

The concentration of selenium matters: a field study on mercury accumulation in rice by selenite treatment in qingzhen, Guizhou, China

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Abstract

Aims The consumption of rice grain produced in mercury (Hg) contaminated soil was identified as a major route of dietary Hg exposure. The aims were 1) to determine the most suitable concentration of Se that can lead to least Hg accumulation in rice grain in real Hg contaminated paddy field in Qingzhen, and 2) to elucidate the possible mechanism of the protection against the phytotoxicity of Hg in rice by Se.

Methods Rice plants were treated with different concentrations (0, 0.01, 0.1, 0.5, 1 and 5 $\mu\text{g}/\text{mL}$) of sodium selenite in a real paddy field in Qingzhen, Guizhou,

China. The concentrations of Hg and Se in soil, stream water, rice tissues, and the seed setting rate (SSR) and thousand seed weight (TSW) were checked. The distribution and chemical forms of Hg and Se in rice root were studied by XRF and XAS.

Results Treating the rice plants with 0.5 $\mu\text{g}/\text{mL}$ of sodium selenite achieved the lowest Hg accumulation in rice grain while the highest SSR and TSW. In rice root, XRF found decreased Hg uptake, and XAS found Hg-Se complexes were formed. These findings, together with the formation of biological barriers like iron plaque, could explain the decreased accumulation of Hg in rice grain at Se levels below 0.5 $\mu\text{g}/\text{mL}$. Se concentrations over 0.5 $\mu\text{g}/\text{mL}$ led to increased Hg accumulation and decreased SSR and TSW, which were ascribed to the significantly increased Se accumulation in rice grain.

Conclusions This field study suggest that treatment with appropriate level of Se (0.5 $\mu\text{g}/\text{mL}$ in this study) is an efficient way to reduce Hg accumulation in rice and increase rice yield and quality, thereafter to protect the health of the rice-dependent populations in Hg-contaminated area.

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Introduction

Mercury (Hg) is linked to a number of human health diseases and brings toxic effects to kidneys, livers,

lungs, especially the central nervous systems (Guallar et al. 2002). Due to its high toxicity, persistence in the environment, and bioaccumulation in the food chain, Hg and its compounds are listed as a priority control pollutant by many international organizations (Clarkson and Magos 2006). For the general population, the intake of marine food is the major way of Hg exposure. In recent years, it is found that the consumption of crops, especially the rice grown in Hg-contaminated soil is another way of Hg exposure (Feng et al. 2008; Horvat et al. 2003).

The farmland in Qingzhen, Guizhou of Southwestern China is heavily contaminated by Hg due to the irrigator's using Hg-containing stream water discharged by an organic chemical factory (Matsuyama et al. 2004; Matsuyama et al. 2009; Matsuyama et al. 2005; Qu 1999; Yasuda et al. 2004). The factory applied a similar production process to that of Chisso Corporation, Japan, in which Hg was used as a catalyst to produce acetic acid and finally caused the Minamata Disease. The concentration of Hg in the soil was found to be 0.56 to 390 mg/kg with an average of 68.3 ± 90.8 mg/kg (Zhang et al. 2008), which is far beyond the Chinese national limit for paddy soils (1.5 mg/kg) (Environmental Quality Standard for Soils (GB15618-1995) 1995). It is estimated that about 260 ha of local farmland in Qingzhen was contaminated (Qu 1999; Wang et al. 1990; Xiong 1985; Zhang et al. 2008). Studies found that the methylmercury (MHg) level in rice reached 19.6 ng/g (Wang et al. 1990) and the total Hg level was as high as 33.5 ng/g (Horvat et al. 2003), which is higher than the Chinese national limit for Hg in rice (20 ng/g) (Chinese Ministry of Health 2005). The MHg level in many hair and blood samples from local residents was over 500 ng/g and 50 ng/g, respectively (Wang et al. 1990). Animal study found the Hg contaminated rice could significantly induce the expression of *c-jun* mRNA and its protein in brains of rats (Cheng et al. 2006). All these results suggest that public health risks need be seriously considered in local populations and the remediation of the farmland is urgently necessary.

Several techniques, including soil washing (Lin 2009), low-temperature thermal desorption (Lai 2008; Qiu et al. 2014) and phytoremediation (Long et al. 1994) have been applied to treat the Hg contaminated farmland in Qingzhen. Due to the volatility of elemental Hg, thermal desorption is frequently applied to restore Hg contaminated soils (Wang et al. 2012). It was found

more than 90 % of the total Hg could be removed from the contaminated soil at 350 °C (Qiu et al. 2014). However, the costs of this technique and the effects of the thermal treatment on soil properties should be carefully balanced. Soil washing could remove the soluble and exchangeable Hg in the heavily polluted soil but the essential gradients in soil were also removed (Lin 2009). Besides, the high costs and the disturbance to the soil by soil washing should also be taken into account. Phytoremediation uses various plants to degrade, extract, contain or immobilize contaminants from soil, which is cheap and simple. It was found that *Boehmeria nivea* (L.) Gaudich could extract more Hg than rice and was proposed to grow *Boehmeria nivea* (L.) Gaudich rather than rice in these rice paddy field (Long et al. 1994). Similarly, Zhao et al. found *Rubus L.* and *Artemisia argyi H.* are Hg-hyperaccumulation plants and can survive in Hg contaminated area (Zhao et al. 2014a). However, it should be noted that rice is the staple food for local residents and it is relatively hard to change their dietary structure.

Recently, rice grown aerobically was found to markedly reduce the Hg accumulation as well as lead to much lower proportion of MHg in rice grain (Peng et al. 2012). Further investigation suggests that the decreased sulfate-reducing bacteria numbers and proportion of Hg methylators in the rhizosphere are the main reason for the reduced Hg accumulation (Wang et al. 2014c). These findings provide an alternative way to reduce the Hg uptake through rice consumption while keep the dietary structure for rice-dependent populations. However, the growth of rice and the rice yield were also affected in aerobically condition since flooded paddy ensures increased nitrogen fixation, increased phosphorous levels and minimized competition from weeds and soil-borne pathogens (Bindraban et al. 2006; Peng et al. 2012). Therefore, finding other ways that can keep the growth pattern of rice while reduce the Hg accumulation in rice grain is highly desired.

Selenium (Se) is an essential trace element which has long been found to protect against the toxicity of Hg (Bjerregaard and Christensen 2012; Koeman et al. 1973; Kosta et al. 1975). The possible mechanism for this protection is that, first, Se has high affinity to Hg and can form Hg-Se complexes, which is inert in the body; second, Se is incorporated into important selenoenzymes and their antioxidative properties, which help eliminate the reactive oxygen species induced by Hg *in vivo* (Chen et al. 2006; Ralston and Raymond

2010). Se supplementation trials have been conducted in people suffering from Hg poisoning with confirmed protective role of Se (Li et al. 2012; Seppanen et al. 2000). For plants, recent hydroponic and greenhouse experiments found that selenite could protect against the toxicity of Hg in rice and inhibit Hg accumulation in rice tissues (Wang et al. 2014b; Zhao et al. 2014b). This is in agreement with another investigation about the influence of Se on the uptake and translocation of Hg in the rice-soil system from a Hg mined area, where both Hg and Se occur due to historic Hg mining and retorting activities (Zhang et al. 2012). However, to the best of our knowledge, treatment of rice grown in real Hg contaminated paddy field like in Qingzhen with Se was not reported yet and the reason for the reduced Hg accumulation in rice is still unclear. The aims of this study are: 1) To determine the most suitable concentration of Se that can lead to least Hg accumulation in rice grain while have the highest rice yield and grain quality in real Hg contaminated paddy field in Qingzhen, and 2) To elucidate the possible mechanism of the protection against the phytotoxicity of Hg in rice by Se.

Materials and methods

Experimental design

A rice paddy field in Qingzhen was chosen for this study, which uses the Hg-containing stream water to flood the paddy field. A widely grown rice cultivars in this area, Zhongyou158 (ZY158), was selected in this experiment. To prevent the possible disturbance to the paddy field, the rice was grown in 12 polypropylene (50 cm in length, 35 cm in width, and 35 cm in height) boxes with no drainage holes. The boxes were put into the paddy field and the rice was cultivated using the paddy soil by a local farmer according to normal practice from June to October, 2010. The experimental boxes were divided into two parallel groups, and each group of six boxes was added with different concentrations of sodium selenite solution (0, 0.01, 0.1, 0.5, 1, and 5 $\mu\text{g}/\text{mL}$, diluted with the stream water, expressed as control, 0.01Se, 0.1Se, 0.5Se, 1Se, and 5Se group, respectively) every 30 days, until rice matured.

Sample collection and preparation

The whole rice plants were harvested at the maturity stage. The rice samples were washed with deionized water, then separated to roots, stems, leaves, hulls and grains. After freeze-dried, their dry weight were weighed. Finally, these tissues were ground to powders, and stored at $-20\text{ }^{\circ}\text{C}$ prior to further analysis.

The soil samples were collected from rhizosphere (0–20 cm depth) and placed into the sealed polyethylene bags to prevent cross pollution. The soil samples were ground to pass a 150-mesh sieved for further analysis. The water samples were collected from the stream and the paddy field.

Analytical methods and quality control

Hg and Se concentrations in rice tissues, soil and water samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Elemental X7, USA) (Li et al. 2006; Li et al. 2007). Detailed information about the analysis can be found in supporting information, S1.

Distribution of Hg and Se in rice roots by XRF

The rice roots were cut into 40 μm slices with a freezing microtome (CM1850, Germany). The slices were then fixed onto 3 μm mylar films prior to X-ray Fluorescence (XRF) analysis.

The XRF analysis was performed at beamline BL15U (3.5 GeV, 250 mA) in Shanghai Synchrotron Radiation Facility (SSRF, China). A detailed description about the beamline is given in S2. The counts of the elements of Hg and Se were normalized to that of the I_0 to correct the effect of the SR beam flux variation on the signal intensity and imaged using Igor Pro. 5.01 (Zhao et al. 2014b).

Hg speciation in rice roots by XAS

For synchrotron radiation X-ray absorption spectroscopy (XAS) analysis, the uniform fine powder of freeze-dried rice roots were pressed into 1 mm-thick tablets for XAS analysis. HgCl_2 , $\text{Hg}(\text{GSH})_2$, HgS , CH_3HgCl , HgSe , and $\text{CH}_3\text{Hg-GSH}$ were used as model compounds.

The Hg L_{III} -edge (12,284 eV) spectra of rice roots and model samples were collected at beamline 14 W1 in

SSRF. To avoid the possible interference between Hg L_{III}-edge and Se K-edge (12,658 eV), only the X-ray absorption near-edge spectra (XANES) were collected (Gailer et al. 2000). A detailed description about the beamline is given in S3. Least-squares linear combination fitting (LCF) of the XANES spectra was performed to get the Hg and Se speciation in rice tissues (Li et al. 2010; Ressler et al. 2000). The percentage of each Hg-containing species in total Hg equals the fractional contribution of a model spectrum to the total spectra.

Statistics

Data sets were analyzed using analysis of variance (ANOVA) and Student's *t*-test using the Statistical Package for Social Science (SPSS) for Windows (version 9.05; SPSS Inc., Chicago, IL). For all tests, values of *p* < 0.05 were considered to indicate statistical significance. Where necessary, data sets were transformed to normalize the distribution.

Results

Hg and Se in the paddy soil and stream water

The Hg and Se contents in the paddy field soil were 236 ± 13 and 0.69 ± 0.47 mg/kg, respectively. The Hg level in this study agrees with the results in the same area reported by other researchers, suggesting the Hg contamination is still there (Horvat et al. 2003; Zhang et al. 2008). As for Se levels in soil, a national survey on 21 main types of soils, from purple soil (Se: 0.076 mg/kg), red soil (Se: 0.315 mg/kg), yellow soil (Se: 0.397 mg/kg) to laterite (Se: 0.946 mg/kg) were reported (Tan et al. 2002). The soil in Guizhou belongs to the mixture of laterite, yellow soil and red soil (Qin et al. 2009). This suggests that the Se level in this study falls in the background level of Guizhou province.

After Se treatment, the Hg contents in soil of control, 0.01Se, 0.1Se, 0.5Se, 1Se, and 5Se group were 214, 209, 228, 273, 267 and 254 mg/kg, respectively. The concentrations of Hg in the 0.1Se, 0.5Se, 1Se and 5Se group were higher than the control group, suggesting Se treatment led to concentrated Hg in the rhizosphere.

The Se contents in soil were 0.74, 0.52, 0.41, 0.75, 0.78 and 0.85 mg/kg, respectively. Compared to the Se level in soil before Se treatment, the 0.5Se, 1Se, and 5Se group had a bit higher but not significantly increased Se

level. After Se treatment, no significant difference was found for the Se level soil, suggesting the addition of this small amount of Se did not change the Se status in the paddy field.

The Hg and Se contents in stream water used for irrigation in this study were 0.11 ± 0.04 ng/L and 4.56 ± 2.36 ng/L, respectively. It was reported that the Hg contents in the stream water reached 12.7 ng/mL in 1999 (Horvat et al. 2003). This suggests that the Hg contamination in the stream water is less serious than before. This also means that the Hg contamination in rice is mostly from the paddy soil now. The Se level in the stream water was below the Chinese national maximum contaminant level for surface water (20 ng/L) (Ministry of Environmental Protection 2002).

Mass distribution of Hg and Se in whole rice plant

Mass distribution of Hg and Se in different tissues of rice plant is shown in Fig. 1. The mass of Hg or Se in a tissue was calculated based on the concentration of Hg or Se in a tissue times the dry weight of the tissue. The mass distribution is the mass of Hg or Se in a tissue divided by the total mass of Hg or Se in the whole plant.

From Fig. 1, it can be seen in the control group, Hg in roots, stems, leaves, hulls and grains were 82, 7, 2.5, 3 and 5.5 % of total Hg, respectively. Meng et al. (Meng et al. 2010) found similar distribution of Hg in rice grain but larger proportion of Hg in leaves due to Hg⁰ in ambient air in artisanal Hg mining site. After Se treatment, the proportion of Hg in root increased to 94, 94, 92, 90 and 87 % in 0.01, 0.1, 0.5, 1 and 5Se group, respectively. On the other hand, the Hg proportion in grain were 2, 2, 2.2, 2.5 and 3 % of total Hg, in 0.01, 0.1, 0.5, 1 and 5Se group, respectively. This result shows that Se treatment increased the proportion of Hg in root but decreased the proportion of Hg in rice grain.

For Se, it was 70, 5, 3, 5 and 17 % in roots, stems, leaves, hulls and grains, respectively in the control group. After Se treatment, significantly increased proportion of Se was found in grains, which were 31, 42, 41, 40, 39 and 62 % in 0.01, 0.1, 0.5, 1 and 5Se group, respectively.

The bioaccumulation factors (BAF, rice/soil concentrations ratios) and the transfer factors (TF, rice/root concentrations ratios) are two indicators for the uptake of Hg from soil to rice grain and the transfer of Hg from rice root to grain. The BAFs and TFs of Hg are shown in Table 1. The BAF for Hg is 0.00025 in the control

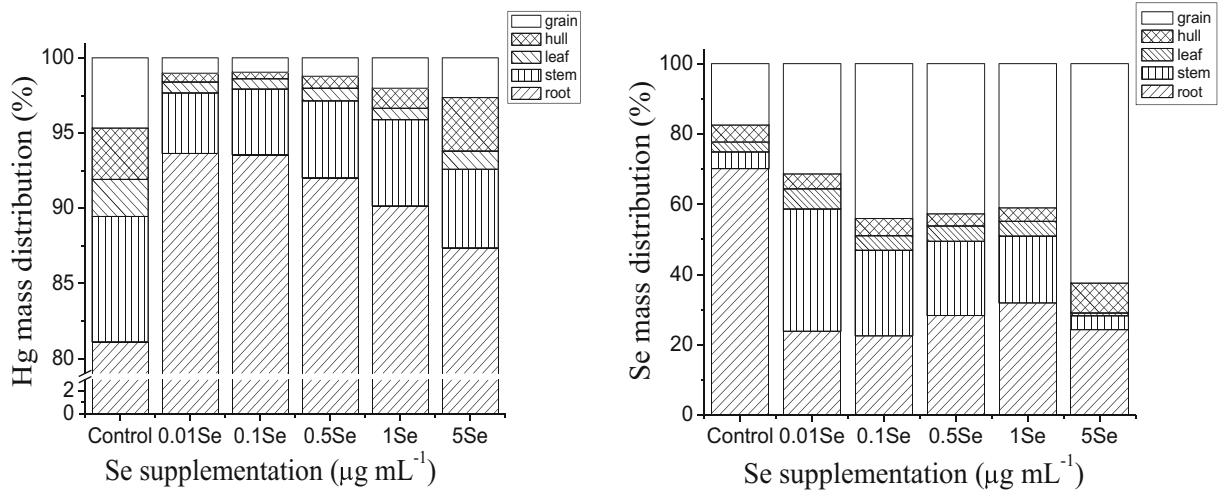


Fig. 1 Mass proportions of Hg and Se in rice tissues of a whole rice plant in different groups

group, while 0.5Se group is 0.00014, approximately half of the control, suggesting Se below 0.5 $\mu\text{g/mL}$ greatly inhibited Hg to enter the rice grain. On the other hand, the BAF of Hg is 0.00026 in 1Se group, 0.00037 in 5Se group, respectively, indicating that Se higher than 0.5 $\mu\text{g/mL}$ promotes the Hg accumulation in rice grain.

The transfer factors (TF, rice/root concentrations ratios) of Hg is 0.0012 in 0.01Se group, 0.0011 in 0.1Se group and 0.0013 in 0.5Se group, compared with 0.0036 in the control group (Table 1). For 1Se and 5Se group, similar transfer factors were found in the control group. This also suggests that Se level below 0.5 $\mu\text{g/mL}$ could reduce the transfer of Se from root to rice grain while Se level higher than 0.5 $\mu\text{g/mL}$ did not change the transfer efficiency from root to rice grain.

Table 1 Bioaccumulation factors of mercury (rice/soil concentrations ratios), and transfer factors of mercury (rice/root concentrations ratios) at different groups

Se concentration	BAF* ^a	TF* ^b
Control	0.00025±2.56E-05	0.0036±3.72E-04
0.01Se	0.00020±1.72E-05	0.0012±1.04E-04
0.1Se	0.00020±1.92E-05	0.0011±1.11E-04
0.5Se	0.00014±8.91E-05	0.0013±0.83E-04
1Se	0.00026±1.42E-05	0.0035±1.91E-04
5Se	0.00037±2.99E-05	0.0035±2.85E-04

* mean ± SD, n=3

^a BAF Bioaccumulation Factors, ^b TF Transfer Factors

Concentration of Hg and Se in rice grain

The mean concentration of Hg in rice grain of the control group is 53.15 ng/g (Fig. 2), which is nearly 2.7 times higher than the Chinese national limit for Hg in rice as 20 ng/g (Chinese Ministry of Health 2005).

The average concentration of Se in the control group is 143 ng/g (Fig. 2), which is in agreement with the previous study by Horvat et al. (Horvat et al. 2003). After Se treatment, the concentrations of Se are 383, 759, 832, 1376, 3313 ng/g in 0.01, 0.1, 0.5, 1Se, 5Se groups, respectively. It can be seen that Se treatment is an easy way to enrich Se in rice grain. Several other studies found that Se treatment could increase Se concentration in grains up to 51 times (Chen et al. 2002; Fang et al. 2009; Wang et al. 2013).

Seed setting rate and thousand seed weight of rice grain

Seed setting rate (SSR) is the percentage of cereal crops plump grains accounted for the total number of spikelets, and thousand seed weight (TSW) or thousand kernel weight (TKW) is the weight of one thousand seeds, both of which are important indexes for rice yield and quality (Li et al. 2013). The reference values for SSR and TSW of ZY158 are 82.9 and 28.0 g by China Rice Data Center (<http://www.ricedata.cn/variety/varis/603097.htm>). However, due to Hg contamination, the SSR and TSW in the rice grown in Qingzhen were significantly lower than the reference value as 61.4 and 16.5 g (Fig. 3).

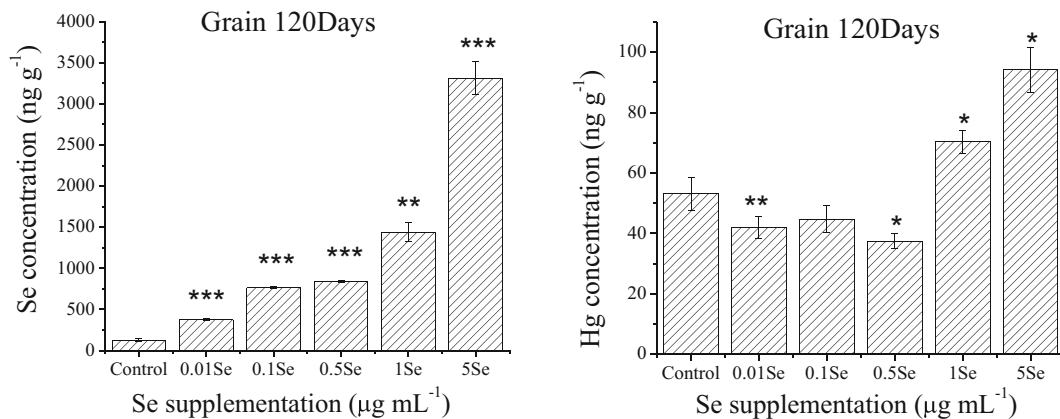


Fig. 2 Concentrations of Se and Hg in rice grains in different groups. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ compared with the control group

After Se treatment, 0.01Se, 0.1Se and 5Se groups have reduced SSR, but 0.5Se and 1Se groups have increased SSR, 69.9 and 69.8 %, respectively. As for TSW, the 0.01, 0.1, 0.5 and 1Se groups have increased TSW but the 5Se group with even lower TSW than the control group, suggesting lowered rice yield. 0.5Se group has the TSW of 21.6 g, which is the best among all the groups.

Spatial distribution and chemical forms of Hg and Se in rice roots

Root is the first biological barrier that protects the rice plant from Hg contamination in soil. Since it was found in this study that root is the major tissue that accumulated Hg, the spatial distribution and chemical forms of Hg and Se in the 0.5Se group were investigated using XRF and XAS.

The spatial distribution of Hg and Se in rice roots is shown in Fig. 4. The normalized X-ray fluorescence intensities are scaled from blue to red, suggesting low to high levels of Hg or Se. In the control group, Hg is distributed not only in the epidermal and endodermal tissues but also in the vascular tissues, which agrees with other studies on mercury distribution in plant roots (Carrasco-Gil et al. 2011; McNear et al. 2012). This is ascribed to the cysteine rich domains in the major root cells and Hg has a high affinity to the thiol in cysteine domain (Zhao et al. 2014b).

After treating with Se, the co-existence of Hg and Se was found in the epidermal and vascular tissues but with decreased accumulation of Hg, compared to the control group. This suggests that Se treatment decreased the uptake of Hg into the root from the soil and the aerial part of rice.

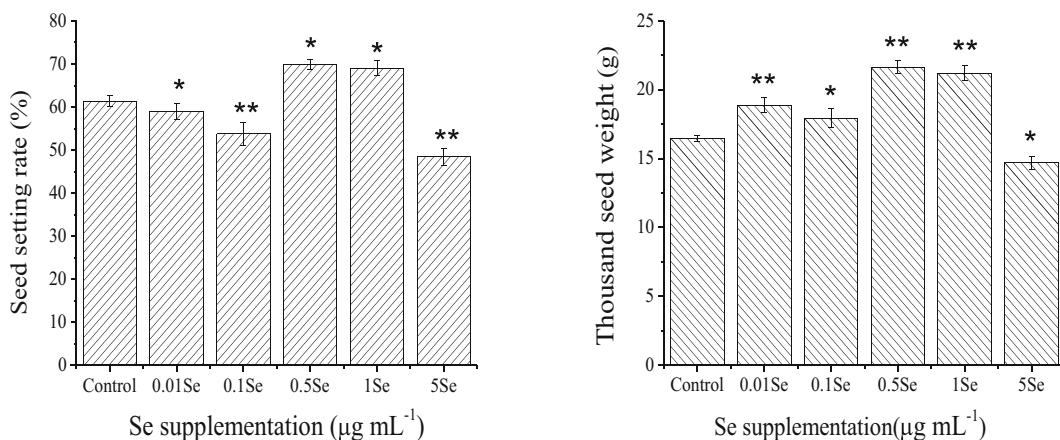
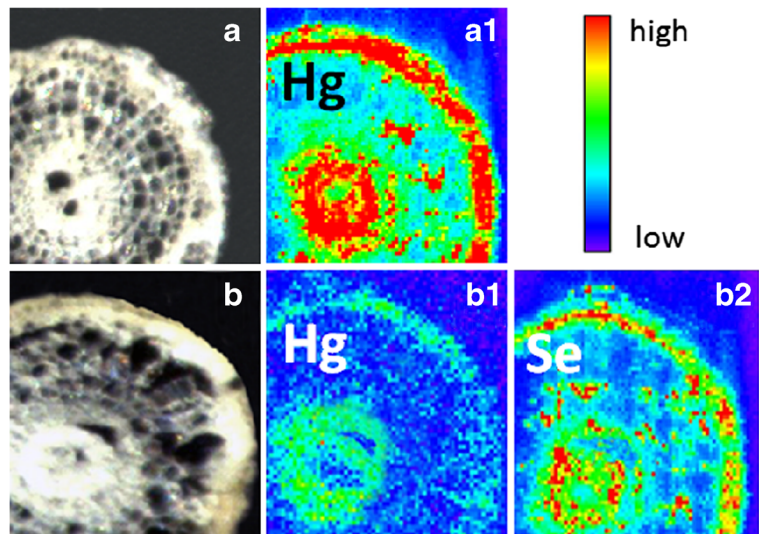


Fig. 3 Seed setting rate (SSR) and thousand seed weight (TSW) of rice grain in different groups. ** $p < 0.01$, * $p < 0.05$ compared with the control group

Fig. 4 The distribution of Hg and Se in rice roots. (a,b) the cross section of the root in the control group and Se-treated group under optical microscope; (a1) the distribution of Hg in the cross section of the root in the control group by XRF; (b1, b2) the distribution of Hg and Se in the cross section of the root in the Se-treated group by XRF



Several studies found that Hg and Se could form Hg–Se complexes in the plant roots. For example, Caruso et al. confirmed the existence of a Hg–Se containing protein (molecular weight ≥ 70 kDa) in the roots of *Brassica juncea* and soybean using size exclusion chromatography (Mounicou et al. 2006; Yathavakilla and Caruso 2007). However, the chemical forms of Hg and Se in rice root are not clear yet. Zhang et al. proposed that Hg–Se complexes may exist in the rhizosphere/rice roots but no further evidence was presented (Zhang et al. 2012). In this study, the chemical forms of Hg and Se were studied by XAS, which applies synchrotron radiation-based X-rays to probe local structural and electronic environment with minimal sample preparation.^{38, 39}

Figure S1 shows the normalized Hg L_{III} spectra of roots in the control and Se-treated groups with a serial of Hg compounds. It can be seen that both CH_3HgCl and $HgCl_2$ have a shoulder peak at 12,284 eV while the control roots, the Se-treated roots, $HgSe$, and $Hg(GSH)_2$ do not exhibit such shoulder peak. This suggests that Hg in the roots may complex with Se or S rather than Cl.

LCF results of the control and Se-treated groups are shown in Table 2 and Figure S2. In the control group, over 81 % of the Hg was in the form of Hg–S using $Hg(GSH)_2$ as a model compound, while in the Se-treated group, the Hg–S forms reduced to 71.9 and 27.8 % was in the form of Hg–Se using $HgSe$ as a model compound. These results clearly show that Hg–Se complexes were formed in roots besides the Hg–S compounds.

Discussions

The most suitable level of Se with least Hg accumulation in rice grain

The average concentration of Se in the rice grain in the control group is 143 ng/g, which indicates that Qingzhen has reasonable Se availability. This was confirmed by the analysis of Se level in paddy soil, which is 0.69 ± 0.47 mg/kg. This may also explain why the soil Hg is certainly high, but the grain Hg is not that much higher than the national limit.

Table 2 Percentage of Hg species in the control and Se-treated groups by LCF fitting. Values in parentheses show the SD

Sample	$HgCl_2$	$Hg(GSH)_2$	HgS	CH_3HgCl	HgSe	CH_3HgGSH
Control	18.1(2.3)	81.5 (3.1)	<1	—	—	—
Se-treated	<1	71.9 (3.1)	—	—	27.8(3.0)	—

Although selenate is usually more effective than selenite to get Se into plant parts beyond the root, our previous studies found that selenate is more phytotoxic than selenite (Zhao et al. 2013). Besides, under the reducing conditions as in rice paddy field, selenate will largely be reduced to selenite and even selenide. Therefore, only selenite was used in this study.

After Se treatment, the lowest Hg accumulation in rice grain is in 0.5Se group, which is 37.36 ng/g. Wang et al. also found that the low Se group (with Se level at 1 mg/kg in soil) through greenhouse experiment, which falls in the same Se range in soil to this study, got similar Hg level in rice grain (Wang et al. 2014b). They found that Se level at 5 mg/kg (high Se group) in soil could reduce the Hg concentration in rice grain to be below 20 ng/g. However, they did not test whether this level of Se in soil affected the rice grain yield and quality or not. Furthermore, Se in soil at 5 mg/kg was considered as Se-excessive and the potential risk of selenosis should be considered when applying to real paddy field (Tan 1996). The 1 and 5Se group got elevated Hg accumulation in rice grain, which was 3.5 and 4.7 times higher than the Chinese national limit. This indicates that Se at 0.5 $\mu\text{g}/\text{mL}$ gets the least accumulation of Hg in rice grain but Se levels over 0.5 $\mu\text{g}/\text{mL}$ can even enhance the accumulation of Hg in rice grain. Besides, when considering the SSR and TSW, this study also suggests that

the 0.5Se group has the highest rice grain yield and rice quality.

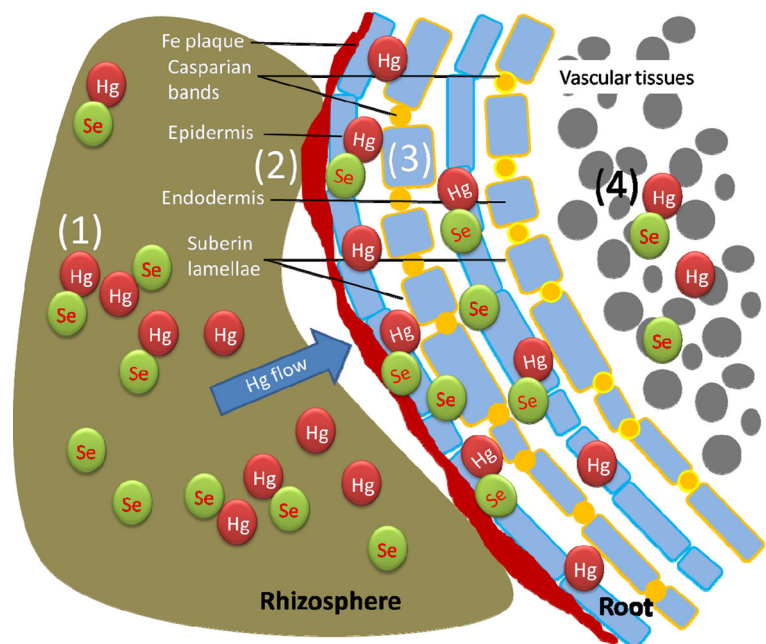
The mechanism on Se protection against Hg toxicity

In this study, it was found that the 0.5Se group has the lowest Hg accumulation in rice grain while the highest yields and quality in a real Hg contaminated paddy field in Qingzhen. This is significant from the view of the protection of the health of the rice-dependent populations. The mechanism for this protection may be ascribed to several reasons. A summarization of the proposed mechanism is shown in Fig. 5.

First, Se could reduce the bioavailability of Hg and the production of MHg in the rhizosphere and thus suppressed the uptake of Hg into the root cells (Wang et al. 2014b). This is because selenite (SeO_3^{2-}) can be reduced to selenide (Se^{2-}) in rhizosphere and to combine with Hg^{2+} in soil to form insoluble HgSe compounds which is less bioavailable to rice (Plant et al. 2003). Besides, HgSe compounds may further react with the abundant dissolved organic matters in rhizosphere to form large molecular weight HgSe complexes to further reduce the bioavailability of Hg (Chiasson-Gould et al. 2014).

Second, Se could promote the formation of iron plaque (Li et al. 2014; Zhou et al. 2014). Iron plaque

Fig. 5 Cross-sectional diagram of the rice root and the rhizosphere, showing the proposed mechanism for the reduced Hg accumulation in rice grains at low Se concentration. (1) The decreased bioavailability of Hg by forming Hg-Se complexes in the rhizosphere; (2) The induction of iron plaque on root surface by Se; (3) The formation of biological barriers like Casparian bands and suberin lamellae; (4) The formation of Hg-Se complexes in root



can sequester metal(loid)s by adsorption and/or co-precipitation. For example, it was found that iron plaque significantly inhibited the uptake of arsenic in rice (Chen et al. 2005). Li et al. found through hydroponic experiment that Se addition enhanced the formation of iron plaque and inhibited the translocation of Hg (Li et al. 2014). Our XRF results (Fig. 4) clearly show that Hg distributed in the epidermis of rice root and Hg in vascular tissues of roots decreased significantly after Se treatment at 0.5 $\mu\text{g}/\text{mL}$.

Third, Se could induce and enhance the development of extracellular barriers. For example, root anatomy found that Casparian bands and suberin lamellae were formed in Se treated rice root (Wang et al. 2014b). Casparian strip is a band of cell wall material deposited on the radial and transverse walls of the endodermis, which can block the passive flow of materials, such as water and solutes into the stele of a plant. Suberin lamellae are formed in the endodermis of many species after Casparian band deposition, which also significantly affects the radial uptake of water, dissolved nutrients and radial loss of oxygen (Perumalla and Peterson 1986).

Fourth, Se could form large molecular weight Hg-Se complex in root that could not be translocated to the aerial part of rice. Wang et al. also found that over 80 % of the total Hg was in root in four different rice cultivars (Wang et al. 2014a). Our results suggest that over 90 % of the Hg was blocked in rice root (Fig. 1) after Se treatment. XANES fitting (Table 2 and Figure S2) found that 27.8 % of the Hg was in the form of Hg-Se complex.

The uptake of Hg from soil to the plants involves symplastic and apoplastic pathways (Patra and Sharma 2000). The block of the symplastic and apoplastic pathways by the formation of Casparian bands and suberin lamellae and other biological barriers like iron plaque will decrease the uptake and translocation of large molecular weight Hg-Se complexes. This will then reduce the Hg accumulation in rice grains after Se treatment at 0.5 $\mu\text{g}/\text{mL}$. However, this could not explain the increased accumulation of Hg in rice grain at Se levels over 0.5 $\mu\text{g}/\text{mL}$, i.e., in the 1Se and 5Se group, the Hg concentration was even higher than the control group.

Our study found that Se treatment could significantly increase Se levels in rice grain (Fig. 2), especially those in 5Se group as 3313 ng/g, which is in agreement with other studies (Carey et al. 2012; Hu et al. 2013). Since Se has high affinity to Hg ($\log K_{\text{sp}} = -64.5$), it is easy to

understand that high Se in grain will promote the translocation and redistribution of Hg and thus lead to the increased Hg accumulation in rice grain. Besides, Hg uptake is found to be via various essential element membrane transporters. For example, Esteban et al. (Esteban et al. 2013) found reduced Hg uptake was observed in the presence of Mn for oilseed rape, suggesting that Hg influx is mediated by Mn transporter. On the other hand, Hu et al. (Hu et al. 2013) found that Se treatment could reduce the uptake and transportation of Mn in rice. Therefore, the presence of Se may promote the Hg influx through Mn transporter and lead to the increased Hg accumulation in rice grain. Furthermore, the chemical forms of Hg and Se with high level of both Hg and Se in rice grain deserve further study. If Hg-Se complex is formed in rice grain, which is less bioavailable, it may in fact bring less health risks to the rice-dependent populations than the control rice.

Conclusions

Since the 1 and 5Se groups have lowered rice yield and quality and increased Hg accumulation, 0.5 $\mu\text{g}/\text{mL}$ of Se is regarded as the most appropriate concentration to treat rice growing in Hg contaminated paddy field in Qingzhen.

This study not only provides an alternative way to reduce Hg in rice grain in real paddy field, but also help elucidating the mechanism about the protection by Se in Hg contaminated rice. It should be noted that although Se treatment at levels of 0.5 $\mu\text{g}/\text{mL}$ could reduce the Hg accumulation in rice grain, it did not change the Hg level in soil. Therefore, it is recommended that the Se treatment should be combined with other remediation techniques to not only reduce the Hg accumulation in rice grains but also decrease the Hg contamination in the paddy field.

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References

- Bindraban PS, Hengsdijk H, Cao W, Shi Q, Thiyagarajan TM, Van Der Krogt W, Wardana IP (2006) Transforming inundated rice cultivation. *Int J Water Res Dev* 22:87–100. doi:10.1080/07900620500405338
- Bjerregaard P, Christensen A (2012) Selenium reduces the retention of methyl mercury in the brown shrimp *Crangon crangon*. *Environ Sci Technol* 46:6324–6329
- Carey A-M et al (2012) Grain accumulation of selenium species in rice (*Oryza sativa* L.). *Environ Sci Technol* 46:5557–5564
- Carrasco-Gil S et al (2011) Complexation of Hg with phytochelatin is important for plant Hg tolerance. *Plant Cell Environ* 34:778–791. doi:10.1111/j.1365-3040.2011.02281.x
- Chen C et al (2006) The roles of serum selenium and selenoproteins on mercury toxicity in environmental and occupational exposure. *Environ Health Perspect* 114:297–301
- Chen L, Yang F, Xu J, Hu Y, Hu Q, Zhang Y, Pan G (2002) Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *J Agric Food Chem* 50:5128–5130. doi:10.1021/jf0201374
- Chen Z, Zhu Y-G, Liu W-J, Meharg AA (2005) Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. *New Phytol* 165:91–97. doi:10.1111/j.1469-8137.2004.01241.x
- Cheng J, Yuan T, Wang W, Jia J, Lin X, Qu L, Ding Z (2006) Mercury pollution in two typical areas in Guizhou Province, China and its neurotoxic effects in the brains of rats fed with local polluted rice. *Environ Geochem Health* 28:499–507
- Chiasson-Gould SA, Blais JM, Poulain AJ (2014) Dissolved organic matter kinetically controls mercury bioavailability to bacteria. *Environ Sci Technol* 48:3153–3161. doi:10.1021/es4038484
- Chinese Ministry of Health (2005) Pollutants limit in food (GB2762-2005). China Standard Press, Beijing
- Clarkson TW, Magos L (2006) The toxicology of mercury and its chemical compounds. *Crit Rev Toxicol* 36:609–662. doi:10.1080/10408440600845619
- Environmental Quality Standard for Soils (GB15618-1995) (1995) Ministry of environmental protection of the People's Republic of China
- Esteban E, Deza M, Zomoza P (2013) Kinetics of mercury uptake by oilseed rape and white lupin: influence of Mn and Cu. *Acta Physiol Plant* 35:2339–2344. doi:10.1007/s11738-013-1253-6
- Fang Y, Zhang Y, Catron B, Chan Q, Hu Q, Caruso JA (2009) Identification of selenium compounds using HPLC-ICPMS and nano-ESI-MS in selenium-enriched rice via foliar application. *J Anal At Spectrom* 24:1657–1664. doi:10.1039/B912538H
- Feng X et al (2008) Human exposure to methylmercury through rice intake in mercury mining areas, Guizhou province, China. *Environ Sci Technol* 42:326–332. doi:10.1021/es071948x
- Gailer J et al (2000) Structural basis of the antagonism between inorganic mercury and selenium in mammals. *Chem Res Toxicol* 13:1135–1142
- Guallar E et al (2002) Mercury, fish oils, and the risk of myocardial infarction. *N Engl J Med* 347:1747–1754. doi:10.1056/NEJMoa020157
- Horvat M et al (2003) Total mercury, methylmercury and selenium in mercury polluted areas in the province Guizhou. *China Sci Total Environ* 304:231–256. <http://www.ricedata.cn/variety/varis/603097.htm>
- Hu Y, Y-z H, Y-c H, Y-x L, J-h L (2013) Effect of selenium on the uptake and translocation of manganese, iron, phosphorus and selenium in rice (*Oryza sativa* L.). *Environ Sci* 34:4119–4125
- Koeman JH, Peeters WHM, Koudstaal-Hol CHM, Tjioe PS, De Goeij JJM (1973) Mercury-selenium correlations in marine mammals. *Nature* 245:385–386
- Kosta L, Byrne AR, Zelenko V (1975) Correlation between selenium and mercury in man following exposure to inorganic mercury. *Nature* 254:238–239
- Lai L (2008) Remediation of mercury-contaminated soils by using low temperature thermal desorption in qingzhen, guizhou master thesis, Guizhou Normal University
- Li S et al (2013) Natural variation in PTB1 regulates rice seed setting rate by controlling pollen tube growth. *Nat Commun* 4:Article number: 2793 doi:10.1038/ncomms3793
- Li Y-F et al (2006) Elimination efficiency of different reagents for the memory effect of mercury using ICP-MS. *J Anal At Spectrom* 21:94–96. doi:10.1039/b511367a
- Li Y-F et al (2007) Simultaneous speciation of selenium and mercury in human urine samples from long-term mercury-exposed populations with supplementation of selenium-enriched yeast by HPLC-ICP-MS. *J Anal At Spectrom* 22:925–930
- Li Y-F et al (2012) Organic selenium supplementation increases mercury excretion and decreases oxidative damage in long-term mercury-exposed residents from Wanshan, China. *Environ Sci Technol* 46:11313–11318
- Li Y-F, Wang X, Wang L, Li B, Gao Y, Chen C (2010) Direct quantitative speciation of selenium in selenium-enriched yeast and yeast-based products by X-ray absorption spectroscopy confirmed by HPLC-ICP-MS. *J Anal At Spectrom* 25:426–430
- Li Y, Zhao J, Gao Y, Li Y-F, Li B, Zhao Y, Chai Z (2014) Effects of iron plaque and selenium on the absorption and translocation of inorganic mercury and methylmercury in rice (*Oryza sativa* L.). *Asian J Ecotoxicol* 9:972–977
- Lin K (2009) Studies on the leaching efficacy of heavily hg polluted paddy soil. Guizhou University
- Long Y, Liu S, Xiong J, Yang J, Li K (1994) Study on the removal efficiency of mercury in paddy soil using *Boehmeria nivea* (L.) *Gaudich*. *Agro-Environ Protect* 13:30–33
- Matsuyama A et al (2004) Distribution of methylmercury in an area polluted by mercury containing wastewater from an organic chemical factory in China. *Bull Environ Contam Toxicol* 73:846–852
- Matsuyama A, Taniguchi Y, Yasuda Y (2009) Relationships between leaching of methylmercury from the soil and the basic characteristics of alkali soil polluted by mercury in Guizhou China. *Bull Environ Contam Toxicol* 82:363–366
- Matsuyama A et al (2005) Relationship between leached total mercury and leached methylmercury from soil polluted by mercury in wastewater from an organic chemical factory in the People's Republic of China. *Bull Environ Contam Toxicol* 75:1234–1240

- McNear DH, Afton SE, Caruso JA (2012) Exploring the structural basis for selenium/mercury antagonism in *Allium fistulosum*. *Metallomics* 4:267–276
- Meng B et al (2010) Distribution patterns of inorganic mercury and methylmercury in tissues of rice (*Oryza sativa* L.) plants and possible bioaccumulation pathways. *J Agric Food Chem* 58:4951–4958
- Ministry of Environmental Protection (2002) Environmental standard for surface water (GB3838-2002). China Environmental Science Press, Beijing
- Mounicou S, Shah M, Meija J, Caruso JA, Vonderheide AP, Shann J (2006) Localization and speciation of selenium and mercury in *Brassica juncea*-implications for Se-Hg antagonism. *J Anal At Spectrom* 21:404–412. doi:10.1039/B514954A
- Patra M, Sharma A (2000) Mercury toxicity in plants. *Bot Rev* 66: 379–422. doi:10.1007/BF02868923
- Peng X, Liu F, Wang W-X, Ye Z (2012) Reducing total mercury and methylmercury accumulation in rice grains through water management and deliberate selection of rice cultivars. *Environ Pollut* 162:202–208. doi:10.1016/j.envpol.2011.11.024
- Perumalla CJ, Peterson CA (1986) Deposition of Casparian bands and suberin lamellae in the exodermis and endodermis of young corn and onion roots. *Can J Bot* 64:1873–1878. doi:10.1139/b86-248
- Plant JA, Kinniburgh DG, Smedley PL, Fordyce FM, Klinck BA (2003) Arsenic and selenium. In: Turekian HDHK (ed) *Treatise on geochemistry*. Pergamon, Oxford, pp 17–66. doi:10.1016/B0-08-043751-6/09047-2
- Qin S, Fan C, Sun R (2009) Characteristic, problems and utilization countermeasures of soil resource in Guizhou. *Guizhou Agric Sci* 37:94–98
- Qiu R, Zhang J, Dong Z, Yu Z, Feng H, Lai L (2014) Low-temperature thermal desorption of farmland soil contaminated by mercury. *Chin Environ Sci Technol* 37:48–52
- Qu L (1999) Mercury pollution caused by guizhou organic chemical factory in environment. *J Guizhou Norm Univ* 17:25–28
- Ralston NVC, Raymond LJ (2010) Dietary selenium's protective effects against methylmercury toxicity. *Toxicology* 278:112–123
- Ressler T, Wong J, Roos J, Smith IL (2000) Quantitative speciation of Mn-bearing particulates emitted from autos burning (methylcyclopentadienyl)manganese tricarbonyl-added gasolines using XANES spectroscopy. *Environ Sci Technol* 34: 950–958. doi:10.1021/es990787x
- Seppanen K, Kantola M, Laatikainen R, Nyysönen K, Valkonen VP, Kaarloop V, Salonen JT (2000) Effect of supplementation with organic selenium on mercury status as measured by mercury in pubic hair. *J Trace Elem Med Biol* 14:84–87
- Tan J (1996) *Chronic keshan disease and environmental elements of life*. Chinese Medicine Science and Technology Press, Beijing
- Tan J, Zhu W, Wang W, Li R, Hou S, Wang D, Yang L (2002) Selenium in soil and endemic diseases in China. *Sci Total Environ* 284:227–235. doi:10.1016/S0048-9697(01)00889-0
- Wang J, Feng X, Anderson CWN, Xing Y, Shang L (2012) Remediation of mercury contaminated sites—a review. *J Hazard Mater* 221–222:1–18
- Wang S, Wang R, Liu Y, Liu J, Song D, Huang Q, Du Z (1990) Studies on methylmercury pollution in Qingzhen district of Guizhou Province. *Sci Geogr Sin* 10:168–176
- Wang X et al (2014a) Radial oxygen loss has different effects on the accumulation of total mercury and methylmercury in rice. *Plant Soil* 385:343–355. doi:10.1007/s11104-014-2239-x
- Wang X, Tam NF-Y, Fu S, Ametkhan A, Ouyang Y, Ye Z (2014b) Selenium addition alters mercury uptake, bioavailability in the rhizosphere and root anatomy of rice (*Oryza sativa*). *Ann Bot* 114:271–278. doi:10.1093/aob/mcu117
- Wang X, Ye Z, Li B, Huang L, Meng M, Shi J, Jiang G (2014c) Growing rice aerobically markedly decreases mercury accumulation by reducing both Hg bioavailability and the production of MeHg. *Environ Sci Technol* 48:1878–1885. doi:10.1021/es4038929
- Wang Y-D, Wang X, Wong Y-S (2013) Generation of selenium-enriched rice with enhanced grain yield, selenium content and bioavailability through fertilisation with selenite. *Food Chem* 141:2385–2393. doi:10.1016/j.foodchem.2013.05.095
- Xiong J (1985) Effect of mercury-containing waste water from an organic chemical plant on the environmental quality of local farmland. Guizhou People's Publishing House, Guiyang
- Yasuda Y et al (2004) Mercury distribution in farmlands downstream from an acetaldehyde producing chemical company in Qingzhen city, Guizhou, People's Republic of China. *Bull Environ Contam Toxicol* 72:445–451
- Yathavakilla S, Caruso J (2007) A study of Se-Hg antagonism in *Glycine max* (soybean) roots by size exclusion and reversed phase HPLC–ICPMS. *Anal Bioanal Chem* 389:715–723. doi:10.1007/s00216-007-1458-x
- Zhang H et al (2012) Selenium in soil inhibits mercury uptake and translocation in rice (*Oryza sativa* L.). *Environ Sci Technol* 46:10040–10046
- Zhang J, Qu L, Feng X, Zhang W, Guo Y, Lin K, Li M (2008) Farmland mercury contamination in the vicinity of an organic chemical factory in Guizhou, China. *Chin J Geochem* 27: 424–430
- Zhao J et al (2013) Selenium inhibits the phytotoxicity of mercury in garlic (*Allium Sativum*). *Environ Res* 125:75–81
- Zhao J, Li Y, Gao Y, Li B, Li Y-F, Zhao Y, Chai Z (2014a) Study on mercury resistant wild plants growing in mercury mine area of Wanshan District, Guizhou Province. *Asian J Ecotoxicol* 9:881–887
- Zhao J et al (2014b) Selenium modulates mercury uptake and distribution in rice (*Oryza sativa* L.), in correlation with mercury species and exposure level. *Metallomics* 6:1951–1957
- Zhou X-b, Yu S-h, Wang W-h, Chang H, Zhou Y-X (2014) Effects of application of selenium in soil on the formation of root surface iron plaque and mercury uptake by rice plant. *J Southwest Univ* 36:103–107