the mixtures were centrifuged at 3000 r/min for 30 min and washed twice by ultrapure water to remove unbound organic matter. Both uncoated and coated clay particles were then placed into centrifuge tubes and spiked with IHg or MeHg solutions to achieve a spiking concentration of 5  $\mu\text{g}$  IHg/g or 0.05  $\mu\text{g}$  MeHg/g. Background total mercury (IHg and MeHg) levels in clay particles (vermiculite: 10.75 ng/g, kaolinite: 12.06 ng/g, montmorillonite: 5.60 ng/g) were less than 1% of spiked IHg concentrations (5  $\mu\text{g}$ /g), and could thus be ignored. Background MeHg levels in clay were below the detection limit. The spiked particles in capped centrifuge tubes were incubated for 3 days in an incubator shaker at 25°C and 300 r/min. Afterwards, the mixtures were centrifuged at 3000 r/min for 30 min and washed once by ultrapure water to remove any unbound Hg. Methylation of Hg should be negligible within the incubation period (i.e., 3 days) according to our preliminary experiment. Subsequently, the Hg-spiked particles were evaluated for THg or MeHg concentrations (details below) and used for chemical extraction or sequential extraction.

### 1.3. Extraction of Hg from Hg-spiked particles

Mercury extraction by  $\text{CaCl}_2$  (1.47 g/L or 0.01 mol/L, 0.1 g soil/1 mL extractant, Gupta and Sinha, 2007) or BSA (10 g/L, 0.1 g soil/0.3 mL extractant, Zhong and Wang, 2006a) was used to assess potential bioavailability of soil-bound Hg to plants (Gupta and Sinha, 2007; Jing et al., 2008) or deposit-feeders (Lawrence et al., 1999; Voparil and Mayer, 2004; Zhong and Wang, 2006a) respectively. Hg-spiked particles were mixed with either  $\text{CaCl}_2$  or BSA solution in capped polypropylene centrifuge tubes and shaken at 300 r/min in an incubator shaker at 25°C for 2 hr ( $\text{CaCl}_2$ , Gupta and Sinha, 2007) or 4 hr (BSA, Zhong and Wang, 2006a,b). Three replicates were performed for each treatment. After that, the mixtures were centrifuged at 3000 r/min for 30 min and the supernatants were filtered through 0.45  $\mu\text{m}$  membrane filters. Concentrations of THg or MeHg in the filtrates and in the remaining particles were then measured (details below). Concentrations of IHg were calculated by subtracting the MeHg concentrations from the measured THg concentrations. The mercury (IHg or MeHg) extraction rate (%), by  $\text{CaCl}_2$  or BSA, quantifying the potential bioavailability of Hg in particles, was calculated as:  $100 \times \text{Hg in filtrate} / (\text{Hg in filtrate} + \text{Hg remained in particle after extraction})$ . The recoveries of mercury extraction, i.e.,  $100 \times (\text{Hg in filtrate} + \text{Hg remained in particle after extraction}) / \text{Hg in particle before extraction}$ , were 96.7%–101.5%.

### 1.4. Quantification of Hg geochemical fractionation in particles

To quantify Hg association with various biogeochemically relevant pools in Hg-spiked particles, the commonly used five-step sequential chemical extraction method (designed specifically for Hg, Bloom et al., 2003) was used. The five geochemical fractions were operationally defined as water soluble (WATF, e.g.,  $\text{HgCl}_2$ , extracted by deionized water), stomach acid soluble (STOF, e.g., HgO and  $\text{HgSO}_4$ , extracted by 0.01 mol/L HCl + 0.10 mol/L  $\text{CH}_3\text{COOH}$ ), organo-complexed (ORGF, e.g., Hg-organic matter, MeHg and  $\text{Hg}_2\text{Cl}_2$ , extracted by 1 mol/L KOH), strongly complexed (STRF, e.g., Hg bound up in

Fe/Mn oxide, amorphous organosulfur and mineral lattices, extracted by 12 mol/L  $\text{HNO}_3$ ) and residue Hg (RESF, *e.g.*, HgS and HgSe, Zhong and Wang, 2006b). Three replicate samples were measured for each treatment. More details can be found in our previous publications (Zhong and Wang, 2008a, 2009). The recoveries of sequentially extracted mercury (*i.e.*, 100 × Hg extracted in five fractions/Hg in particle before sequential extraction) were 89.1%–94.9%.

### 1.5. Determination of THg and MeHg concentrations

All aqueous samples (*e.g.*, extractant solutions) were immediately acidified with  $\text{HNO}_3$  (for THg determination) or HCl (for MeHg determination) and stored in the dark at  $-20^\circ\text{C}$  until THg or MeHg determination within 7 days. All solid samples (*e.g.*, particles after spiking or extraction) were immediately analyzed for THg or MeHg concentrations.

Both solid (*i.e.*, particles) and aqueous (*i.e.*, extractant solutions) samples were measured by a DMA-80 direct Hg analyzer (Milestone, Italy) without sample digestion (US EPA, 1998). The minimum detection level (MDL) for THg is 0.005 ng. Standard solutions of IHg and standard soil GBW07423 (THg:  $32 \pm 3$  ng/g) were analyzed for THg concentrations for each batch of samples, and the recovery rates were between 90% and 110%. A matrix spike experiment was done for IHg: various clay particles were spiked with IHg, equilibrated overnight and then evaluated for IHg by DMA-80. The recovery rates of IHg were between 90%–110%.

Concentrations of MeHg were measured by an automatic Brooks Rand model III MeHg analyzer (cold vapor atomic fluorescence spectrophotometry or CVAFS, Brooks Rand, USA) using EPA method 1630. The minimum detection level for MeHg is 0.002 ng/L. Both solid and aqueous samples were digested by 25% KOH-methanol and kept in an incubator shaker at  $60^\circ\text{C}$  for 4 hr in the dark (Bloom, 1989; Gagnon et al., 1997). Then the digested samples were diluted with ultrapure water and stored in the dark at  $-20^\circ\text{C}$  for less than 24 hr before MeHg determination. Standard sediment (ERM-cc58, MeHg:  $75.5 \pm 4.0$  ng/g) was digested with each batch of sample digestion following the same procedure. The recoveries were between 80% and 120%.

Background concentrations of THg or MeHg in all extractant solutions were less than 1% of measured THg or MeHg levels in sample solutions, and were ignored when calculating Hg concentrations in samples.

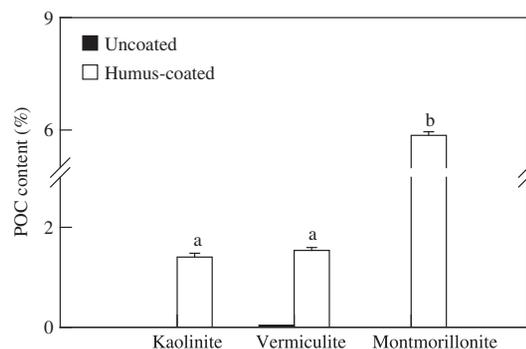
### 1.6. Statistics

Any significant differences in Hg concentrations in particles, Hg extraction rates, or Hg distribution in different geochemical fractions among different treatments were analyzed using one-way analysis of variance (ANOVA) and tested with LSD comparisons ( $p < 0.05$ ).

## 2. Results and discussion

### 2.1. Humus coating of various clay minerals

Particulate organic carbon (POC) levels in various clay minerals with or without humus coating are shown in Fig. 1.



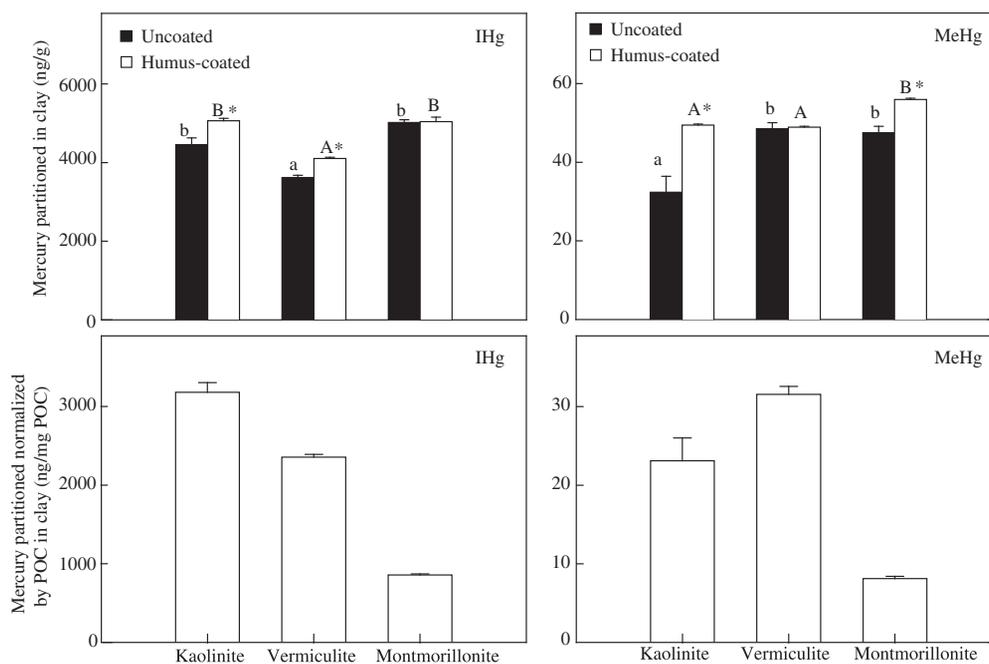
**Fig. 1 – Particulate organic carbon (POC) levels in different clay minerals with or without humus coating. Mean  $\pm$  SD ( $n = 3$  for humus-coated clay and  $n = 1$  for uncoated clay). Letters indicate significant differences among different treatments ( $p < 0.05$ , one-way ANOVA).**

The POC levels in uncoated clay particles were extremely low (0%–0.04%). Humus coating resulted in an increase of POC levels in clay particles. Such increase should be attributed to sorption of humus onto clay particles, *e.g.*, through coulombic interaction, complexation, hydrophobic effects, cation-bridging, ligand exchange, van der Waals interaction or hydrogen bonding (Abate and Masini, 2005; Arnarson and Keil, 2000; Baham and Sposito, 1994; Greenland, 1971; Liu and Gonzalez, 1999). Notably, the concentration of POC was significantly higher in montmorillonite (5.86%) than that in kaolinite or vermiculite. This could possibly be attributed to the larger specific surface area (*e.g.*, 700–800  $\text{m}^2/\text{g}$ , Bernaus et al., 2005) of montmorillonite compared to that of kaolinite (*e.g.*, 7–30  $\text{m}^2/\text{g}$ , Bernaus et al., 2005) or vermiculite (*e.g.*, 55–672  $\text{m}^2/\text{g}$ , Ravichandran and Sivasankar, 1997; Suquet et al., 1991; Temuujin et al., 2003). A previous study also reported that montmorillonite could adsorb more organic matter than kaolinite (Satterberg et al., 2003).

### 2.2. Partitioning of Hg in various clay minerals

Partitioning of IHg or MeHg in various clay minerals coated or not with humus and partitioning of IHg or MeHg normalized by POC content in coated clay minerals is shown in Fig. 2. For uncoated particles, IHg partitioning was higher in montmorillonite than that in kaolinite and vermiculite, which was consistent with the results in previous studies (Bernaus et al., 2005; Obukhovskaya, 1982). Partitioning of MeHg in montmorillonite and vermiculite was comparable and higher than that in kaolinite. The relatively high partitioning of IHg or MeHg in montmorillonite could be attributed to the larger specific surface area and cation exchange capacity of montmorillonite (Lothenbach et al., 1998).

Humus coating resulted in a significant increase in mercury partitioning in most treatments. This may be because humus adsorbed onto clay particles provides more strong binding sites for mercury, *e.g.*, thiol groups (Benoit et al., 2001; Haitzer et al., 2002; Skyllberg, 2008), carboxylic groups (Abollino et al., 2008; Malandrino et al., 2006; Schuster, 1991), phenolic groups (Arnarson and Keil, 2000; Bernaus et al., 2005), and amino groups (Bernaus et al., 2005; Malferrari et al.,



**Fig. 2** – Partitioning of spiked inorganic mercury (IHg) or methylmercury (MeHg) in different clay minerals with or without humus coating and partitioning of IHg or MeHg normalized by POC content in coated clay minerals. Mean  $\pm$  SD ( $n = 3$ ). \* indicates significant differences between minerals with or without humus coating ( $p < 0.05$ , one-way ANOVA). Lowercase or capital letters indicate significant differences among uncoated or coated treatments, respectively ( $p < 0.05$ , one-way ANOVA).

2010). In particular, IHg or MeHg partitioning in humus-coated montmorillonite was higher than that in the other coated particles. This could be attributed to the fact that humus adsorption was highest in montmorillonite (Fig. 1), resulting in a large increase in mercury binding sites in humus-coated montmorillonite.

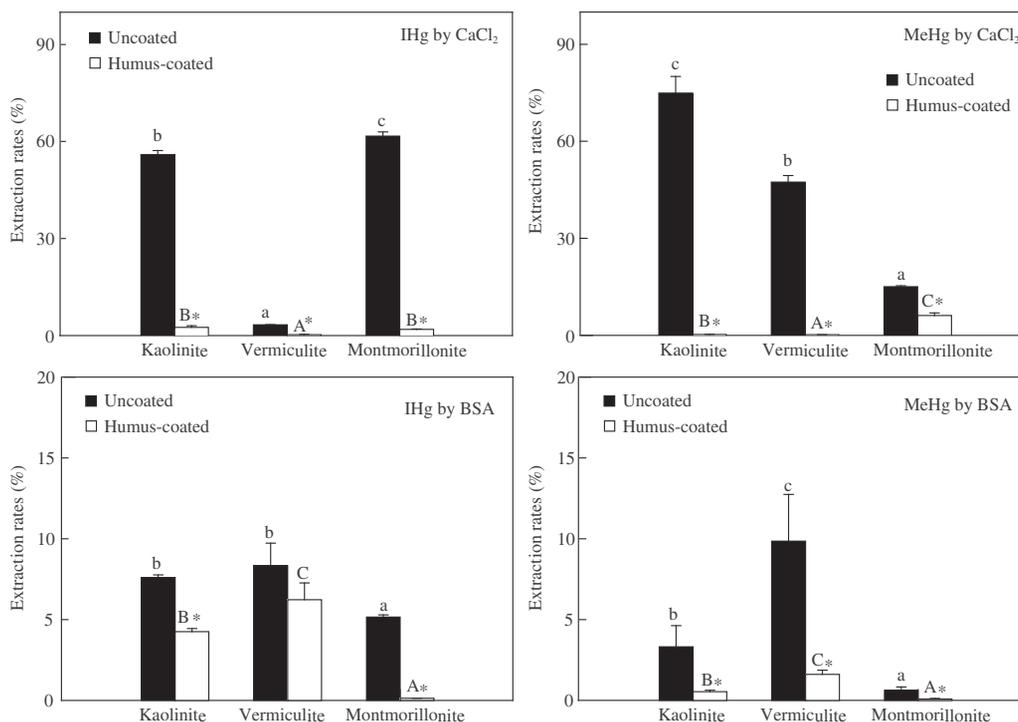
Concentrations of IHg or MeHg in coated clay normalized by POC levels (ng Hg/mg POC) were calculated as: IHg or MeHg concentration in coated clay (ng Hg/g clay)/POC content in coated clay (mg POC/g clay). Normalized Hg concentrations were more comparable in coated kaolinite and vermiculite, but much lower in coated montmorillonite, suggesting that less Hg could be bound by humus in the presence of montmorillonite. The decreased Hg-humus binding could probably be due to competition between montmorillonite and humus for mercury binding.

### 2.3. Extraction of Hg by $\text{CaCl}_2$ from various clay minerals

Calcium chloride could exchange  $\text{Ca}^{2+}$  with metal ions in soils, and extraction of metals by  $\text{CaCl}_2$  is commonly used to assess availability of soil-bound metals to plants (Gupta and Sinha, 2007; Rauret, 1998). Therefore, extraction of Hg by  $\text{CaCl}_2$  was applied in this study to assess potential phyto-availability of soil-bound Hg. Extraction rates of IHg by  $\text{CaCl}_2$  from humus-coated or uncoated clay are shown in Fig. 3. For uncoated particles, the IHg extraction rate was much lower in vermiculite than that in montmorillonite or kaolinite. It was reported that metal adsorption on montmorillonite (2:1 layer mineral) or kaolinite (1:1 layer mineral) was mostly on the outer layers (Baham and Sposito, 1994; Bhattacharyya and

Gupta, 2008), and thus  $\text{Hg}^{2+}$  could probably be more easily exchanged by  $\text{Ca}^{2+}$ . In contrast, IHg tends to enter the inter-layer of vermiculite by either cation exchange or formation of inner-sphere complexes with functional groups (Abollino et al., 2008; Malandrino et al., 2006), and becomes more difficult to be extracted by  $\text{CaCl}_2$ . In addition, EXAFS analyses indicated that IHg in montmorillonite was preferentially sorbed as Hg-OH<sub>2</sub> complexes, which were weakly bound to the 2:1 layers (Brigatti et al., 2005). By contrast, IHg could be strongly sorbed as Hg-O complexes (in addition to Hg-OH<sub>2</sub> complexes) in vermiculite, resulting in more stable binding between IHg and vermiculite (Brigatti et al., 2005).

Mercury could be sorbed on humus by either cation exchange or complexation (Schuster, 1991). Our results showed that humus coating led to a significant decrease in IHg extraction rates, which could be attributed to strong complexation of IHg with functional groups within humus (Abate and Masini, 2005; Benoit et al., 2001), e.g., thiol groups (Graham et al., 2013; Haitzer et al., 2002), carboxylic groups (Abollino et al., 2008; Malandrino et al., 2006; Schuster, 1991), phenolic groups (Arnarson and Keil, 2000; Bernaus et al., 2005), and amino groups (Bernaus et al., 2005; Malferrari et al., 2010). For humus-coated clay, the extraction rate of IHg by  $\text{CaCl}_2$  was much higher in montmorillonite (1.9%) and kaolinite (2.5%) than that in vermiculite (0.3%). It was thought that organic molecules were mostly sorbed on the outer layers or edges of either montmorillonite or kaolinite (Baham and Sposito, 1994; Thimsen and Keil, 1998). This may result in relatively weak binding between humus or IHg-humus complexes and montmorillonite or kaolinite. In addition, our results about Hg distribution in different geochemical fractions (Fig. 4) could

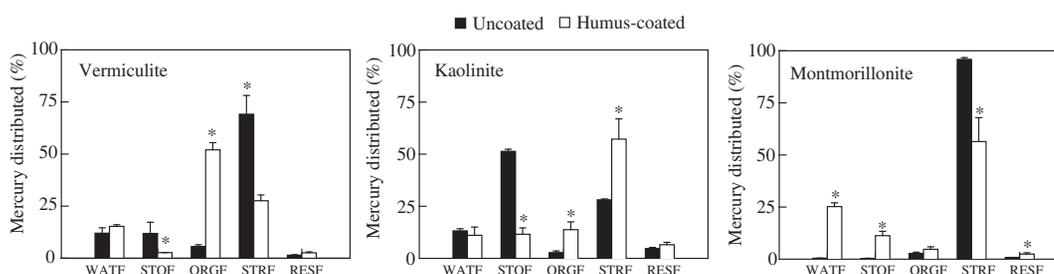


**Fig. 3** – IHg or MeHg extraction rates by calcium chloride (CaCl<sub>2</sub>) or bovine serum albumin (BSA) from different clay minerals with or without humus coating. Mean ± SD (n = 3). \* indicates significant differences between clay minerals with or without humus coating ( $p < 0.05$ , one-way ANOVA). Lowercase or capital letters indicate significant differences among uncoated or coated treatments, respectively ( $p < 0.05$ , one-way ANOVA).

provide further evidence on the different binding strength between IHg-humus and various clay minerals. Distribution of IHg in the organo-complexed fraction (ORGF), in which IHg was considered rather refractory (Zhong and Wang, 2008a), increased substantially in vermiculite after humus coating. In contrast, distribution of IHg in the water soluble fraction (WATF) and stomach acid soluble fraction (STOF), the two most mobile fractions of Hg, increased significantly in montmorillonite after humus coating. Such increase is possibly due to desorption of small-molecular-weight Hg-humus complexes, which are relatively hydrophilic and have lower affinity to clay particles (Abate and Masini, 2005; Arnarson and Keil, 2000). The observed differences in Hg geochemical fractionation may indicate that IHg-humus complexes could

be bound more strongly in vermiculite, and explained the relatively low IHg extraction from humus-coated vermiculite. Unlike vermiculite, the major increase in kaolinite after coating was IHg in the strongly-complexed fraction (STRF), in which IHg was relatively mobile compared to that in ORGF (Zhong and Wang, 2006b). This could possibly explain why IHg extraction from coated kaolinite was much higher than that from coated vermiculite.

Extraction rates of MeHg by CaCl<sub>2</sub> from humus-coated or uncoated clay are shown in Fig. 3. The extraction rate of MeHg from uncoated kaolinite was relatively high (74.9%). The higher MeHg extraction from kaolinite compared to that from montmorillonite or vermiculite could be attributed to the 1:1 structure of kaolinite and metal sorption on the outer layers



**Fig. 4** – Distribution of spiked IHg in different geochemical fractions (WATF: water soluble fraction; STOF: stomach acid soluble fraction; ORGF: organo-complexed fraction; STRF: strongly complexed fraction; RESF: residual fraction) in different clay minerals with or without humus coating. Mean ± SD (n = 3). \* indicates significant differences of IHg distribution in a certain fraction between clay minerals with or without humus coating ( $p < 0.05$ , one-way ANOVA).

(Bhattacharyya and Gupta, 2008). Meanwhile, extraction of MeHg from montmorillonite (15.1%) was much lower than that from vermiculite (47.4%), which was different from the result for IHg. Such discrepancy could be explained by the differences in binding preferences between MeHg and IHg (Gagnon and Fisher, 1997; Morel et al., 1998), originating from differences in charge, ionic radius, and other properties (Speight, 2005).

Similar to IHg, MeHg extraction rates from humus-coated clay were significantly lower than those from uncoated clay, which could be explained by the strong binding between MeHg and humus (*e.g.*, with thiol groups, Skyllberg, 2008; Benoit et al., 2001; Haitzer et al., 2002). A previous study using XANES indicated that MeHg-clay binding was more dependent on ionic interaction, while MeHg-humic acid binding exhibited a higher covalent bond character, leading to more stable binding between MeHg and humic acid (Bernaus et al., 2005). Such difference in covalent bond character could result in lower bioavailability of humus-bound MeHg. The extraction rate of MeHg was lowest in humus-coated vermiculite, probably due to the higher binding strength between humus and vermiculite (discussed above).

#### 2.4. Extraction of Hg by BSA from various clay minerals

Previous studies reported that Hg extraction by BSA (*i.e.*, complexation of soil-bound metals with BSA and subsequent solubilization of metals from soils) could simulate digestive solubilization of Hg in deposit-feeders (Lawrence et al., 1999; Voparil and Mayer, 2004; Zhong and Wang, 2006a,b), which could be the limiting step of the metal bioavailability process (Zhong and Wang, 2006c). Therefore, BSA extraction is usually used to assess bioavailability of metals (including Hg) in soils or sediments (Chen and Mayer, 1998; Lawrence et al., 1999; Voparil and Mayer, 2004; Zhong et al., 2012; Zhong and Wang, 2006a,b, 2008b).

Extraction rates of IHg or MeHg by BSA from humus-coated or uncoated clay are shown in Fig. 3. For uncoated particles, extraction of IHg or MeHg from montmorillonite was lowest. This could be attributed to the large number of functional groups (*e.g.*, hydroxyl groups, and silanol groups) on the surface (Bhattacharyya and Gupta, 2008; Guerra et al., 2009) of montmorillonite. Those functional groups could complex

with Hg in particles and inhibit Hg complexation by BSA. Another possibility is that BSA itself could be substantially adsorbed by montmorillonite during extraction, possibly due to the larger relative surface area of montmorillonite (Babel and Kurniawan, 2003; Bailey et al., 1999), and result in higher partitioning of Hg in montmorillonite.

Coating of clay particles with humus led to a significant decrease of Hg extraction by BSA in most treatments (Fig. 3). This could also be explained by the strong binding between Hg and humus sorbed on clay particles (Bono, 1997; Wang et al., 1997). Similar to that from uncoated particles, Hg extraction from humus-coated particles was lowest in montmorillonite. The much lower extraction rate of Hg from humus-coated montmorillonite could be attributed to its much higher POC level (5.9%, Fig. 1) and thus higher affinity to Hg.

The decrease of Hg extraction rates after humus coating (2.1%–5.0% for IHg and 0.5%–8.2% for MeHg) was much smaller for BSA extraction than  $\text{CaCl}_2$  extraction (3.0%–59.8% for IHg and 8.9%–74.6% for MeHg). This is because the complexation of Hg ions with humus would greatly reduce the number of Hg ions available for cation exchange reaction during  $\text{CaCl}_2$  extraction. In contrast, BSA contains a large amount of strong binding sites for Hg (*e.g.*, thiol groups, Benoit et al., 2001; Haitzer et al., 2002), and could compete with particle-sorbed humus for Hg binding. Therefore, extraction of Hg by BSA was less affected by humus in the particles. In fact, a significant negative relationship was found between IHg extraction rate by  $\text{CaCl}_2$  and IHg distribution in the organo-complexed fraction (ORGF, Fig. 5), indicating that IHg extraction by  $\text{CaCl}_2$  was strongly affected by its complexation with humus. However, such a relation was not found between IHg extraction by BSA and IHg in ORGF, suggesting that the effect of organic complexation on BSA extraction was much weaker.

### 3. Conclusions and implications

Our results indicated that various clay minerals and humus coating could have significant effects on the partitioning and chemical extraction of Hg. Due to differences in characteristics, *e.g.*, specific surface area, cation exchange capacity, structure (*i.e.*, 1:1 or 2:1), and mechanism of humus or Hg sorption (*e.g.*, cation exchange, and complexation), various

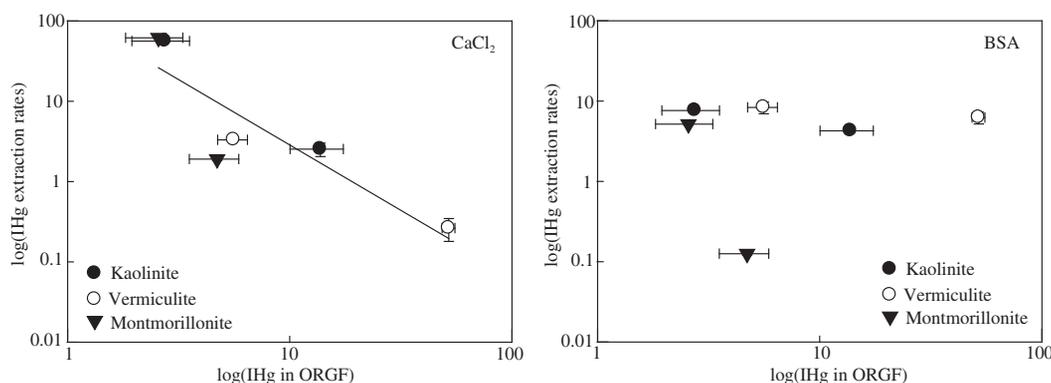


Fig. 5 – Relationship (log–log scale) between IHg extraction rates (%) by  $\text{CaCl}_2$  or BSA and IHg distribution (%) in organo-complexed fraction. Mean  $\pm$  SD ( $n = 3$ ).

clay minerals would have different binding affinity toward Hg, as we observed in the present study. Furthermore, our data suggested that although Hg sorbed on uncoated clay particles could be extracted at a relatively high rate by either CaCl<sub>2</sub> or BSA, Hg extraction decreased sharply after humus coating, and such decrease was attributed to the strong binding between Hg and humus sorbed on clay particles. Considering that a large proportion of Hg is associated with soil organic matter in the natural environment (Coufalík et al., 2011; Han et al., 2006) as well as the higher affinity of organic matter toward Hg (Yu et al., 2006), we suspected that organic-clay could have a larger effect on Hg bioavailability than clay itself. More studies (*e.g.*, using EXAFS, and XRD) should be carried out to fully understand the mechanism of the Hg-clay-organic interaction.

Extraction of Hg by either CaCl<sub>2</sub> or BSA was used to assess the potential bioavailability of particle-bound Hg to plants or deposit-feeders. Our data suggested that humus-clay may have quite different effects on the potential bioavailability of Hg to plants and that to deposit-feeders. Such differences could possibly be attributed to the different mechanisms of solubilizing Hg from particles: Hg was extracted by CaCl<sub>2</sub> mainly by cation exchange, while Hg could be strongly complexed with BSA and be desorbed from particles. Consequently, humus coating had a larger effect on CaCl<sub>2</sub> extraction than on BSA extraction. Such laboratory results using chemical extraction methods should be extrapolated to field investigations with caution, before being further confirmed by biological uptake or bioaccumulation experiments.

Based on our results, it was found that clay could have different effects on the potential bioavailability of IHg and MeHg. For example for uncoated clay, IHg extraction by CaCl<sub>2</sub> was highest in montmorillonite, while MeHg extraction was lowest. Such differences between IHg and MeHg could be due to differences in charge, ionic radius, and other properties, which were not examined in this study. More investigations comparing the effects of clay on sorption of IHg and MeHg in soils are needed.

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## REFERENCES

- Abate, G., Masini, J.C., 2005. Influence of pH, ionic strength and humic acid on adsorption of Cd(II) and Pb(II) onto vermiculite. *Colloids Surf. A Physicochem. Eng. Asp.* 262 (1–3), 33–39.
- Abollino, O., Giacomino, A., Malandrino, M., Mentasti, E., 2008. Interaction of metal ions with montmorillonite and vermiculite. *Appl. Clay Sci.* 38 (3–4), 227–236.
- Arnarson, T.S., Keil, R.G., 2000. Mechanisms of pore water organic matter adsorption to montmorillonite. *Mar. Chem.* 71 (3–4), 309–320.
- Babel, S., Kurniawan, T.A., 2003. Low-cost adsorbents for heavy metals uptake from contaminated water: a review. *J. Hazard. Mater.* 97 (1–3), 219–243.
- Baham, J., Sposito, G., 1994. Adsorption of dissolved organic carbon extracted from sewage sludge on montmorillonite and kaolinite in the presence of metal ions. *J. Environ. Qual.* 23 (1), 147–153.
- Bailey, S.E., Olin, T.J., Bricka, R.M., Adrian, D.D., 1999. A review of potentially low-cost sorbents for heavy metals. *Water Res.* 33 (11), 2469–2479.
- Benoit, J.M., Mason, R.P., Gilmour, C.C., Aiken, G.R., 2001. Constants for mercury binding by dissolved organic matter isolates from the Florida Everglades. *Geochim. Cosmochim. Acta* 65 (24), 4445–4451.
- Bernaus, A., Gaona, X., Ivask, A., Kahru, A., Valiente, M., 2005. Analysis of sorption and bioavailability of different species of mercury on model soil components using XAS techniques and sensor bacteria. *Anal. Bioanal. Chem.* 382 (7), 1541–1548.
- Bhattacharyya, K.G., Gupta, S.S., 2008. Adsorption of a few heavy metals on natural and modified kaolinite and montmorillonite: a review. *Adv. Colloid Interf. Sci.* 140 (2), 114–131.
- Bloom, N., 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. *Can. J. Fish. Aquat. Sci.* 46 (7), 1131–1140.
- Bloom, N.S., Preus, E., Katon, J., Hiltner, M., 2003. Selective extractions to assess the biogeochemically relevant fractionation of inorganic mercury in sediments and soils. *Anal. Chim. Acta* 479 (2), 233–248.
- Bono, A.B., 1997. The Partitioning of Mercury Among the Solid Components of Sediments of the Saguenay Fjord. McGill University, Québec.
- Brigatti, M.F., Colonna, S., Malferrari, D., Medici, L., Poppi, L., 2005. Mercury adsorption by montmorillonite and vermiculite: a combined XRD, TG-MS, and EXAFS study. *Appl. Clay Sci.* 28 (1–4), 1–8.
- Chen, Z., Mayer, L.M., 1998. Mechanisms of Cu solubilization during deposit feeding. *Environ. Sci. Technol.* 32 (6), 770–775.
- Chen, L.G., Xu, Z.C., Ding, X.Y., Zhang, W.D., Huang, Y.M., Fan, R.F., et al., 2012. Spatial trend and pollution assessment of total mercury and methylmercury pollution in the Pearl River Delta soil, South China. *Chemosphere* 88 (5), 612–619.
- Coufalík, P., Červenka, R., Komárek, J., 2011. Mercury speciation in soil in vicinity of coal beds using sequential extraction. *Environ. Earth Sci.* 62 (2), 421–427.
- Cruz-Guzmán, M., Celis, R., Hermosín, M.C., Koskinen, W.C., Nater, E.A., Cornejo, J., 2006. Heavy metal adsorption by montmorillonites modified with natural organic cations. *Soil Sci. Soc. Am. J.* 70 (1), 215–221.
- Dai, Z.H., Feng, X.B., Zhang, C., Wang, J.F., Jiang, T.M., Xiao, H.J., et al., 2013. Assessing anthropogenic sources of mercury in soil in Wanshan Hg mining area, Guizhou, China. *Environ. Sci. Pollut. Res.* 20 (11), 7560–7569.
- do Nascimento, F.H., Masini, J.C., 2014. Influence of humic acid on adsorption of Hg(II) by vermiculite. *J. Environ. Manag.* 143, 1–7.
- Gagnon, C., Fisher, N.S., 1997. Bioavailability of sediment-bound methyl and inorganic mercury to a marine bivalve. *Environ. Sci. Technol.* 31 (4), 993–998.
- Gagnon, C., Pelletier, E., Mucci, A., 1997. Behaviour of anthropogenic mercury in coastal marine sediments. *Mar. Chem.* 59 (1–2), 159–176.
- Graham, A.M., Aiken, G.R., Gilmour, C.C., 2013. Effect of dissolved organic matter source and character on microbial Hg methylation in Hg-S-DOM solutions. *Environ. Sci. Technol.* 47 (11), 5746–5754.

- Greenland, D., 1971. Interactions between humic and fulvic acids and clays. *Soil Sci.* 111 (1), 34–41.
- Guerra, D.L., Viana, R.R., Airolidi, C., 2009. Adsorption of mercury cation on chemically modified clay. *Mater. Res. Bull.* 44 (3), 485–491.
- Gupta, A.K., Sinha, S., 2007. Assessment of single extraction methods for the prediction of bioavailability of metals to *Brassica juncea* L. Czern. (var. Vaibhav) grown on tannery waste contaminated soil. *J. Hazard. Mater.* 149 (1), 144–150.
- Haitzer, M., Aiken, G.R., Ryan, J.N., 2002. Binding of mercury(II) to dissolved organic matter: the role of the mercury-to-DOM concentration ratio. *Environ. Sci. Technol.* 36 (16), 3564–3570.
- Han, F.X., Su, Y., Monts, D.L., Waggoner, C.A., Plodinec, M.J., 2006. Binding, distribution, and plant uptake of mercury in a soil from Oak Ridge, Tennessee, USA. *Sci. Total Environ.* 368 (2–3), 753–768.
- Jing, Y.D., He, Z.L., Yang, X.E., Sun, C.Y., 2008. Evaluation of soil tests for plant-available mercury in a soil-crop rotation system. *Commun. Soil Sci. Plant Anal.* 39 (19–20), 3032–3046.
- Kongchum, M., Hudnall, W.H., Delaune, R.D., 2011. Relationship between sediment clay minerals and total mercury. *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.* 46 (5), 534–539.
- Lawrence, A.L., McAloon, K.M., Mason, R.P., Mayer, L.M., 1999. Intestinal solubilization of particle-associated organic and inorganic mercury as a measure of bioavailability to benthic invertebrates. *Environ. Sci. Technol.* 33 (11), 1871–1876.
- Liu, A.G., Gonzalez, R.D., 1999. Adsorption/desorption in a system consisting of humic acid, heavy metals, and clay minerals. *J. Colloid Interface Sci.* 218 (1), 225–232.
- Lothenbach, B., Krebs, R., Furrer, G., Gupta, S.K., Schulin, R., 1998. Immobilization of cadmium and zinc in soil by Al-montmorillonite and gravel sludge. *Eur. J. Soil Sci.* 49 (1), 141–148.
- Malandrino, M., Abollino, O., Giacomino, A., Aceto, M., Mentasti, E., 2006. Adsorption of heavy metals on vermiculite: influence of pH and organic ligands. *J. Colloid Interface Sci.* 299 (2), 537–546.
- Malferrari, D., Brigatti, M.F., Marcelli, A., Chu, W.S., Wu, Z.Y., 2010. Effect of temperature on Hg-cysteine complexes in vermiculite and montmorillonite. *Appl. Clay Sci.* 50 (1), 12–18.
- Morel, F.M.M., Kraepiel, A.M.L., Amyot, M., 1998. The chemical cycle and bioaccumulation of mercury. *Annu. Rev. Ecol. Syst.* 29, 543–566.
- Obukhovskaya, T., 1982. Mercury sorption by soil minerals. *Soviet Soil Sci.* 14 (3), 49–55.
- Rauret, G., 1998. Extraction procedures for the determination of heavy metals in contaminated soil and sediment. *Talanta* 46 (3), 449–455.
- Ravichandran, J., Sivasankar, B., 1997. Properties and catalytic activity of acid-modified montmorillonite and vermiculite. *Clay Clay Miner.* 45 (6), 854–858.
- Satterberg, J., Arnarson, T.S., Lessard, E.J., Keil, R.G., 2003. Sorption of organic matter from four phytoplankton species to montmorillonite, chlorite and kaolinite in seawater. *Mar. Chem.* 81 (1–2), 11–18.
- Schuster, E., 1991. The behavior of mercury in the soil with special emphasis on complexation and adsorption processes—a review of the literature. *Water Air Soil Pollut.* 56 (1), 667–680.
- Sdiri, A.T., Higashi, T., Jamoussi, F., 2014. Adsorption of copper and zinc onto natural clay in single and binary systems. *Int. J. Environ. Sci. Technol.* 11 (4), 1081–1092.
- Skyllberg, U., 2008. Competition among thiols and inorganic sulfides and polysulfides for Hg and MeHg in wetland soils and sediments under suboxic conditions: illumination of controversies and implications for MeHg net production. *J. Geophys. Res.* 113 (G2). <http://dx.doi.org/10.1029/2008JG000745>.
- Speight, J.G., 2005. *Lange's Handbook of Chemistry*. 16th ed. McGraw-Hill, New York.
- Suquet, H., Chevalier, S., Marcilly, C., Barthomeuf, D., 1991. Preparation of porous materials by chemical activation of the Llano vermiculite. *Clay Miner.* 26 (1), 49–60.
- Temuujin, J., Okada, K., MacKenzie, K.J.D., 2003. Preparation of porous silica from vermiculite by selective leaching. *Appl. Clay Sci.* 22 (4), 187–195.
- Thimsen, C.A., Keil, R.G., 1998. Potential interactions between sedimentary dissolved organic matter and mineral surfaces. *Mar. Chem.* 62 (1–2), 65–76.
- US EPA, 1998. Method 7473 Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry. U.S. Environmental Protection Agency, US.
- Voparil, I.M., Mayer, L.M., 2004. Commercially available chemicals that mimic a deposit feeder's (*Arenicola marina*) digestive solubilization of lipids. *Environ. Sci. Technol.* 38 (16), 4334–4339.
- Wang, D.Y., Qing, C.L., Guo, T.Y., Guo, Y.J., 1997. Effects of humic acid on transport and transformation of mercury in soil-plant systems. *Water Air Soil Pollut.* 95 (1–4), 35–43.
- Yu, G.F., Wu, H.T., Jiang, X., He, W.X., Qing, C.L., 2006. Relationships between humic substance-bound mercury contents and soil properties in subtropical zone. *J. Environ. Sci.* 18 (5), 951–957.
- Zhong, H., Wang, W.X., 2006a. Sediment-bound inorganic Hg extraction mechanisms in the gut fluids of marine deposit feeders. *Environ. Sci. Technol.* 40 (19), 6181–6186.
- Zhong, H., Wang, W.X., 2006b. Metal–solid interactions controlling the bioavailability of mercury from sediments to clams and sipunculans. *Environ. Sci. Technol.* 40 (12), 3794–3799.
- Zhong, H., Wang, W.X., 2006c. Influences of aging on the bioavailability of sediment-bound Cd and Zn to deposit-feeding sipunculans and soldier crabs. *Environ. Toxicol. Chem.* 25 (10), 2775–2780.
- Zhong, H., Wang, W.X., 2008a. Effects of sediment composition on inorganic mercury partitioning, speciation and bioavailability in oxic surficial sediments. *Environ. Pollut.* 151 (1), 222–230.
- Zhong, H., Wang, W.X., 2008b. Methylmercury extraction from artificial sediments by the gut juice of the sipunculan, *Sipunculus nudus*. *Environ. Toxicol. Chem.* 27 (1), 138–145.
- Zhong, H., Wang, W.X., 2009. Inorganic mercury binding with different sulfur species in anoxic sediments and their gut juice extractions. *Environ. Toxicol. Chem.* 28 (9), 1851–1857.
- Zhong, H., Kraemer, L., Evans, D., 2012. Effects of aging on the digestive solubilization of Cu from sediments. *Environ. Pollut.* 164, 195–203.