

# Selenium in Soil Inhibits Mercury Uptake and Translocation in Rice (*Oryza sativa* L.)

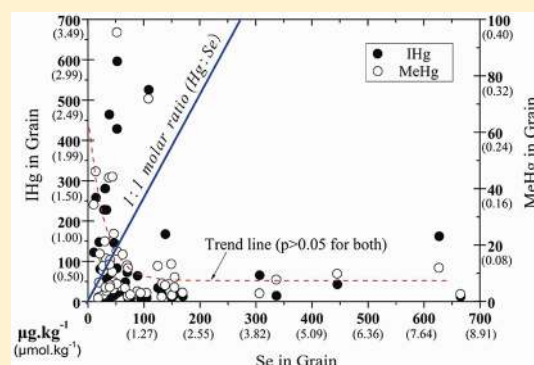
Hua Zhang,<sup>†,‡</sup> Xinbin Feng,<sup>\*,†</sup> Jianming Zhu,<sup>†</sup> Atindra Sapkota,<sup>†</sup> Bo Meng,<sup>†</sup> Heng Yao,<sup>†</sup> Haibo Qin,<sup>†</sup> and Thorjörn Larssen<sup>‡</sup>

<sup>†</sup>State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, 550002, China

<sup>‡</sup>Norwegian Institute for Water Research, Gaustadalléen 21, 0349 Oslo, Norway

**S** Supporting Information

**ABSTRACT:** A great number of studies have confirmed that mercury–selenium (Hg–Se) antagonism is a widespread phenomenon in microorganisms, fish, poultry, humans, and other mammals. However, by comparison, little attention has been paid to plants. To investigate the influence of Se on the uptake and translocation of methylHg/inorganic Hg (MeHg/IHg) in the rice–soil system, we determined the levels of Se, IHg, and MeHg in different parts of rice plants (including the root, stem, leaf, husk, and grain (brown rice)) and corresponding soils of root zones collected from a Hg mined area, where Hg and Se co-occur due to historic Hg mining and retorting activities. The results showed that, in general, the Se levels were inversely related to the levels of both IHg and MeHg in the grains. In addition, a consistent reduction in translocation of both IHg and MeHg in the aerial shoots (i.e., the stem, leaf, husk, and grain) with increasing Se levels in the soils was observed. Furthermore, the Se levels were positively correlated with the IHg levels in the soils and the roots. These results suggest that Se may play an important role in limiting the bioaccessibility, absorption, and translocation/bioaccumulation of both IHg and MeHg in the aerial rice plant, which may be related to the formation of an Hg–Se insoluble complex in the rhizospheres and/or roots.



## INTRODUCTION

Mercury (Hg) is known to be a toxic trace element. Its organic form, such as methyl-Hg (MeHg), is of great concern due to the high toxicity and potentially severe effects on humans.<sup>1</sup> MeHg can be generated from inorganic Hg (IHg) in the environment by micro-organisms under special conditions and can be bioaccumulated and biomagnified in the food chain,<sup>2</sup> eventually posing a serious threat to the ecosystems and humans.

Selenium (Se) is an essential trace element for human beings and animals which can alleviate the toxicity of many heavy metals, including Hg,<sup>3</sup> since it is incorporated in the active center of antioxidant seleno-enzymes (glutathione peroxidase and thioredoxin reductase).<sup>4</sup> However, it can also be toxic to humans and animals due to a very narrow margin between its essentiality, deficiency, and toxicity amount to living organisms.<sup>5</sup>

The antagonism by Se of the toxicity of Hg in organisms has been known since an initial study by Pařízek and Ošťádalová in 1967<sup>6</sup> showed that Se effectively prevented mortality caused by high doses of IHg in rats. In 1972, Ganther et al.<sup>7</sup> further demonstrated that Se decreased the toxicity of MeHg in fish (tuna). Since then, a large number of subsequent studies have confirmed that Hg–Se antagonism is a widespread phenomenon in microorganisms, fish, poultry, humans, and other

mammals, and most of this abundant literature has been well summarized by several articles,<sup>8–15</sup> suggesting that a universal Se–Hg antagonism may exist among various species.

However, by comparison, there have been few related studies conducted for plants, which were mostly conducted in laboratory-controlled experiments (rather than in natural fields with more complex conditions because they vary both spatially and temporally), and were only focused on the detoxifying effect of Se on IHg but not MeHg.<sup>16–20</sup> From the few available reports on plants, however, an increased Se supply could reduce the accumulation of Hg in the aerial portions of plants, which may be primarily restricted to the formation of an insoluble Hg–Se-metabolizing complex in the plant roots that precludes further metabolism or translocation of Hg to aerial plant tissues.<sup>17–20</sup>

Rice, the most popular staple food for more than half of the world's population, is one of the dominant Se sources, especially for people in Asian countries.<sup>21</sup> However, our recent studies have indicated that rice is also a major dietary source of MeHg exposure for the rural population in some inland areas in

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China.<sup>22–24</sup> To date, although several studies have been individually conducted on the speciation, distribution, and translocation of Hg or Se in the rice plant,<sup>21,24,25</sup> few realized the potential influence of Se on Hg.

The Wanshan Hg mined area located in southwestern China (Figure S1), is a unique, ideal field laboratory for studying Se–Hg antagonism because Se is an important element coexisting with Hg. Tiemannite (HgSe), which occurs in hydrothermal veins associated with other selenides, and other Hg minerals, such as cinnabar, which are often associated with calcite, has been reported in Wanshan since 1975.<sup>26</sup> The chemical composition of the tiemannite from this area was measured to be 70–74 wt % of Hg and 24–27 wt % of Se,<sup>27</sup> which was very close to the theoretical value for tiemannite (HgSe) (Hg: 71.70 wt %; Se: 28.30 wt %).<sup>26</sup> Therefore, the concentrations of Se were positively correlated with the Hg concentrations in the cinnabar in Wanshan ( $R^2 = 0.52–0.86$ ).<sup>26,28</sup>

In general, the majority of the Se in the study area is scattered in cinnabar (HgS), in addition to the formation of a small quantity of tiemannite and metacinnabar. Se in common cinnabar in Wanshan area was observed to be 0.02–0.87 wt % and reached 2.7 wt % in metacinnabar because the S in the cinnabar can be replaced by Se to form an isomorphous series of HgS–HgSe.<sup>27</sup> The historic large-scale mining and retorting activities of high Se-containing cinnabar and ores have caused an increased loading of both Hg and Se in the surrounding terrestrial and aquatic ecosystems, e.g., Hg in the paddy soil and rice grain has been reported to reach 311 and 0.46 mg/kg,<sup>24</sup> respectively, with the corresponding Se reported as being up to 16 and 1 mg/kg, respectively.<sup>29</sup>

Although a great number of studies about Hg pollution have been conducted in Wanshan,<sup>22,24,29–32</sup> the study by Horvat et al.<sup>29</sup> was the only one to mention the influence of Se in the local environment. They did not, however, discuss the antagonistic effects of Se on Hg in the rice plant.

This study was designed to test the hypothesis that levels of Se in the soil may affect the uptake and translocation of IHg and MeHg in the rice plant (*Oryza sativa* L.) and explore the possible underlying mechanisms.

## MATERIALS AND METHODS

**Study Area.** The Wanshan area is a typical hilly and Karstic terrain with elevations ranging from 205 to 1149 m above sea level. The region has a subtropical humid climate characterized by abundant precipitation and mild temperature. The annual average rainfall is 1200–1400 mm, and the annual mean temperature is 17 °C.

The Wanshan Hg mined area, which has been called the “mercury capital” of China, is the largest cinnabar deposit in Asia and a typical large-scale Hg mine. Hg mining started in this region more than 2000 years ago, although mining was most intensive during the last half of the 20th century. Approximately 22 000 tons of Hg, 6000 tons of cinnabar, and large quantities of mine wastes have been produced in Wanshan.<sup>32</sup>

There are five major river valleys in the Wanshan area (Figure S1). Interestingly, most Hg of the mines and retorting facilities is situated in the upstream part of the rivers. Rice paddies are located throughout the valleys, from just downstream of the calcine piles to the side valleys and tributaries. Hence, there are strong gradients in the levels of Hg and Se within a limited area, making the region particularly suitable for case studies of the interactive relationship between Se and Hg in the environment.

**Sample Collection and Preparation.** Building upon our previous study, 59 rice grain samples and the corresponding soils from root zones collected from the Wanshan area (covering about 700 km<sup>2</sup>) (Figure S1), in which the bioaccumulation of MeHg versus IHg in the rice grain was previously reported,<sup>24</sup> were directly used for Se measurements. To better explore the potential relationship of Se/Hg in different parts of rice, e.g., root, leaf, stem, husk, and grain, we reconducted a sampling campaign for the whole rice plant from nine typical sites (Figure S1) covering “extremely elevated”, “elevated”, and “less-impacted” levels of Se versus Hg in the soils. The typical sites were identified according to the observed data in the first sampling campaign. All rice samples were collected during the harvest period (late August). In the second sampling, for the purpose of studying the correlation between rice plant and the associated soil from the root zone, at each site, a cluster of 15–20 intact rice plants was uprooted, and about 500 g (wet weight) of soil surrounding the root (10–20 cm depth) was collected, which contained the rhizosphere (i.e., the zone of soil surrounding root within only a few mm wide where the biology and chemistry of the soil are influenced by root). In the first sampling, the grains and corresponding soils of root zones (5–10 cm depth) were directly collected (the rice plant was not uprooted), and a composite sample was composed of five subsamples (for regional distribution study). All soil samples were individually sealed into three successive polyethylene bags to avoid cross-contamination and transferred (with ice packs) to the laboratory on the same day of sampling. The detailed sampling sites are shown in Figure S1.

The rice plant samples were successively washed with distilled water and deionized water. Special attention was paid to the cleaning of rice samples, especially for the root parts (in order to avoid strong attachment of soil). Then the samples were dried at 40 °C until a constant weight. Afterward, the samples were processed by first removing the husk from the grains, and then the grains (brown rice) were crushed and ground using a grinder (for whole rice plant samples, after separating the grain (brown rice), the other parts were divided into four fractions, including the root, stem, leaf, and husk). Similarly, the soil samples, free of plant residue or stones, were dried at 40 °C until a constant weight, ground in an agate mortar, and then sieved with a minus 200 mesh (0.074 mm) prior to digestion.

**Analytical Methods for Hg.** For the total Hg concentration (THg) analysis, the soil samples were digested in a water bath (95 °C) using a fresh mixture of HCl (12 mol/L) and HNO<sub>3</sub> (16 mol/L) (1:3, v/v), and the rice samples were digested with a mixture of HNO<sub>3</sub> (16 mol/L) and H<sub>2</sub>SO<sub>4</sub> (18.4 mol/L) (4:1, v/v).<sup>33</sup> THg was measured in the soil samples using cold vapor atomic absorption spectrometry (CVAAS), and THg in the rice samples was determined using the dual-stage gold amalgamation method and cold vapor atomic fluorescence spectrometry (CVAFS) detection, following method 1631.<sup>34</sup> For the MeHg analysis, the rice samples were prepared using the KOH–methanol/solvent extraction method,<sup>35</sup> and the soil samples were prepared using the CuSO<sub>4</sub>–methanol/solvent extraction method.<sup>36</sup> For both sample types, MeHg was determined using aqueous ethylation, purge, trap, and GC CVAFS detection,<sup>37</sup> following method 1630.<sup>38</sup> IHg was obtained by subtracting MeHg from THg.

**Analytical Methods for Se.** The total Se in all samples was determined by hydride generation-atomic fluorescence spec-

trometry (HG-AFS).<sup>39</sup> Briefly, 0.05–0.10 g of sample was placed in a 30-mL PTFE (Teflon) bomb with 2.0 mL of concentrated HNO<sub>3</sub> (16 mol/L) and 1.0 mL of HF (57 mol/L) for soil, and with 2.5 mL of concentrated HNO<sub>3</sub> (16 mol/L) and 0.5 mL of HF (57 mol/L) for rice samples. The mixture was then allowed to stand for 2 h to degas and was sealed in a stainless-steel container and heated in an oven at 150 °C for 16 h. After the samples cooled, 2 mL of H<sub>2</sub>O<sub>2</sub> (30%) was added, and the bomb was once again heated at 90 °C for about 45 min. The solution was transferred into a 15-mL PFA beaker and evaporated to near dryness at 70 °C. The Se was transformed into Se(IV) by adding 3 mL of 5 mol/L HCl followed by incubation at 95–100 °C for 45 min. The solution was diluted for the final analysis. All reagents were subdistilled to minimize the blank level. Recovery and quality assurance were validated by using different reference materials. All samples in our data set were measured at least twice. All concentrations of Hg and Se in this report were based on dry weight (dw).

Data are generally described by the geometric/arithmetic mean  $\pm$  standard deviation (SD) (due to the log-normal distribution pattern of the data). Correlations between covariant sets of data were subjected to regression analysis. Correlation coefficients ( $r^2$ ) and significance probabilities ( $p$ ) were computed for the linear or exponential regression fits according to the scatter characteristics. Significant differences were declared at  $p < 0.01$  (or  $p < 0.05$  if the data is not significant at 0.01 level). Statistical analyses were done using SPSS 13. Information on QA/QC of our measurement data is available in the Supporting Information.

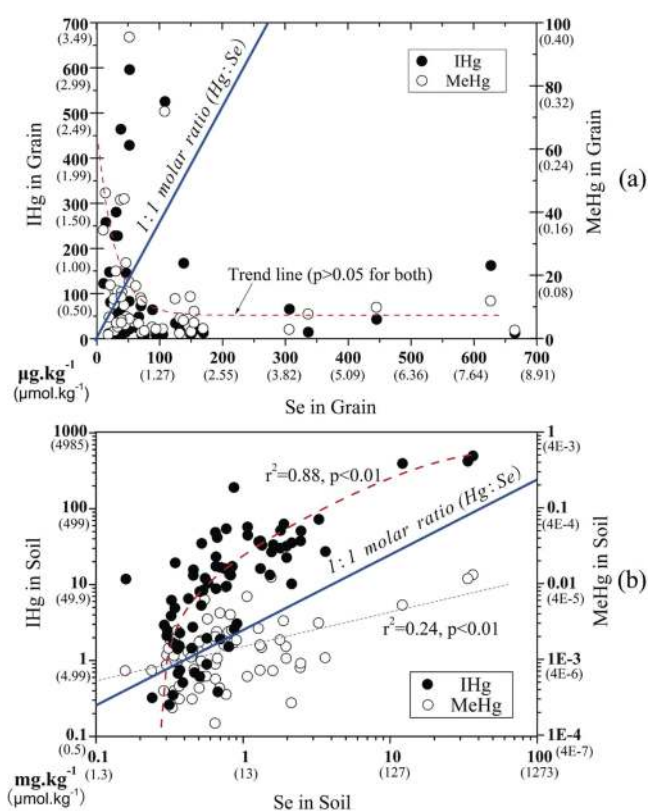
## RESULTS

**Se Levels versus Hg Levels in Grains.** The concentrations (dw) of Se in the rice grain ranged widely from 0.02 to 0.67 mg/kg, with a geometric/arithmetic mean of 0.065/0.10  $\pm$  0.15 mg/kg ( $n = 59$ ). These values were generally higher than the 0.040–0.070 mg/kg recommended for agriculture products by the Chinese Nutrition Society<sup>40</sup> (approximately 10% of all analyzed samples exceeded the maximum food standard limit of 0.3 mg/kg Se in cereal<sup>41</sup>) but much lower than those in the Enshi seleniferous region, which is one of the most typical Se-rich areas in China (range: 0.084–9.67 mg/kg; generally higher than 0.30 mg/kg).<sup>21</sup>

The geometric/arithmetic mean of concentrations (dw) of IHg and MeHg in the corresponding grain samples were 0.044/0.096  $\pm$  0.13 mg/kg and 8.2/13.5  $\pm$  17  $\mu$ g/kg, respectively, as previously reported,<sup>24</sup> with more than 70% having THg exceeding 0.020 mg/kg, the maximum value recommended for consumption by the Standardization Administration of the People's Republic of China.<sup>42</sup>

In general, the concentrations of Se were inversely correlated to the concentrations of both IHg and MeHg in the grain samples, although this relationship was not significant ( $p > 0.05$  for both) (Figure 1a). Furthermore, more than 70% of the rice grain samples had molar ratios of Se:Hg  $> 1$  (Figure S2).

**Se Levels versus Hg Levels in the Soil.** The Se concentrations (dw) in the soil samples ranged widely from 0.16 to 36.6 mg/kg, with a geometric/arithmetic mean of 1.1/2.1  $\pm$  6.6 mg/kg ( $n = 59$ ). Approximately 90% of the sites were between the range of Se-rich soil (0.45–2.0 mg/kg) and Se-optimum soil (0.2–0.45 mg/kg) (Figure 1b), and approximately 10% of the sites exceeded the baseline of 3.0 mg/kg for Se-excessive soil, according to the soil Se classification



**Figure 1.** Relationship between Se and IHg/MeHg in the rice grain (a) and in the associated soil from root zone (b).

standards established by epidemiological surveys of “Keshan disease” (associated with Se deficiency) in China.<sup>43</sup>

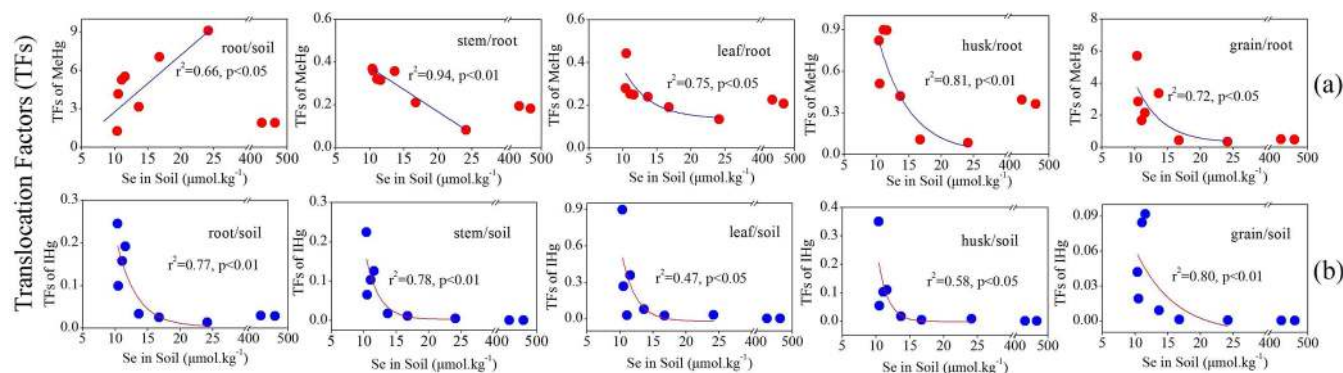
These paddy soil Se values (0.16–36.6 mg/kg) were comparable with reports from the Enshi seleniferous region (0.5–47.7 mg/kg),<sup>21</sup> although the corresponding amount of rice grain Se in the present study area (range: 0.02–0.67 mg/kg; only 10% of sites exceeded 0.3 mg/kg) was much lower than that in the Enshi district (range: 0.084–9.67 mg/kg; 75% higher than 0.30 mg/kg),<sup>21</sup> as mentioned above. The variation in Se concentrations in the rice grains between the two regions might be related to the occurrence of elevated Hg levels in the soils in Wanshan compared with that in Enshi (which has no known Hg contamination).

The IHg in the soils in Wanshan was 0.26–496 mg/kg, with a geometric/arithmetic mean of 7.2/16.4  $\pm$  98 mg/kg, and more than 70% of its sites exceeded the Chinese national standard limit for paddy soils of 1.5 mg/kg;<sup>24</sup> the corresponding MeHg levels were 0.14–13.3  $\mu$ g/kg, with a geometric/arithmetic mean of 1.4/2.5  $\pm$  3.3  $\mu$ g/kg, as we previously reported.<sup>24</sup>

Se concentrations were positively correlated with concentrations of both IHg and MeHg in the soils ( $r^2 = 0.88$  for IHg and 0.24 for MeHg; log-transformed;  $p < 0.01$  for both) (Figure 1b). But only approximately 20% of the soil samples in the study area had molar ratios of Se:Hg  $> 1$ , which was much lower than that of the corresponding rice grain samples (over 70%) as mentioned above (Figure S2). No significant correlations were observed between the molar ratio of Se/Hg in grains and that in the corresponding soils (Figure S2).

The geometric mean of translocation factors from the soil to grain (i.e., TF (grain/soil) = concentration<sub>grain</sub>/concentration<sub>soil</sub>) of Se (0.093  $\pm$  0.33) were approximately 15 $\times$  greater





**Figure 2.** Concentrations of Se in the soil compared with the TFs of IHg (and TFs of MeHg) in different parts of rice plant (e.g., root, stem, leaf, husk, and grain). The two “extreme value sites” on the right side were not included in the regression analysis; all correlation coefficients were estimated by an exponential regression except for the top-left first two subfigures which were estimated by linear regression.

than the TFs of IHg ( $0.006 \pm 0.025$ ) but 62 times lower than the TFs of MeHg ( $5.8 \pm 8.7$ ) (Figure S3). The rice plant can therefore be categorized as an accumulator of MeHg but not of Se or IHg due to their generally low TFs (TFs <1 are usually not classified as intensive bioaccumulators<sup>44</sup>). There was no significant ( $p > 0.05$ ) correlation between TFs of Se and TFs of IHg (or TFs of MeHg) in the rice grains (Figure S3).

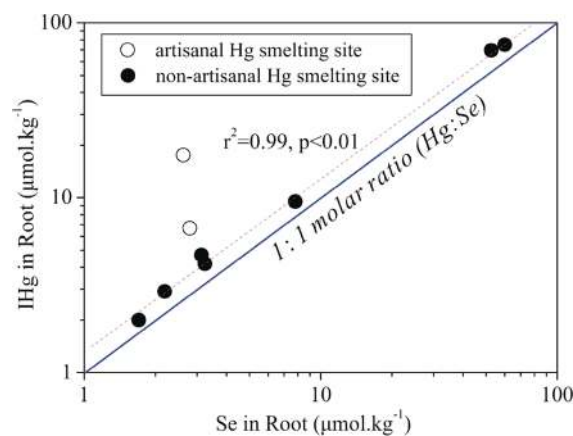
**Distribution Pattern of Se versus Hg in Different Parts of Rice from Typical Sites.** To better elucidate the antagonistic effects of Se on Hg in different rice tissues, the typical sampling sites for whole rice plant collection were divided into three groups based on the Hg levels and/or Se levels (shown with arithmetic mean  $\pm$  SD) in the soil (Figure S4), i.e., “extreme sites” (IHg:  $460 \pm 52$  mg/kg; Se:  $35 \pm 2.0$  mg/kg) (D12-a,b in Figure S1), “elevated sites” (IHg:  $31 \pm 25$  mg/kg; Se:  $1.14 \pm 0.42$  mg/kg) (all sites for whole rice plant collection except for D12-a,b and D8–D9 in Figure S1), and “control sites” (IHg:  $2.8 \pm 0.37$  mg/kg; Se:  $0.89 \pm 0.13$  mg/kg) (D8–D9 in Figure S1). It is clearly shown that even the Hg concentrations in “control sites” are elevated when compared with the corresponding Chinese paddy soil Hg standard limit of 1.5 mg/kg. The corresponding MeHg levels in the three groups were  $12.3 \pm 2.5$ ,  $5.1 \pm 4.4$ , and  $1.6 \pm 0.2$   $\mu$ g/kg, respectively.

In general, all three groups share similar characteristics of the distribution pattern of MeHg, Se, and IHg for the aboveground parts (i.e., MeHg: grain > husk > leaf  $\approx$  stem; Se: leaf > stem  $\approx$  husk  $\approx$  grain; IHg: leaf > stem  $\approx$  husk > grain) if not including the root (Figure S4). However, for “extreme sites” in which the aerial parts were compared with the root, the highest levels of MeHg were observed in the roots rather than in the grains. This observation was in contrast with the results from two other groups (Figure S4), which showed that the grains were the highest bioaccumulator of MeHg. Similarly, the highest levels of IHg in “extreme sites” were observed in the root rather than in the leaf, which was the opposite of the results observed by two groups (Figure S4-g, h, i).

A consistently negative correlation was observed between the Se levels in the soils and the TFs of MeHg (shoot/root) (Figure 2a) or the TFs of IHg (shoot/soil) (Figure 2b) in all of the aerial shoots, with the shoot representing the stem, leaf, husk, and grain ( $R^2 = 0.94, 0.75, 0.81,$  and  $0.72$ , respectively, for the TFs of MeHg, and  $0.78, 0.47, 0.58,$  and  $0.80$ , respectively, for the TFs of IHg); two “extreme value sites” were not included in this regression analysis, which however, also showed a similar behavior with other sites for TFs of IHg (but not TFs of MeHg). A similarly consistent relationship,

therefore, was also observed between the Se levels in the soils and the concentrations of IHg or MeHg in the aerial shoots ( $R^2 = 0.21–0.57$  for MeHg and  $0.15–0.69$  for IHg), although most of these relationship were not significant at <0.05 level (except for husk MeHg and grain IHg) (Figure S5-a,b).

There was a significantly positive correlation between Se and IHg in the roots ( $r^2 = 0.99, p < 0.01$ , log-transformed), which showed a pattern similar to a 1:1 (IHg:Se) molar ratio (Figure 3). Two sites (A18, A20; Figure S1) close to artisanal Hg



**Figure 3.** IHg versus Se in root samples collected from typical sites (the artisanal Hg smelting sites were not included in the regression analysis).

mining activities in the Gouxi valley showed molar ratios of Hg:Se > 1 (2 and 6, respectively). No approximately 1:1 molar ratios of IHg:Se were found in the aerial shoots.

## DISCUSSION

**Possible Mechanisms of Formation of Hg–Se Complex in the Rhizosphere/Root.** A 1:1 molar ratio of an Hg–Se complex containing macromolecules with a very high stability in the roots that were unavailable to the aerial parts of plant has been previously proposed in studies on Hg and Se coexposure in Se-accumulating plants, e.g., soybean (*Glycine max*),<sup>19</sup> Indian mustard (*B. juncea*),<sup>17,18</sup> and *A. fistulosum*,<sup>20</sup> which all had a significant reduction in the Hg uptake with an increasing Se concentration.<sup>17,20</sup> A recent study by McNear et al.<sup>45</sup> using the techniques of capillary reversed phase chromatography coupled with inductively coupled plasma mass spectrometry (capRPLC-ICPMS) together with X-ray

absorption near edge structure (XANES) and synchrotron based X-ray fluorescence spectroscopies (SXRF) has further identified the formation of a Hg–Se complex on the root surface of *A. fistulosum* supplemented with  $\text{Hg}^{2+}$  and  $\text{SeO}_3^{2-}$ .

In the present study, the positive relationship between Se concentrations and IHg/MeHg concentrations in the soils from root zones (Figure 1b), and the approximately 1:1 molar ratio of Hg:Se in the roots (Figure 3), may also indicate the formation of an HgSe insoluble complex occurred in the rhizospheres and/or roots.

In highly reducing paddy systems with prolonged flooding, the reduction of selenite to elemental Se or selenide metal complexes (or mercuric chloride/mercuric hydroxide to elemental Hg) is likely to occur, because the rhizosphere of the flooded paddy is unique from the bulk soil that provides the enhanced microbial activity, lower pH, and release of carbon rich root exudates that could facilitate the reduction of  $\text{SeO}_4^{2-}$  and  $\text{SeO}_3^{2-}$  into  $\text{Se}^0$  or  $\text{Se}^{2-}$  (or  $\text{Hg}^{2+}\text{Cl}_2$  and  $\text{Hg}^{2+}[\text{OH}]_2$  into  $\text{Hg}^0$ ).<sup>45</sup> Usually, Hg and Se within soil can undergo the reduction courses of  $\text{Hg}^0 \rightleftharpoons \text{Hg}_2^{2+} \rightleftharpoons \text{Hg}^{2+} \rightleftharpoons (\text{CH}_2)\text{Hg} \rightleftharpoons (\text{CH}_2)_2\text{Hg}$  and  $\text{SeO}_4^{2-} \rightarrow \text{SeO}_3^{2-} \rightarrow \text{Se}^0 \rightarrow \text{Se}^{2-}$ .<sup>16</sup>

As mentioned earlier, S in the cinnabar ( $\text{HgS}$ ) can be replaced by Se to form an isomorphous series of  $\text{HgS}$ – $\text{HgSe}$ .<sup>27</sup> Besides, the solubility product constant ( $K_{\text{sp}}$ ) of HgSe is about  $10^6$  lower ( $\sim 10^{-58}$ ) than that of HgS ( $\sim 10^{-52}$ ).<sup>46</sup> Therefore, it is possible that Hg could react first with Se to form an inert precipitate of HgSe under certain conditions in the paddy soil or at the root surface according to the equations of  $\text{Hg}^0 + \text{Se}^0 \rightarrow \text{HgSe}$  and/or  $\text{Hg}^{2+} + \text{Se}^{2-} \rightarrow \text{HgSe}$ .<sup>10,45</sup> If this is the case, it is not hard to speculate here that the occurrence of a precipitate of a covalent HgSe complex would restrict the amount of bioavailable  $\text{Hg}^{2+}$  in the soil, thereby stopping or at least greatly restricting the Hg methylation, which would finally cause a reduction in translocation of both  $\text{Hg}^{2+}$  and MeHg in different aerial tissues of rice plant with increasing Se in the soil. This has been confirmed by the consistent observations in the present study that Se concentrations in the soil samples were found to be significantly and negatively correlated with both TFs of IHg and TFs of MeHg in different aerial shoots (Figure 2a,b).

**Suppression Effect of Se on Uptake and Translocation of Hg in Rice.** The inverse relationship between the Se concentrations and concentrations of IHg/MeHg in the grain samples (Figure 1a) also suggest that Se may have a protective effect against the bioaccumulation of both IHg and MeHg in rice plants, although some sites still showed relatively elevated levels of IHg and MeHg with increasing Se levels in the grains due to the corresponding elevated levels in the soils. However, there appears to be a certain threshold value for Se for it to exert its antagonistic effect against Hg bioaccumulation in the rice plant. When the concentrations of Se in the rice grains were relatively low ( $<100 \mu\text{g}/\text{kg}$  or  $1.27 \mu\text{mol}/\text{kg}$ ), there was more variation in both the IHg and MeHg concentrations, and the antagonistic effect was somehow less evident. When the Se concentrations were higher than  $100 \mu\text{g}/\text{kg}$ , relatively low levels of both IHg and MeHg bioaccumulation were consistently observed in all rice grain samples and vice versa, resulting in their inverse relationships.

A very similar antagonistic effect has been observed in fish samples by Peterson et al.,<sup>47</sup> who observed that high Hg concentrations in fish tissues (collected across 12 western U.S. states) were found only when Se concentrations in the same tissue were low. In addition, Belzile et al.<sup>48</sup> reported a consistently inverse relationship between the concentrations

of IHg/MeHg and Se at all trophic levels (including fish such as perch and walleye) in freshwater lakes of Sudbury, Ontario, Canada. These results were consistent with our observation in rice plants, which demonstrated a remarkable suppression effect of Se on Hg bioaccumulation. However, it is worthy of remark that the metabolism of Se and Hg between plants and animals should be different, hence the mechanisms of the suppression effect of Se on Hg bioaccumulation between them should also be different, which needs to be scrutinized in future study. Besides, it also deserves to be noted here that Hg and Se were usually not inversely correlated (and even showed a positive correlation) in tissues and/or natural waters.<sup>8–13</sup>

Additionally, our previous risk assessment of Hg exposure for the population in this area may be overestimated as more than 70% of the rice grain samples had molar ratios of Se:Hg  $> 1$  in the present study (Figure S2), considering that Se may counteract the toxicity of Hg exposure, particularly when the Se:Hg molar ratios approach or exceed 1.<sup>45</sup> Ganther et al.<sup>7</sup> have previously proposed the Se:Hg molar ratio  $>1$  as protective against Hg toxicity, which is an important value for risk assessment rather than relying only on the level of Hg, although the actual ratio that is protective is unclear.

Interestingly, similar to the observation of inhibited bioaccumulation of both IHg and MeHg in all different aerial shoots of rice plant with increasing Se concentrations in paddy soils in the present study, Belzile et al.<sup>49</sup> have also observed a reduced bioassimilation of both THg and MeHg by aquatic biota throughout the whole food web with increasing Se concentrations in freshwater lakes of Sudbury, Ontario, Canada. These findings are of great importance as they may shed light on the possible use of Se as a remediation agent against Hg contamination in natural paddy/lake systems. However, the very narrow margin between Se's essentiality, deficiency, and toxicity amount to humans and animals<sup>5</sup> should be always kept in mind before Se is used as a remediation agent to mitigate Hg toxicity.

The root may act as an effective barrier or a buffer for the suppression of Hg absorption and translocation to the aboveground shoots.<sup>17–20</sup> Therefore, it was expected that the TFs of Hg from soil to root would show a reduction with increasing Se in the soil. However, the Se levels in the soils were positively correlated with the TFs of MeHg (root/soil) ( $R^2 = 0.66$ ,  $P < 0.01$ ) but negatively correlated with the TFs of IHg (root/soil) ( $R^2 = 0.77$ ,  $P < 0.01$ ) (Figure 2a,b); similar results were also observed between the Se levels in the soils and the MeHg (or IHg) levels in roots (Figure S5-a,b). These contrary results implied that increasing Se in the soil could inhibit the absorption of IHg in the root, but not that of MeHg.

The TFs of MeHg (root/soil) ( $4.2 \pm 2.5$ ) were observed to be over  $10\times$  greater than the TFs of IHg (root/soil) ( $0.15 \pm 0.17$ ) and TFs of Se (root/soil) ( $0.30 \pm 0.18$ ) (Figure S3), which may be related to the ability of phytochelatin, small peptides that detoxify heavy metals in rice roots, to sequester IHg (most likely also Se) but not MeHg.<sup>50</sup> In other words, the suppression effect of Se on IHg uptake and translocation to the aerial parts of the rice plant may be linked to certain processes occurring in both the soils and roots but may be mainly linked to the roots for MeHg.

The higher molar ratios of Hg:Se in roots from Hg artisanal smelting sites (6:1 and 2:1) than other sites (approximately 1:1) (Figure 3) may be related to the Hg artisanal smelting activities, suggesting that newly formed Hg in soil in Hg artisanal smelting sites could be more readily absorbed and

translocated in roots than Hg with extended residence time in soils in other sites.<sup>30,51</sup> Besides, it should be noted that IHg in rice plant can be originated from air, in addition to soil, especially for areas where atmospheric Hg is elevated.<sup>24,52</sup> Although it cannot be excluded that methylation may occur inside the plant,<sup>53</sup> literature as well as our recently published data<sup>51</sup> suggest that MeHg in rice plant should mainly be absorbed and translocated from the soil. It is proven that reducing bacteria (SRB) actively occur in rice paddy soil,<sup>54</sup> which is known to be the main contributor of Hg methylation in the environment.<sup>2</sup>

In contrast with our expectations, Se levels in different rice tissues were positively correlated with IHg levels (or MeHg levels) in the soils ( $r^2 = 0.72-0.83$  for IHg and  $0.48-0.64$  for MeHg,  $p < 0.01$  for all) (Figure S6-a,b) but no significant relationship was observed between TFs of Se in different rice tissues and IHg levels (or MeHg levels) in the soils (Figure S6-c,d). It appears that the absorption and translocation of Se in rice plant may not be significantly affected by the Hg levels in the soils, which was different from the suppressed effect of Se on Hg in the rice plant. This contrast may be related with the higher absorption and translocation capacity of Se in rice plant than IHg, as more than 70% of the grains samples in the study area had molar ratios of Se:Hg  $> 1$  which were much higher than that in the corresponding soils (only approximately 20%) (Figure S2).

As discussed earlier, the formation of covalent Hg–Se insoluble bonds occurring in the rhizospheres and/or roots would limit the amount of bioavailable IHg and MeHg in the soils of root zones. In addition, the increase of Se in the soils could cause a reduction in translocation of both IHg and MeHg from soils to grains, but simultaneously also cause a relatively increased Se in the grains (Figure S7-b), finally causing a disproportionate uptake and translocation of IHg/MeHg versus Se in grains. This process may be the most plausible mechanism to explain why the Se levels were inversely correlated with both IHg and MeHg in grains, as previously mentioned.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Additional text and figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Phone: 86-851-5891356; fax: 86-851-5891609; e-mail: [fengxinbin@vip.skleg.cn](mailto:fengxinbin@vip.skleg.cn).

### Notes

The authors declare no competing financial interest.

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