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Prediction of methylmercury accumulation in rice grains by chemical extraction methods

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ABSTRACT

To explore the possibility of using chemical extraction methods to predict phytoavailability/bioaccumulation of soil-bound MeHg, MeHg extractions by three widely-used extractants ($CaCl_2$, DTPA, and $(NH_4)_2S_2O_3$) were compared with MeHg accumulation in rice grains. Despite of variations in characteristics of different soils, MeHg extracted by $(NH_4)_2S_2O_3$ (highly affinitive to MeHg) correlated well with grain MeHg levels. Thus $(NH_4)_2S_2O_3$ extraction, solubilizing not only weakly-bound and but also strongly-bound MeHg, may provide a measure of 'phytoavailable MeHg pool' for rice plants. Besides, a better prediction of grain MeHg levels was obtained when growing condition of rice plants was also considered. However, MeHg extracted by $CaCl_2$ or DTPA, possibly quantifying 'exchangeable MeHg pool' or 'weakly-complexed MeHg pool' in soils, may not indicate phytoavailable MeHg or predict grain MeHg levels. Our results provided the possibility of predicting MeHg phytoavailability/bioaccumulation by $(NH_4)_2S_2O_3$ extraction, which could be useful in screening soils for rice cultivation in contaminated areas.

1. Introduction

Recent studies revealed that elevated production of methylmercury (MeHg) in contaminated paddy soils could result in high MeHg levels in rice grains, posing a potential health risk to human beings (Qiu et al., 2008). Therefore, it is of great importance to assess the phytoavailability of soil-bound MeHg to rice plants and predict its accumulation in rice grains.

Chemical extraction methods, in which extracted metals are considered mobile, or bioavailable, have long been used to assess bioavailability/phytoavailability of metals in soils or sediments (Jing et al., 2008; Rauret, 1998; Zhong and Wang, 2006a). Although 'bioavailable metal' quantified by those extraction methods is operationally defined, good correlations between metal extraction and its bioavailability/bioaccumulation in plants or organisms have been frequently reported (e.g., Gupta and Sinha, 2007; Zhong and Wang, 2006b). One possible reason for such relationship was that desorption of metal from soils/sediments (e.g., in the rhizosphere or within digestive tracts of organisms) could be a limiting step of metal bioaccumulation process (Zhong and Wang, 2006c), especially for metal having high affinity to particles. In view of the strong binding between MeHg and soil particles (Ullrich et al., 2001), especially soil organic contents (Lee et al., 2001), prediction of MeHg accumulation in plants by chemical extraction methods warrants investigation.

Extractions of mercury from soils by various extractants, e.g., diethylene triamine pentaacetate acid (DTPA) and calcium chloride ($CaCl_2$), have long been used to quantify phytoavailability of soil-bound mercury to plants (Harsh and Doner, 1981; Jing et al., 2008; Zagury et al., 2006). However, these methods have rarely been validated by correlating mercury extraction and its bioaccumulation. Besides, few methods have been tested for their validities for predicting mercury bioavailability in soils of different properties. Furthermore, most of those previous researches focused on total mercury (THg). Few attempts were taken to assess phytoavailability of soil-bound MeHg using chemical extraction methods, although MeHg is more bioaccumulative and toxic.

To explore the possibility of predicting phytoavailability of MeHg in paddy soils and its accumulation in rice grains, MeHg was extracted from eight soils of different properties (e.g., pH, organic content, and clay content). Three extractants were used for MeHg extraction, including $CaCl_2$, DTPA and ammonium thiosulfate ($(NH_4)_2S_2O_3$), commonly used to increase mobility and

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phytoavailability of soil-bound mercury in phytoextraction, e.g., Moreno et al., 2005a, 2005b). Soils were planted with rice plants, and MeHg concentrations in harvested rice grains were correlated with MeHg extractions by the three extractants. Consequently, validities of different chemical extraction methods for predicting grain MeHg levels were compared.

2. Materials and methods

2.1. Soils and chemicals

Eight types of soils were collected from different provinces of China (Table 1). The soils were air-dried, ground, and sieved through a 150 μm mesh before use.

Mercury chloride ($HgCl_2$), DTPA, $CaCl_2$, and $(NH_4)_2S_2O_3$ were purchased from Sigma–Aldrich (USA). Methylmercury Chloride (CH_3ClHg) was obtained from Brooks Rand (USA). Trace metal grade nitric acid (HNO_3) and hydrochloric acid (HCl) was purchased from Sinopharm Chemical Reagent (China) and Nanjing Chemical Reagent (China), respectively. Potassium hydroxide (KOH) was from Chinasun Specialty Products (China). Methanol (CH_3OH) and NaBet4, used for MeHg digestion and determination, was from TEDIA (USA) and Brooks Rand Lab (USA), respectively. All chemicals were of analytical reagent grade or higher. Background mercury levels in all chemicals were tested and found to be extremely low.

2.2. Growing rice plants in mercury-spiked soils

Different types of soils were spiked with inorganic mercury (IHg, 5 mg/kg) (Peng et al., 2012) at the start of the experiment (day 0). To achieve higher MeHg levels in soils, IHg-spiked soils instead of contaminated soils were used in this study, considering that newly spiked IHg could be more bioavailable to mercury methylation bacteria than aged IHg (Hintelmann et al., 2002). Mercury spiked soils were placed into pots (polypropylene) for equilibration (2.8 kg soil/pot) in a greenhouse. Three replicate pots were used for each soil type (totally 24 pots). On day 5, all pots were flooded with deionized water. On day 17, thirty day-old rice seedlings of Wufengyou2168 (*indica* WFY2168) were transplanted (2 seedlings/pot). Granulated fertilizer was added in the form of phosphorus (P) as $CaHPO_4 \cdot H_2O$ (321 mg/kg), potassium (K) as KCl (179 mg/kg), and nitrogen (N) as $CO(NH_2)_2$ (214 mg/kg) as a basal fertilizer on day 5. Addition of fertilizer was repeated on day 60 at panicle initiation stage and day 90 at flowering stage. Soils were flooded with deionized water (3 cm above the soil surface) during the entire growth period. And rice plants were grown for 115 days (July 1 to October 24, 2013) in the greenhouse.

Soils were sampled at the start (day 26, i.e., 9th day after transplanting) and at the end (day 132, i.e., the day of rice harvest, 115th day after transplanting) of the growing period. Surface soil (5–20 cm) was collected into a 50 mL polypropylene centrifuge tube, vacuum sealed, and then transported to the laboratory in an ice-cooled container within 3 h.

At harvest, rice plants were cut 4 cm above the soil surface, and separated into rice grains, flag leaves and stems. The plant samples were first cleaned with tap water and then rinsed with cysteine solution (8 mmol/L) to remove surface sorbed mercury (Zhong and Wang, 2009). After that, plant samples were rinsed thoroughly with deionized water, dried at 40 °C and weighed. The aboveground biomass was calculated by summing the dry weights of rice grains, flag leaves and stems. Rice grains were dehulled manually, and the obtained brown rice samples were freeze-dried (Labconco, USA), ground in a ceramic mortar and sieved to less than 150 μm.

2.3. Extractions of MeHg by three extractants

The collected soil samples were centrifuged at 3000 rpm for 30 min to collect porewater, and then extracted by $CaCl_2$ (0.01 mol/L, Pueyo et al., 2004), DTPA (0.005 mol/L, Jing et al., 2008), or $(NH_4)_2S_2O_3$ (0.0135 mol/L, Yin et al., 2013). About two grams (wet weight) of soil was placed into a 15 mL polypropylene tube and extractant solution was added at a ratio of 2 mL/g (solution volume per soil wet weight). The capped tubes were sealed with parafilm and shaken at 300 rpm in a incubating shaker at 25 °C for 2 h ($CaCl_2$, Gupta and Sinha, 2007; DTPA, Feng et al., 2005) or 12 h ($(NH_4)_2S_2O_3$, Yin et al., 2013). Afterward, the mixtures were centrifuged at 3000 rpm for 30 min and the supernatant solutions were filtered through 0.45 μm membrane filters. To avoid loss of MeHg, HCl was added to the supernatant solutions (Meng et al., 2011). To minimize oxidation of soils during shaking, extractant solutions were prepared with deoxygenated water. And all operations except centrifugation, filtration and shaking were conducted in a glove box filled with nitrogen gas.

2.4. Measurement of MeHg concentrations

Concentrations of MeHg were measured by an automatic Brooks Rand model III MeHg analyzer (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 incubating shaker at 60 °C for 4 h (Bloom, 1989; Gagnon et al., 1997). Then the digested samples were diluted with ultrapure water and stored in the dark at −20 °C for less than 24 h before MeHg determination. Standard sediment (ERM-cc58) was digested with each batch of sample digestion following the same procedure. And the recoveries were between 80% and 120%. Quality control (QC) was assured by method/reagent blanks, matrix spikes (>74% for all extractant solutions), certified

Table 1
Soil properties, background mercury concentrations in soils, as well as aboveground biomass and relative growth index of rice plants.

Soil	Sampling site	Soil properties			Background mercury concentrations (ng/g)		Rice plants	
		pH	POC (%)	Clay (%)	THg	MeHg	Aboveground biomass(g)	Relative growth index (RGI)[a]
A	Heilongjiang Province	7.5	2.0	37.6	93.4 ± 10.5	0.097 ± 0.045	70.1 ± 5.1	1.07 ± 0.08
B	Sichuan Province	7.8	1.6	41.9	78.9 ± 4.4	0.117 ± 0.000	81.6 ± 6.7	1.25 ± 0.10
C	Shanxi Province	8.0	0.7	41.4	39.8 ± 3.4	0.080 ± 0.002	64.4 ± 4.3	0.98 ± 0.07
D	Hubei Province	7.8	0.5	35.3	27.8 ± 0.4	0.055 ± 0.000	60.9 ± 9.9	0.93 ± 0.15
E	Henan Province	7.7	0.9	30.2	33.0 ± 3.5	0.066 ± 0.002	75.0 ± 5.3	1.14 ± 0.08
F	Jiangsu Province	6.9	0.9	45.7	32.5 ± 2.8	0.041 ± 0.002	71.2 ± 8.9	1.09 ± 0.14
G	Guizhou Province	7.9	4.3	41.3	130.7 ± 11.5	0.132 ± 0.000	79.2 ± 10.9	1.21 ± 0.17
H	Jiangxi Province	4.7	0.3	15.5	52.5 ± 10.6	0.025 ± 0.001	21.8 ± 2.8	0.33 ± 0.04

[a] Relative growth index (RHI) = Aboveground biomass of rice plants grown in a certain soil/Average aboveground biomass of all rice plants grown in different soils (i.e., 65.5 g).