



# Impacts of selenium supplementation on soil mercury speciation, and inorganic mercury and methylmercury uptake in rice (*Oryza sativa* L.)

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## ABSTRACT

Rice grain is known to accumulate methylmercury (MeHg) and has been confirmed to be the major pathway of MeHg exposure to residents in mercury (Hg) mining areas in China. Selenium (Se) supplementation has been proven to be effective in mitigating the toxicity of Hg. To understand how Se supplementation influences soil Hg speciation, a wide range of Se (0–500 mg/kg) was applied to Hg polluted paddy soils in this study, which decreased MeHg concentration in soil from  $2.95 \pm 0.36$  to  $0.69 \pm 0.16$   $\mu\text{g}/\text{kg}$  (or 77%). After Se addition, humic acid state Hg (F4) was transformed into strong-complexed state Hg (F5), indicating that Hg bound up to the non-sulfur functional groups of humic acid (non-RSH) was released and reabsorbed by strong binding Se functional group (F5). As a result, inorganic Hg (IHg) was reduced by >48%, 18%, and 80% in root, stem, and grain, respectively, however, the reduction was not apparent in leaf. Substantial reductions were also found for MeHg in grain and root, but not in stem and leaf. Soil is suggested to be the main source of both MeHg and IHg in rice grain. Such a finding may provide an idea for improving Hg-polluted paddies through controlling soil IHg and MeHg. Further research on the molecular structure of the strong-complexed Hg in F5 should be conducted to elucidate the mechanism of Hg-Se antagonism.

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## 1. Introduction

Methylmercury (MeHg) is known to be the most toxic Hg compound form because it can bioaccumulate and biomagnify through the food chain and pose health risks to humans (Ha et al., 2017; Hong et al., 2012; Obrist et al., 2018). As the Minamata Convention on Mercury went into effect on 16th August 2017, increased attention and efforts will be directed toward the control of Hg in different environmental media (Lin et al., 2017).

Rice (*Oryza sativa* L.) cultivated in mercury (Hg) contaminated soil has been extensively reported to accumulate MeHg (Horvat et al., 2003; Qiu et al., 2008; Qiu et al., 2006), and the consumption of contaminated rice has been confirmed to be the dominant

pathway of MeHg exposure for inhabitants living in mercury mining areas (Feng et al., 2008; Zhang et al., 2010a). Mitigating Hg levels in rice is needed in order to protect rice-consuming populations from Hg exposure, which is the one of the aims of the Minamata Convention on Mercury (UNEP, 2017).

Selenium (Se) is an essential nutrient for both humans and animals (Rayman, 2000), and it can protect organisms from Hg toxicity (Parížek and Ostádalová, 1967). The phenomenon of Hg-Se antagonism widely exists in a variety of organisms and environments (Paulsson and Lundbergh, 1991; Ralston et al., 2008; Turner and Swick, 1983). Studies conducted on soil-rice systems indicated that Se addition to soil can effectively reduce inorganic Hg (IHg) and MeHg in rice (Li et al., 2014; Wang et al., 2014; Zhou et al., 2014). Se addition can enhance the development of iron plaque and apoplastic barriers in the endodermis, which hinder both IHg and MeHg uptake (Li et al., 2014; Wang et al., 2014; Zhou et al., 2014). Both MeHg-Se and IHg-Se antagonism have been found to occur in soil, and the formation of nontoxic Hg-Se complexes and

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nanoparticles have been found to reduce the Hg bioavailability to microbial methylators, thus restricting MeHg production in soil (Tang et al., 2017; Wang et al., 2016a; Wang et al. 2016b; Wang et al. 2016c; Wang et al. 2016d). Meanwhile, the Hg-Se complexes could restrict the uptake of Hg into plant roots and shoots (Zhang et al., 2012). Meanwhile, the large molecular weights of IHg-Se and MeHg-Se are difficult to be translocated and metabolized (Li et al., 2015; Wang et al., 2014; Zhao et al., 2014). These studies unanimously suggested that soil plays a dominant role in Hg-Se antagonism in soil-rice systems.

Previous studies revealed the Hg-Se mechanism for IHg and MeHg in rice plant, however, how Se influence soil Hg is poorly understood. Hg in soil exists in different chemical forms, which can be bound to a variety of matrices (Reis et al., 2010; Yu et al., 2012). Chemical and biological reactions can change Hg speciation and binding to different chemical species in soil, which in turn change solubility, toxicity and bioavailability of Hg-bound chemicals (Biester et al., 2002; Reis et al., 2010; Xu et al., 2017). Owing to the different Hg bioavailabilities that result from different soil Hg speciation, no correlation was found between IHg and MeHg concentrations within plants and soil THg (Rothenberg and Feng, 2012; Zhang et al., 2012). Hg speciation in soil are generally in equilibrium status (Benoit et al., 1999), however, such an equilibrium will be disturbed after Se addition and formation of HgSe. Therefore, understanding the mechanism by which Se addition influences the soil Hg speciation, which is likely a key factor in preventing the transport of Hg into rice plants, is needed for developing Hg mitigation methods in crops. Though previous study revealed organo-chelated Hg was the dominant source for bioavailable Hg in paddy soil (Yan et al., 2015), however, the mechanisms that how Se supplementation influence the change of soil Hg speciation and bioavailable Hg are still not clear. Thus, we firstly revealed the fulvic acid state Hg and humic acid state Hg in Se amended soil to identify the role of them in Hg-Se antagonism in paddy soil. The present study aims to explore the influencing mechanisms of Se treatment on the change of Hg speciation in paddy soil and the uptake and translocation of IHg and MeHg in rice.

## 2. Material and methods

### 2.1. Experimental design

Pot experiments were conducted in an abandoned artisanal Hg-retorting site in Gouxu Village in the Wanshan Hg mining region of Guizhou province in southwestern China. Compared with other Hg-mining sites in this area, Gouxu is a typical site that is primarily impacted by atmospheric Hg released nearby during artisanal Hg retorting nearby (Zhao et al., 2016). Rice plants were grown in plastic containers (5 L) filled with 5 kg of soil from a Hg contaminated paddy in Gouxu. Before being added into the containers, the collected soil was air-dried and sieved through an 80-mesh screen (size: 177  $\mu\text{m}$ ). The characteristics of the soil are presented in Table 1.

Various amounts of Se ( $\text{Na}_2\text{SeO}_3$ ) were diluted into distilled deionized water (DDW, 18.2  $\Omega\text{cm}$  water, Milli-Q, Millipore, USA). These solutions were then mixed into the soil one month before rice growing (Zhao et al., 2014). Se concentrations in soils from Enshi, a region of significant geogenic Se enrichment in China, can

reach up to 943 mg/g (Qin et al., 2012). The levels of spiked Hg could be 390 mg/kg in Hg contaminated soil (Zhang et al., 2008). Also, previous studies conducted Se supplementation from 0.5 to 50 mg/kg, nearly all studies have their own effective ranges (Tang et al., 2017; Wang et al., 2016a; Zhang et al., 2017; Zhao et al., 2014; Zhou et al., 2014). Therefore, under consideration of Se bioavailability, the highest Se treatment level for the current work was set at 500 mg/kg (500Se), which was less than the highest Se level in geogenic Se enrichment region (Qin et al., 2012), with addition levels of 20 mg/kg (20Se), 40 mg/kg (40Se), 60 mg/kg (60Se), 100 mg/kg (100Se), and 300 mg/kg (300Se). Control soil without Se treatment (0Se) was also applied. It should be noted that Se supplementation levels in this study could result in toxic levels of Se in grain (Rani et al., 2005), all the soils and rice plants were collected for experiment.

Experiments were maintained in the field for the entire growing season of rice. After harvesting, rice samples were rinsed with deionized water, and then rice plants were separated into root, stem, leaf, and grain with stainless steel scissors. Additionally, rhizosphere soils were also collected simultaneously.

All the rice tissues were weighed and freeze-dried at  $-80^\circ\text{C}$  (EYELA FDU-1100, Tokyo Rikakikai Co. Ltd, Japan). The different rice plant samples were ground to a 200 mesh size (IKA-A11 basic, IKA, Germany). The grinder was thoroughly cleaned before and after each sample with ethanol to avoid cross-contamination. All the parts of the rice plants were then stored at  $4^\circ\text{C}$  for further analysis. Soil samples were freeze-dried, ground in an agate mortar, and then passed through 200 mesh sieves (Xu et al., 2017).

### 2.2. Analysis

For soil THg, approximately 0.1 g dry sample was digested with freshly prepared *Aqua regia* ( $\text{HCl}:\text{HNO}_3 = 3:1, \text{v/v}$ ) in a water bath at  $95^\circ\text{C}$ . An aliquot of the digested solution was measured using cold vapor atomic fluorescence spectroscopy (CVAFS) (USEPA, 2002). For soil MeHg, approximately 0.2 g dry sample was extracted with methylene chloride ( $\text{CH}_2\text{Cl}_2$ ) after leaching with  $\text{HNO}_3$  (Liang et al., 1994; Liang et al., 1996). An aliquot of the extracted solution was measured by gas chromatography-cold vapor atomic fluorescence spectroscopy (GC-CVAFS) following method 1630 (USEPA, 2001). The limits of detection were 0.01  $\mu\text{g/kg}$  for THg and 0.002  $\mu\text{g/kg}$  for MeHg.

For rice THg, approximately 0.5 g dry sample was digested using a mixture of  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  (4:1, v/v) for 2 h in a water bath at  $95^\circ\text{C}$ , followed by CVAFS detection. For rice MeHg, approximately 0.1–0.3 g dry sample was digested using 25% KOH in methanol for 3 h in an oven at  $75\text{--}80^\circ\text{C}$ . MeHg in the samples was extracted with  $\text{CH}_2\text{Cl}_2$  and back-extracted into water, and then measured following method 1630 (Liang et al., 1994; USEPA, 2001). IHg and MeHg in rice samples were discussed below with IHg obtained from THg subtracting MeHg (Xu et al., 2017).

The Hg speciation in the soil was obtained mainly according to a Hg sequential extraction procedure (Bloom et al., 2003; Shoham-Frider et al., 2007) with slight modifications, for which the organic-chelated state was divided into the fulvic (F3) and humic (F4) states based on the sequential fractionation of organic matter (Preston et al., 1994) (shown in Table 2).

**Table 1**  
Characteristics of the experimental soil.

| Soil collection site | pH        | Total carbon (g/kg) | Total nitrogen (g/kg) | Organic matter (g/kg) | Total Se (mg/kg) | IHg ( $\mu\text{g/kg}$ ) | MeHg ( $\mu\text{g/kg}$ ) |
|----------------------|-----------|---------------------|-----------------------|-----------------------|------------------|--------------------------|---------------------------|
|                      | 6.95–7.26 | 28 $\pm$ 0.25       | 3.2 $\pm$ 0.04        | 3.88–4.32             | 0.17–0.37        | 3405 $\pm$ 332           | 1.1–2.4                   |

**Table 2**  
Modified scheme of Hg sequential extraction procedure.

| Fraction | Fraction definition                | Extraction reagent (s)                 | Chemical extraction process  |
|----------|------------------------------------|--|--|
| F1       | Water soluble                      | DDW                                    | 1 g of soil was mixed with 50 ml DDW in a 50 ml centrifuge tube and was shaken for 24 h in an end-over-end shaker at 120 rpm at room temperature. The separation of extract from solid residue was conducted by centrifugation at 3500 rpm for 20 min. The extracts were filtered and 1 ml of 0.2M BrCl was added. Then re-add 25 ml DDW into the centrifuge tube containing the solid residue, re-shake and re-centrifuged and filtered. Finally the combined extracts were diluted into 100 ml in borosilicate glass bottles for analysis. |
| F2       | Human stomach acid soluble         | 0.1 M CH <sub>3</sub> COOH +0.01 M HCl | The residue from step 1 was extracted with 50 ml 0.1 M CH <sub>3</sub> COOH +0.01 M HCl (pH = 2), shaken for 24 h, and then centrifuged at 3500 rpm for 20 min. Same filtering process was conducted and same amount of BrCl was added as step 1. For the rinse step, 25 ml 0.1 M CH <sub>3</sub> COOH +0.01 M HCl was added into the residue, re-shaken, re-centrifuged and filtered. The final extracts were also diluted into 100 ml in borosilicate glass bottle.  |
| F3       | Fulvic acid state                  | 1 M KOH +6 M HCl                       | The residue from step 2 was extracted with 50 ml 1 M KOH, shaken for 24 h, and then centrifuged, filtered, rinsed as those before, and solid residue B was left. Then combined extract were acidified to pH 1 with 6 M HCl, allowed to fully react to precipitation, and then centrifuged as before. The final supernatants were collected and diluted into 100 ml in borosilicate glass bottle, with solid residue C left.  |
| F4       | Humic acid state                   | 1 M KOH                                | Solid residue C from step 3 was re-alkalized to pH 8 with 1 M KOH. In this way the dissolved humic acid was obtained.  |
| F5       | Strong-complexed state             | 12 M HNO <sub>3</sub>                  | Solid residue B from step 3 was re-filled with 25 ml 12 M HNO <sub>3</sub> . The detailed processes were same as before, but without filtering since the acid can solve the filter membranes.  |
| F6       | Mercuric sulfide                   | <i>Aqua regia</i>                      | Solid residue from step 5 was extracted with 25 ml <i>aqua regia</i> , and then same processes as step 5 were conducted.   |
| F7       | Hydrofluoric acid extraction state | HCl + HF                               | Solid residue from step 6 was extracted with 25 ml, then same processes were conducted. But before dilution, the supernatants were alkalinized to pH 7.  |

### 2.3. QA/QC

Both the THg and MeHg analyses were validated using duplicates, method blanks, matrix spikes, and certified reference materials (CRM). The limits of determination were 0.012 µg/kg for THg and 0.003 µg/kg for MeHg.

For soil THg, GBW07405 was employed, and the detected mean concentration of THg was 0.30 ± 0.01 mg/kg (n = 5), which is comparable to the certified value of 0.29 ± 0.04 mg/kg. For rice plants, GBW10020 (Citrus leaves) was used, and the determined value of THg was 148 ± 7 µg/kg, which is comparable to the reference value of 150 ± 20 µg/kg. The relative standard deviations of the duplicates were in the range of 1.6–7.8% for the soil and 2.2–19% for the rice. Recoveries of the matrix spikes in rice were 90–110% for rice.

For Hg speciation in rhizosphere soils, the recoveries were 93.6–102.2% for the total soil Hg speciation (F1+F2+F3+F4+F5+F6+F7).

For MeHg, ERM-CC580 (estuarine sediment) and TORT-2 (Lobster, *Hepatopancreas*) were used for both the soil and rice plants. The obtained values were 76 ± 6 µg/kg (n = 5) for ERM-CC580 and 151 ± 7.1 µg/kg (n = 5) for TORT-2, both of which are comparable to the certified values of 75 ± 4 µg/kg for ERM-CC580 and 152 ± 13 µg/kg for TORT-2. The relative standard deviations in duplicates were less than 9% for MeHg in soil. The recoveries of MeHg in the matrix spikes were between 90% and 120%. The relative standard deviations of the duplicates in the rice sample were less than 15%.

### 2.4. Calculations and statistics

Statistical analyses were performed using the one-way ANOVA with SPSS 22 (Standard, California, USA). All figures were plotted using Origin 9 (©OriginLab Corporation). Significant differences at the 1% level are indicated by capital letters; and significant differences at the 5% level are indicated by lower case letters.

## 3. Results

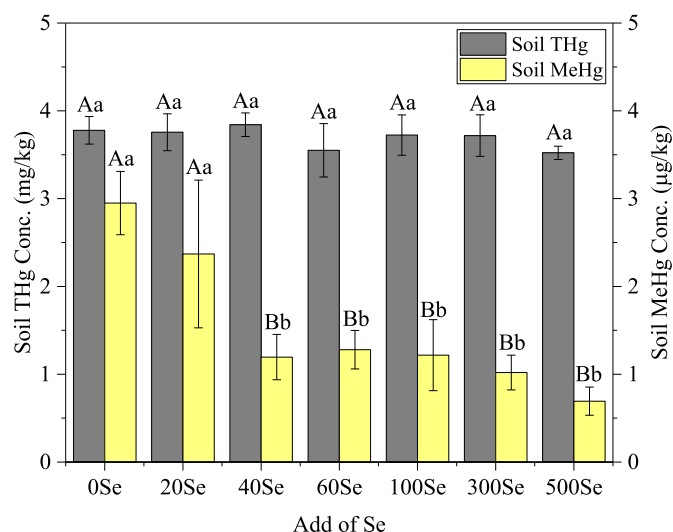
### 3.1. Rhizosphere soil Hg and MeHg

For soil THg, there were no significant differences between the treated groups and control group, and the concentrations of THg in

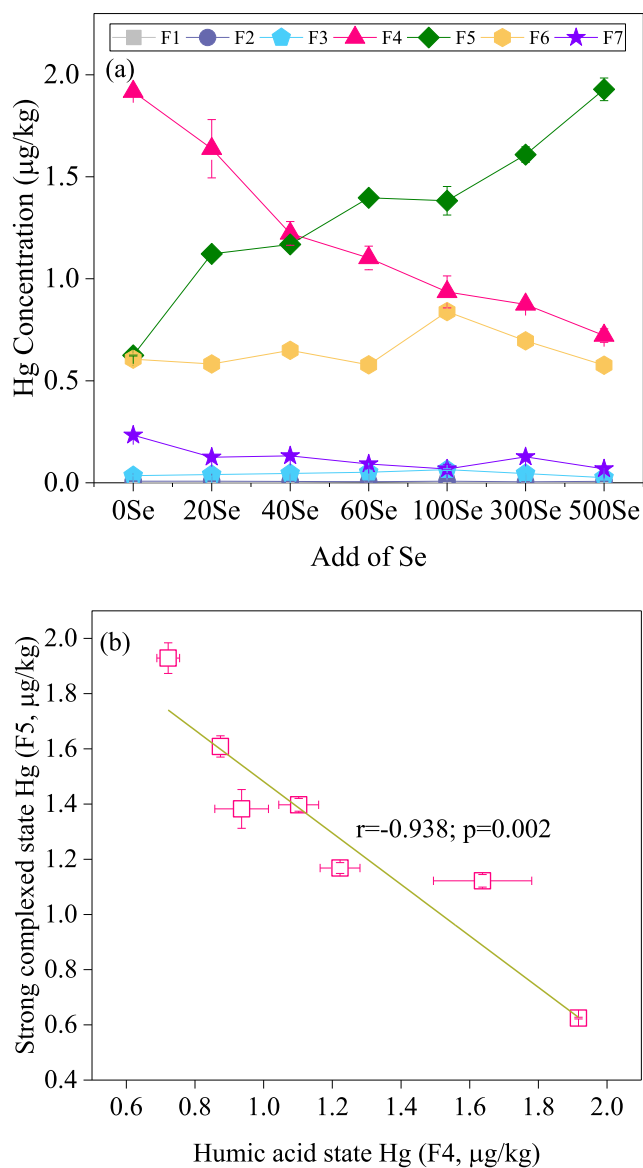
all groups were in the range of 3.5–3.8 mg/kg (Fig. 1). For soil MeHg, all the Se treated groups except the 20Se had remarkably reduced MeHg concentrations in comparison with that of the control group (2.95 ± 0.36 µg/kg). In the Se treatments above 40Se, the reduction rates of soil MeHg were in the range of 57%–77%. Although the lowest soil MeHg of 0.69 ± 0.16 µg/kg was obtained at 500 Se, no significant differences were observed among these groups in the Se treatment above 40Se.

### 3.2. Hg speciation in rhizosphere soil

In all the treatments, the humic acid Hg (F4), strong-complexed state Hg (F5: includes elemental Hg and Hg bound up in amorphous organio-sulfur, Hg-Ag amalgamates, and crystalline Fe/Mn oxides), and mercuric sulfide state Hg (F6) accounted for the majority of the soil Hg speciation, with their total percentage added up to >90% (Fig. 2). Without the Se supplementation, Hg speciation had the following order of F4 (1.9 ± 0.0021 mg/kg, 56%) > F5 (0.62 ± 0.0033 mg/kg; 18%) > F6 (0.61 ± 0.026 mg/kg; 18%) > F7



**Fig. 1.** Effect of Se treatment on Hg and MeHg in soil.



**Fig. 2.** (a) The distribution characteristics of different Hg fractions under different Se treatments; and (b) correlations between humic acid Hg (F4) and strong-complexed state Hg (F5).

( $0.23 \pm 0.0002$  mg/kg; 6.8%) > F3 ( $0.0035 \pm 0.0001$  mg/kg; 1%) > F2 ( $0.0008 \pm 0.0001$  mg/kg; 0.23%) > F1 ( $0.0002 \pm 0.00002$  mg/kg; 0.06%).

As Se supplementation increased from 0Se to 500Se, F4 showed a downward trend from 56% in the control group (0Se) to 22% in the 500Se group. In contrast, F5 showed an upward trend from 18% at 0Se to 58% at 500Se. A significantly negative correlation ( $r = -0.938$ ;  $p = 0.002$ ) was observed between F4 and F5. The THg between the different treatment groups showed no significant differences.

### 3.3. IHg and MeHg in grain, root, stem, and leaf

The effects of soil amendment with variable rates of Se on the IHg concentrations in grain, root, stem, and leaf of rice plants are shown in Fig. 3. For grain IHg, the lowest value of  $37 \pm 13$  µg/kg was obtained at 40Se, which is a reduction of 80% compared with that of control group. For root and stem IHg, although no obvious trends

were observed, all the treatment groups had notably decreased IHg levels ( $p < 0.05$ ), with the exception of 20Se, compared with those of the control group. The lowest root IHg at the level of  $345 \pm 14$  µg/kg from the case of 100Se was >48% less than that of the control group. The lowest stem IHg at the level of  $1026 \pm 15$  µg/kg from the case of 300Se was approximately 18% less than that of the control group. For leaf IHg, no obvious trend was observed, and the lowest value of  $10,933 \pm 450$  µg/kg from the case of 20Se was not significantly lower than that of the control group, whereas the highest value of  $15,930 \pm 57$  µg/kg from the case of 300Se was significantly higher ( $p < 0.01$ ) than that of the control group.

Fig. 3 also shows the results of Se effect on MeHg. All the Se treatment groups had remarkably reduced MeHg levels ( $p < 0.01$ ) than that of the control group in grain and root, but such a trend was not found in stem or leaf. The maximum reduction rates of MeHg were 71% and 53% in the grain and root, respectively. The lowest MeHg values were  $6.9 \pm 0.61$  µg/kg,  $12 \pm 0.1$  µg/kg,  $11 \pm 0.02$  µg/kg, and  $14 \pm 0.16$  µg/kg in the grain, root, stem, and leaf, respectively.

### 3.4. Correlations between humic acid state Hg (F4), strong complexed state Hg (F5) and soil, root, and grain MeHg

Fig. 4 shows that F4 is positively correlated with soil MeHg ( $r = 0.975$ ,  $p = 0$ ), root MeHg ( $r = 0.941$ ,  $p = 0.002$ ), and grain MeHg ( $r = 0.972$ ,  $p = 0$ ). This finding suggests that with the increasing amounts of Se addition, MeHg in the soil, root, and grain decreased along with humic acid state Hg. Nevertheless, F5 has a strongly negative correlation with soil MeHg ( $r = 0.9$ ,  $p = 0.006$ ), root MeHg ( $r = 0.87$ ,  $p = 0.011$ ), and grain MeHg ( $r = 0.866$ ,  $p = 0.012$ ), suggesting that with increasing amounts of Se addition, MeHg in the soil, root, and grain decreased with the increasing F5.

## 4. Discussion

### 4.1. Possible mechanism of Se additions on Hg speciation and Hg bioavailability in rhizosphere soil

The downward trend in soil MeHg levels suggests that Se supplementation can reduce Hg methylation in soil. Previous studies have indicated that soil is the action point of Hg-Se antagonism (Rothenberg et al., 2014; Tang et al., 2017; Wang et al., 2016b; Wang et al., 2018; Yin et al., 2013). In literature, only HgSe has been used to understand the mechanism of Hg-Se antagonism in the soil of rice plants. It was proposed that the formation of HgSe in the soil resulted in less bioavailable IHg that can be used by the Hg-methylating microbes (such as sulfate reducing bacteria) in soil (Truong et al., 2014; Yang et al., 2008), which in turn reduced Hg methylation and thus MeHg levels in soils (Wang et al., 2014; Wang et al., 2016a).

In this study, approximately 57% of the total Hg comprises the fulvic acid state Hg (F3) and humic acid state Hg (F4), which are the dominant Hg speciation in soil, similar to the results obtained at Northern Haifa Bay (Ramasamy et al., 2012; Shoham-Frider et al., 2007). High amounts of organic matter can also cause the high ratio of organics-bound Hg speciation (Wu et al., 2016). In this study, the high levels of humic acid state Hg (55.96%) and low levels of fulvic state Hg (1.02%) may be due to the higher transport ability of fulvic acid than humic acid, resulting in low fulvic acid levels and high humic acid levels in the soil (Yao et al., 2000).

In a flooded rice paddy, long-term periods of overlying water cause low pH values and anoxic conditions (Begg et al., 1994; Rothenberg and Feng, 2012). After being added to this anoxic conditions,  $\text{SeO}_3^{2-}$  will be reduced to  $\text{HSe}^-$  and  $\text{Se}^{2-}$  under reduced flood condition (Khan and Wang, 2009; Mayland and Wilkinson,





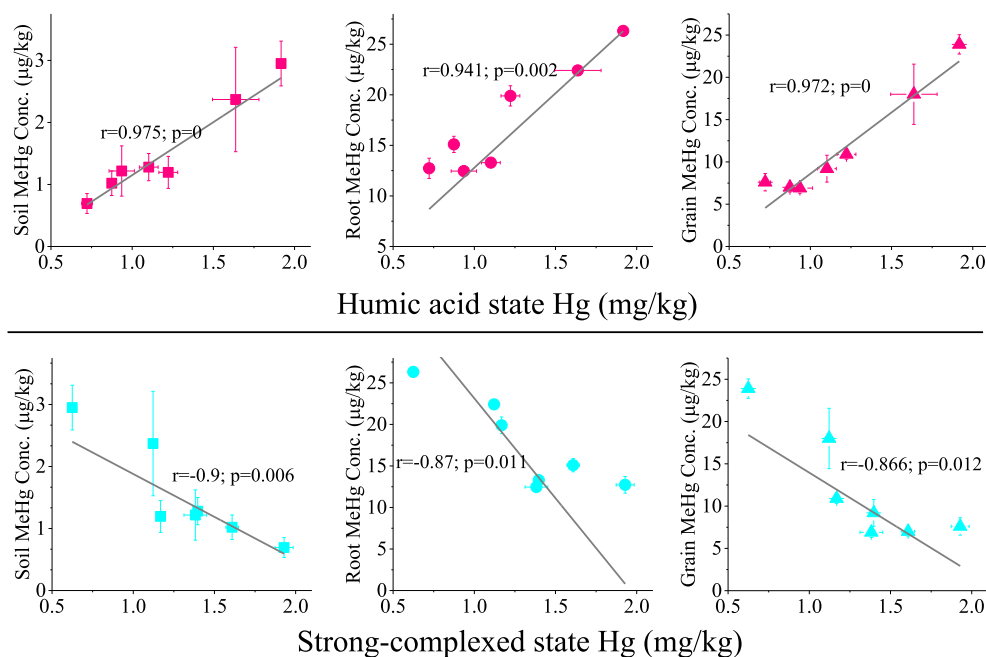


Fig. 4. Correlations between humic acid state Hg (F4) or strong-complexed state Hg (F5) and MeHg in soil, root, or grain.

MeHgSeR, and MeHgSeSR (Khan and Wang, 2009, 2010). In our study, after the Se supplementation to soil, HgSeR, RSHgSeR, MeHgSeR, and MeHgSeSR may play a dominant role in soil Hg levels.

The formation of the above compounds can limit the availability of Hg to microbes (Truong et al., 2014), which will decrease Hg methylation in soil. Because MeHg in the rice grain originates from soil (Rothenberg et al., 2014; Zhang et al., 2010b), MeHg in the grain decreased with the Se supplementation.

Furthermore, the supplementation of Se can activate Mn and Fe in soil, contributing to the development of iron plaque in root (Li et al., 2015; Zhou et al., 2014). Se and Hg compete for Hg-binding sites, protecting plants from the high toxicity of Hg (Feng et al., 2013). Additionally, Se can restrict the uptake of Hg into root cells through the symplastic pathway by decreasing the activity of membrane proteins (Wang et al., 2014). Se addition can increase the formation of apoplastic barriers in the endodermis (Meyer et al., 2009), which controls the Hg uptake through the apoplastic pathway (Wang et al., 2014). Since Hg absorption by the root is an active process (Esteban et al., 2008), Se substantially reduces the uptake and translocation of IHg in rice.

#### 4.2. IHg and MeHg in grain primarily originated from soil

In this study, grain IHg showed a downward trend with the increasing Se supplementation. The highest percentage decrease could reach to 80% compared with the IHg levels of the control group, indicating that grain IHg was influenced by Se in soil. Additionally, another plausible explanation for IHg levels is that the root and leaf reflect soil and atmospheric source, respectively, however, grain IHg shows a similar trend to that of root IHg, which is quite different from that of leaf IHg (Fig. 3). Foliar Se application was conducted, but no significant decrease in grain IHg occurred compared with that of soil Se application (Tang et al., 2017), suggesting that IHg-Se antagonism mainly occurs in the soil. At different growing stages, no significant differences in grain IHg, although significant difference of Se in the plant tissues, can be detected with the increasing foliar Se application (Zhang et al.,

2017). Besides, Se supplementation can contribute to the formation of iron plaque on the root surface, which will hinder Hg uptake from the soil and decrease Hg in the grain (Li et al., 2016; Zhou et al., 2014). The results of X-ray absorption near-edge structure indicated that the formation of HgSe in the soil, after observing reactions between soil IHg and Se, is the prevailing mechanism for Hg-Se antagonism mechanism in a rice paddy system (Wang et al., 2016a). Stable Hg isotope techniques showed that approximately 20% of Hg in grains was from the atmosphere, which also suggests that soil contributes the majority of the grain Hg (Feng et al., 2016; Yin et al., 2013). Therefore, we speculate that at least 80% of grain IHg originates from the soil, suggesting that the soil is the dominant IHg source for the rice grain.

In the present study, with the Se application to soil, the largest reduction in the grain MeHg was 71%, implying that the soil is the key factor influencing grain MeHg. This finding is similar to the results of Wang et al. (2016b). Grain MeHg showed the same trend as root MeHg, indicating that soil is the MeHg source for rice grain (Zhang et al., 2010b). From Figs. 1 and 3, downward trends of soil, root, and grain MeHg levels were seen with increasing Se application to the soil, suggesting soil is the location of MeHg-Se antagonism. Since grain MeHg originated from the soil MeHg, grain MeHg was notably reduced under soil rather than foliar Se application, indicating that no Hg methylation occurred within the rice plants (Wang et al., 2016b). Hg-Se nanoparticles (Hg/Se = 1) have been found in soil, which also confirmed that MeHg-Se antagonism occurs in soil (Wang et al., 2016b). It is noted that after the effects of soil Se supplementation (71% decrease), approximately 29% of the MeHg can be transferred into the rice grain, suggesting that further attention should be paid to MeHg transportation within rice plants to elucidate the pathway by which MeHg enters the grain after the occurrence of the above-mentioned synergistic effects.

Decreased IHg and MeHg levels in the stem were observed, implying that IHg and MeHg primarily originate from soil. During the milk stage, the translocation of IHg and MeHg from leaf and stem to grain may weaken the Se effects on IHg and MeHg in both the leaf and stem. Because IHg in leaf is primarily from the atmosphere (Fay and Gustin, 2007; Frescholtz et al., 2003), no obvious

decrease in leaf IHg was observed with the addition of Se.

## 5. Implication and perspectives

The present study identified that humic acid state Hg (F4) in soils with Se supplementation was converted into strong-complexed state Hg (F5), and thus reduced the bioavailability of IHg and MeHg in soil. The effects of Hg-Se antagonism increased rapidly with increasing Se levels until 40 ppm, after which such effects became much weaker. The formation of strongly complexed state Hg was suspected to be the most likely driving force of Hg speciation in soil, which acts as an Hg pool for rice plants. Further studies on molecular structures of strongly complexed state Hg in F5 should be conducted to elucidate the mechanism of Hg-Se antagonism.

Soil was proven to be the dominant source of IHg in rice grains, shedding the light on Hg remediation in soil, since MeHg in rice grain has been proven to primarily originate from soil. Further work on the remediation of rice IHg and MeHg should focus on *in situ* soil remediation. Before proposing large-scale application of Se amendments in Hg-polluted paddies, the following outstanding issues should be investigated, such as Hg speciation equilibrium after Se application, the most suitable soil type for Se application, and the effective duration of the effects after Se application.

## Declaration of interests

The authors declare that there are no competing financial interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2019.03.095>.

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