Spectral insight into thiosulfate-induced mercury speciation transformation in a historically polluted soil

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HIGHLIGHTS

- Addition of thiosulfate to soil did not increase the mobility of Hg in the soils over 191 days.
- Addition of thiosulfate to soil promoted the Hg immobilization.
- $Hg(SR)_2$ was converted to nano-particulated HgS in the thiosulfate-treated soils.
- Mercury in the sweet potato was less affected by thiosulfate.

GRAPHICAL ABSTRACT
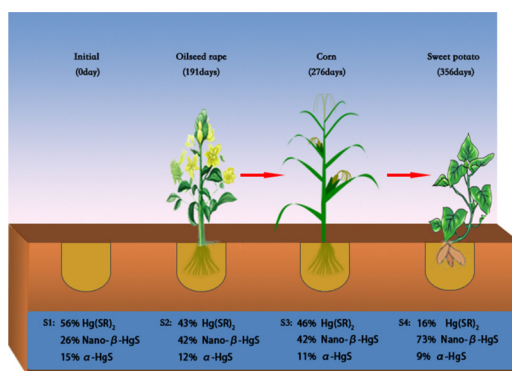
ARTICLE INFO

ABSTRACT

We studied the effect of different doses (0.5%, 2% and 5% (w/w)) of ammonium thiosulfate on mercury (Hg) speciation fractionation following its addition to the soil, as well as its accumulation by oilseed rape (*Brassica napus* L.), corn (*Zea mays* L.), and sweet potato (*Ipomoea batatas* L.), and compared them to a non-treated control in a historically polluted soil. The oilseed rape, corn, and sweet potato were planted consecutively in the same soils on days 30, 191, and 276, respectively after the addition of thiosulfate to the soil. The key results showed that bioavailable Hg contents in the rhizosphere soils ranged from 0.18 to 2.54 µg kg^{-1}, 0.28 to 2.77 µg kg^{-1}, and 0.24 to 2.22 µg kg^{-1}, respectively, for the 0.5%, 2% and 5% thiosulfate treatments, which were close to the control soil (0.25 to 1.98 µg kg^{-1}). The Hg L$_3$-edge X-ray absorption near edge structure (XANES) results showed a tendency of the Hg speciation to transform from the $Hg(SR)_2$ (initial soil, 56%; day-191 soil, 43%; day-276 soil, 46%, and day-356 soil, 16%) to nano particulated HgS (initial soil, 26%; day-191 soil, 42%; day-276 soil, 42%, and day-356 soil, 73%) with time in the soil treated with a 5% dose of thiosulfate. The Hg contents in the tissues of the crops, except for oilseed rape, were slightly affected by the addition of thiosulfate to the soil at all dosages, compared to the control. The addition of thiosulfate did not induce the movement of bioavailable Hg to the lower layer of the soil profile. We conclude a promotion of Hg immobilization by thiosulfate in the soil for over one year, offering a promising method for in-situ Hg remediation at Hg mining regions in China.

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1. Introduction

Mercury (Hg) and its compounds are highly toxic to humans because exposure to these pollutants can adversely affect or damage the central nervous system (Nance et al., 2012). The toxic effects of Hg are generally more severe for fetuses and children than adults (Holmes et al., 2009). There is a growing awareness of the significance of Hg and its effects on human health and ecological safety (Evers et al., 2016). The Minamata Convention on Mercury, which has been ratified by over 84 countries, has been adopted by international parties as a strategy to mitigate the adverse effects of Hg on human health (Wu et al., 2018).

Both natural and anthropogenic sources release Hg and its compounds into soils, while the anthropogenic sources include coal combustion, Hg and gold-mining activities, excessive pesticide application and waste water irrigation in agriculture, as well as industrial activities (Liu et al., 2018; Zhou et al., 2018). Inorganic Hg can be converted to methylmercury (MeHg) by bacteria under anaerobic condition (Zhang et al., 2010). Soil can either receive atmospheric Hg^0 via its deposition or emit Hg^0 depending on environmental conditions, and soil organic matter play a crucial role in binding with Hg (Zhu et al., 2018).

The transfer of inorganic Hg and MeHg in the soil-plant system threatens the health of consumers and the safety of the ecosystem (Gabriel and Williamson, 2004).The widespread contamination of soil with Hg attracts public concerns because consumers are aware that they may be exposed to Hg by eating polluted food products (Beckers and Rinklebe, 2017). This situation may be worse in the less-developed regions of the world, such as Asian and African countries, where Hg and its products are still widely used (Hilson et al., 2018).

Therefore, the development of sustainable methods to mitigate the risk of this metal in soils is needed. Methods, including excavation and disposal, stabilization and solidification, electroremediation, soil washing, thermal desorption (Wang et al., 2012a; Xu et al., 2015) have been used for Hg remediation. However, these methods are generally too expensive and environmentally disruptive to be used for farmlands (Rodriguez et al., 2012). As gentle remediation options (GROs), phytoremediation and in situ immobilization (Shu et al., 2016a, 2016b) may be good options to treat marginal polluted lands, which are difficult for hard development (Antoniadis et al., 2017; Cundy et al., 2016).

Thiosulfate has been used for Hg GROs by promoting phytoextraction efficacy, it was applied to the soil 5 to 7 days immediately before harvest, resulting in the solubilization of a significant amount of Hg for plant uptake in a short period (Moreno et al., 2005b). For instance, previous research found that bioavailable Hg (extracted by $Mg(NO_3)_2$ and CH_3COONa) content in the soil treated with thiosulfate at a dose of 8 g kg^{-1} was significantly increased, compared to the non treated soil after the phytoextraction (Wang et al., 2014). However, an apparent risk associated with this Hg mobilization is the potential movement of Hg to the surroundings in case they are not completely accumulated by the remedial plants. Although many studies have been conducted on thiosulfate-assisted phytoextraction, the behavior of Hg in the soil after the phytoextraction is poorly understood, particularly with the extension of time. This knowledge is critical for the application of thiosulfate as a sustainable amendment for Hg remediation, and the safe use of the land after remediation.

To add to this knowledge, we conducted a field trial in a Hg-polluted farmland from the Wanshan Hg mine of China to investigate the bioavialable Hg and Hg fractionation in the soil, as well as the Hg movement in a soil-crop system in the thiosulfate-treated soils, and compared these to the nontreated control, with an extension of time. The movement of the bioavailable Hg with the soil profile was also studied. We selected a Hg-polluted farmland around the Wanshan Hg mine, which was considered to be the mercury "capital" of China. It has been mined for >2000 years. It is roughly estimated that approximately 10,000 ha of farmland around the Hg mine in Wanshan has been contaminated with Hg, but most of lands are still cultivated for agricultural production. The local residents are at risk of exposure to Hg (Zhang et al., 2010). Therefore, the development of eco-friendly, sustainable, cost-effective methods to remediate those contaminated soils is particularly urgent and important.

The use of thiosulfate as an amendment for Hg remediation at the Wanshan Hg mine is recommended, and thus the study of the behavior of Hg in the soils with thiosulfate amendment could help to build an optimum method for sustainable Hg remediation. Specially, we aimed (1) to study the dynamics of bioavailable Hg in thiosulfate-treated soils; (2) to quantify the change of Hg speciation in the thiosulfate-treated soils using X-ray absorption near edge structure (XANES) spectroscopy, with time extending; (3) to characterize the effect of thiosulfate addition to the soil on Hg accumulation by oilseed rape (Brassica napus L.), corn (Zea mays L.), and sweet potato (Ipomoea batatas L.), and (4) to elucidate the effect of the addition of thiosulfate to soil on the bioavailable Hg movement along with the soil profiles.

2. Material and methods

2.1. Experimental design

The field trial was conducted at a Hg-contaminated farmland at Da Shuixi village located in the vicinity of the Wanshan Hg mine of China (Fig. S1). The farmland was contaminated with Hg due to the extensive mining and retorting activities that have occurred in this area (Wang et al., 2012a). The soil was slightly alkaline (pH, 7.45), with an average content of organic matter (SOM) of 46.08 g kg^{-1}. Its density was 1.12 g cm^{-3}, and its texture was characterized as sandy loam. The total Hg content in the soil was 168 mg kg^{-1} (calculated as dry weight). Detailed information for the physical-chemical properties of the studied soil is shown in Table S1.

Approximately 20 m^2 of contaminated farmland was selected, and divided into four equal-sized plots with an area of 5 m^2 of each (Fig. S1). The first plot was treated with thiosulfate at a dose of 0.5 g per kilogram of soil (denoted Ts0.5); the second plot was treated with thiosulfate at a dose of 2 g per kilogram of soil (denoted Ts2), and the third plot was treated with thiosulfate at a dose of 5 g per kilogram of soil (denoted Ts5). One plot was designated as a non-treated control. Each plot was divided further into three subplots with an area of approximately 1.5 m^2 of each. A drainage channel (width: 35 cm; height: 30 cm) was established to separate the different treatments to avoid their potential cross-impacts. A calculated amount of ammonium thiosulfate was dissolved in de-ionized water, and carefully added to each of the treated plots. The soil mass of each treated plot was calculated by:

$$\text{Soil mass (kg)} = \text{soil bulk density (kg m}^{-3}) \times \text{soil depth(m)} \times \text{area of plot (m}^2)$$

A target treatment of 20 cm soil depth was assumed based on our investigation that farmers at Wanshan Hg mine usually ploughed the soil to a depth of about 20 cm. The soil mass is 345 kg for each subplot (1.5 m^2). The treated soils were maintained for 29 days for the thiosulfate and the soil constituents to chemical equilibrate. Oilseed rape seeds were sown directly in the soil on day 30 after amendment with thiosulfate. The seedlings were maintained for 161 days, and harvested on day 191; After the harvested of the oilseed plants, corn seeds were sown directly in the soil on day 191. The seedlings were maintained for 84 days, and harvested on day 276. Subsequently, sweet potato seedlings were planted, maintained for 80 days, and harvested on day 356. Agronomic management protocols for each treatment were performed manually as required. Three individual plant samples and paired soils were randomly collected from each of the three thiosulfate-treated plots and the control plot. One bulk soil profile (20 cm in depth) from each of the control or thiosulfate-treated plots was sampled using a corer after the sweet potato harvest, and it was divided into 5.0-cm intervals.

The roots and shoots of plants were separated, and washed with running tap water, followed by deionized water. The plant materials were freeze-dried and ground to powder. The dry biomass of plants was recorded. Soil samples were freeze-dried, ground in a ceramic disk mill, and sieved to 200-mesh.

2.2. Sample analysis

To determine the total Hg in the soil, approximately 0.1 g powdered soils were digested in a water bath (95 °C) using a fresh mixture of concentrated HCl and HNO_3 (3:1, v/v), and the Hg concentration in the digested solution was measured by cold vapor atomic absorption spectrometry (CVAAS) using a spectrophotometer (F732-VJ, Huaguang Instruments, China). To determine the total Hg in the plants, approximately 0.1 to 0.2 g powdered plants were digested with concentrated HNO_3, and the Hg in the solution was trapped using the gold amalgamation method, and Hg concentration was determined by cold vapor atomic fluorescence spectrometry (CVAFS) using a Tekran 2500 Hg analyzer (Tekran Ltd., Canada).

The bioavailable Hg contents in the soils were determined as previously described (Wang et al., 2011a). Briefly, 1 g of a soil sample was extracted consecutively using 1 M $Mg(NO_3)_2$ and 1 M CH_3COONa (adjusted to pH 5 with CH_3COOH). After each extraction, the extracts were centrifuged at 3500 rpm for 15 min and the supernatant was separated by passage through a 0.45-μm micro-filter. The sum of the concentration of Hg from the two fractions was defined as the bioavailable Hg content.

The standard reference material GBW10020 (orange foliage, manufactured by Institute of Geophysical and Geochemical Exploration, China) and European-certified reference material ERM-CC580 (estuarine sediment, manufactured by the Institute for Reference Materials and Measurements, Belgium) were used for the plant and soil analytical quality controls, respectively. The averages of the total Hg measured of the plant reference material and soil reference material were 0.15 ± 0.01 mg kg^{-1} (n = 3) and 132 ± 1.0 mg kg^{-1} (n = 3), respectively, which were close to the certified values of 0.15 ± 0.02 mg kg^{-1} and 132 ± 3.0 mg kg^{-1}, respectively. The relative percentage difference for the plant and soil sample replicates was <5% and <3%, respectively.

2.3. X-ray absorption near-edge structure analyses

The soil samples collected from 5 g kg^{-1} thiosulfate treatments were used for X-ray absorption spectroscopic (XAS) analysis. They were freeze-dried, sieved to 200-mesh, and pressed into thin tablets prior to analysis. The reference compounds included cinnabar (α-HgS), meta-cinnabar (β-HgS), the nanoparticles meta-cinnabar (nano particulated HgS) and $Hg(SR)_2$, in which α-HgS and β-HgS were purchased from the Dongxin Chemical Reagent Company, China. While the nano particulated HgS and $Hg(SR)_2$ were synthesized as previously described (Gai et al., 2016; Neville and Drakenberg, 1974), and the detailed information for the synthesis is provided in the SI-1.

All the Hg reference compounds were placed on Kapton taps for the XAS analysis. Mercury L$_3$-edge X-ray absorption spectra were obtained at the EXAFS station (1W1B) of the Beijing Synchrotron Radiation Facility (BSRF) with 2.5 GeV electron energy, 250 mA electron current. An energy range of −200 to 450 eV from the L$_3$ edge of Hg (12.284 keV) was used to acquire the spectra. The data for all the soil samples and the standard $Hg(SR)_2$ were collected in fluorescence mode using a 19-element high-purity Ge solid-state detector under ambient conditions, and the data for the solid Hg reference compounds (α-HgS, β-HgS, and nano particulated HgS) were collected in transmission mode. Data normalization (baseline and background corrections) together with cubic spline interpolation, and linear combination fitting were performed using the IFEFFIT XAS analysis package (Gai et al., 2016).

2.4. Data analysis

Statistical analyses were conducted using SPSS17.0 for Windows. The difference between the treatments was tested by the LSD test (equal variance assumed) or Tamhane's T2 test (equal variance not assumed) of a one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. The biomass and mercury contents of the crops

The comparison of the biomass of crops grown in the non-treated and treated soils could reveal the potential effect of thiosulfate on the growth of crops, which was critical for the use of this chemical as an amendment for remediation. No symptoms of toxicity were visible for the oilseed rape, corn, and sweet potato grown in both control and treated soils during the experiment. As shown in Table 1, the dry biomass of a single seedling (without the seed) and the weight of oilseed rape seed were 43.8 to 62.2 g and 14.9 to 22.4 g, and that of the corn were 55.6 to 81.7 g and 91.4 to 120.4 g, respectively, for the four treatments. As for sweet potato, its aboveground tissue and tuberous root (edible section) had a dry biomass of 23.43 to 31.1 g and 57.5 to 66.3 g, respectively, for four treatments.

It appears that the addition of thiosulfate to soil resulted in the reduction of the biomass of oilseed rape and corn, but did not affect the sweet potato. The difference in the dry biomass of sweet potato between the control and thiosulfate treatments was statistically insignificant (Table 1). The weight of seeds from the oilseed rape grown in the treated soils was smaller than that of the control soil, while that of the corn grown in treated soils was similar to the control, except for 5 g kg^{-1} thiosulfate treatment. The weight of the tuberous roots of sweet potato grown in treated soils was similar to that of the control.

We observed that the biomass and yield of oilseed rape and corn were more affected by the addition of thiosulfate to the soil than those of sweet potato, and we attributed this observation to the stress caused by the high salt contents induced by thiosulfate in the soil. This is consistent with previous studies showing the low levels of plant biomass production in Hg-contaminated Tui mine tailings after one week of treatment with thiosulfate solutions (Moreno et al., 2005a). Thiosulfate addition to the soil did not significantly affect the sweet potato yield. This might be explained by the fact that thiosulfate increased the availability of nitrogen and sulfur in the soil (Cassina et al., 2012), essential nutrients for plants, and might counteract the tendency to reduce the biomass.

As shown in Fig. 1, the addition of thiosulfate to soil did not noticeably affect the Hg contents in the tissues of the corn and sweet potatoes compared to the control plants. The average Hg contents in the edible

Table 1
Dry biomass and yield of crops in different treatments (mean ± sd, n = 3).

Plant species	Treatments	Plant (g)	Yield (g)
Oilseed rape	Control	62.16 ± 4.34a	22.36 ± 0.76a
	Ts0.5	43.75 ± 3.28b	16.40 ± 1.73b
	Ts2	50.40 ± 2.64b	14.97 ± 2.61b
	Ts5	46.63 ± 7.71b	15.62 ± 3.65b
Corn	Control	81.66 ± 5.44a	120.40 ± 4.50a
	Ts0.5	55.62 ± 7.02b	102.60 ± 17.86b
	Ts2	66.78 ± 4.28b	112.93 ± 17.42b
	Ts5	58.21 ± 5.99b	91.40 ± 2.02b
Sweet potato	Control	28.82 ± 12.76a	63.78 ± 8.01a
	Ts0.5	31.09 ± 6.97a	63.33 ± 5.88a
	Ts2	27.33 ± 7.44a	66.30 ± 12.83a
	Ts5	23.43 ± 5.61a	57.52 ± 2.25a

Mean values of biomass for each plant with different lower case letters are significantly different ($p < 0.05$) among the treatments. Plant biomass was calculated by sum of the weights from the different tissues of plants, except the edible section. Yield was the biomass of the edible section of crops.

tissues of these plants grown in both control and thiosulfate-treated soils were lower than the maximum allowable Hg contents (10 ng Hg per gram of fresh biomass) in the edible part of the vegetables established by the Ministry of Environmental Protection of China. This result indicates that effect of thiosulfate application to soils on the accumulation of Hg by corn and sweet potato was of the same magnitude as the effect of these plants grown in non-treated soils, i.e., no significant difference between the treated and non-treated soils.

Unlike corn and sweet potato, the Hg contents in the root, stem, pod, and seed of the treated-oilseed rapes were increased by a factor of up to 1.4, 9.6, 16.2, and 18.1, respectively, in comparison with those of the non-treated control. The enhanced Hg accumulation by the tissues of oilseed rape by thiosulfate was consistent with prior studies, which

Fig. 1. Total mercury contents in the tissues of oilseed rape (A), corn (B) and sweet potato (C). Bars denote standard deviation from the mean of three replicates. Significant differences between the control and thiosulfate treatments are indicated by different lowercase letters (*p* < 0.05).

reported that thiosulfate promoted Hg accumulation by different plants (Wang et al., 2014; Smolinska and Rowe, 2014; Lomonte et al., 2011). For instance, Smolinska and Rowe (2014) reported that thiosulfate promoted Hg translocation to aerial parts of *Lepidium sativum* L., which was 10-fold higher than the unassisted plants. Lomonte et al. (2011) showed that the application of $(NH_4)_2S_2O_3$ to soil enhanced the uptake of Hg by *Atriplex codonocarpa*. The studies showed that the predominant mechanisms of thiosulfate-induced Hg accumulation were linked to the increased Hg mobility in soils, the enhanced permeability of the plasma membrane of roots, and the enhanced sulfur accumulation by plants (Wang et al., 2012b, 2018). The contents of Hg in the leaves of the plants (Oilseed rape, corn and sweet potato) were generally higher than that in other tissues, probably due to the accumulation of Hg^0 from the atmosphere at Wanshan Hg mine where the high concentrations of atmospheric Hg^0 of up 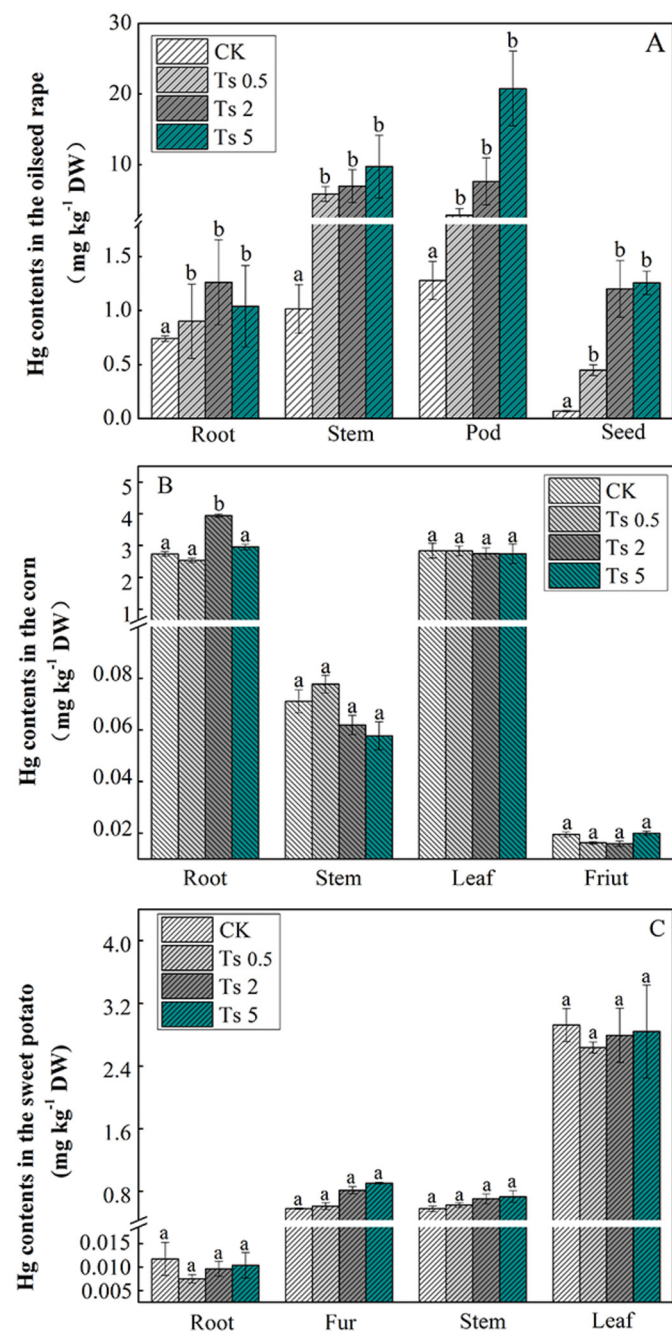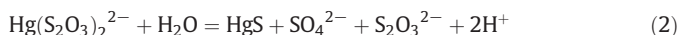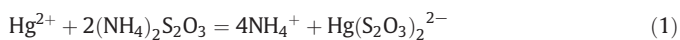to 52,723 ng m^{-3} were recorded (Wang et al., 2011b). To further explain the difference in Hg contents between the crops, the bioavailable Hg content and Hg speciation in the rhizosphere soils from each crop were analyzed.

3.2. The bioavailable Hg contents in the rhizosphere soils

The bioavailable Hg contents in the rhizosphere soils in different treatments were shown in Table 2. The contents of bioavailable Hg in the initial soils ranged from 0.18 to 0.28 ng g^{-1}, which were significantly lower than those in the soils collected on days 191, 276, and 356 (1.60 to 2.77 ng g^{-1}) in both the control and thiosulfate treatments. This indicates that both the growth of plants and application of thiosulfate to the soil resulted in a mobilization of Hg in the soils compared to the initial soils. We further compared the bioavailable Hg contents in the soils between the control and thiosulfate treatments, which were took on days 191, 276, and 356, respectively, and did not observe a significantly increase in bioavailable Hg contents in thiosulfate treatments as compared to that in the control soils at each sampling campaign (except for days 191) (Table 2). It appears that the effect of thiosulfate treatments on Hg mobilization in the soils is of the same magnitude as the effect of the plants grown in the non-treated soils.

Our results differed from prior studies, which found a noticeable increase in bioavailable Hg contents in the thiosulfate treated soils after the addition of thiosulfate for 5–7 days as compared to the control (Pedron et al., 2011; Wang et al., 2014). This difference might be related to the sampling time. Our soil samples were collected after >190 days of thiosulfate amendment, and thus we propose that an immobilization effect for Hg might have happened in the soils after thiosulfate amendments for >190 days.

The speciation of the bioavailable Hg in the pore water of thiosulfate-treated soil is unknown, but is expected to be Hg-thiosulfate (e.g., $Hg(S_2O_3)_2^{2-}$) (Wang et al., 2014). This Hg might be immobilized through the mechanisms of soil mineral adsorption, and geochemical transformation (Zhang et al., 2009; Zhao and Wang, 2010). For instance, the negatively charged complexes could be absorbed by the soil minerals/constituents (e.g., clay particles and organic matter) (Wang et al., 2019). In addition, the Hg-thiosulfate complexes could be converted to Hg sulfides and sulfate upon aging (Eqs. (1)–(2)), resulting in the acidification of the environment (Ullah,

Table 2
Bioavailable Hg content in the rhizosphere soils (ng g^{-1}, mean ± sd, n = 3).

Treatments	Initial soil (0 day)	Rape soil (191 days)	Corn soil (276 days)	Potato soil (365 days)
Control	0.25 ± 0.02a	1.88 ± 0.04a	1.98 ± 0.04a	1.60 ± 0.06a
Ts0.5	0.18 ± 0.03a	2.54 ± 0.05b	1.77 ± 0.03b	1.96 ± 0.04b
Ts2	0.28 ± 0.01a	2.77 ± 0.06c	2.02 ± 0.06a	1.91 ± 0.05b
Ts5	0.24 ± 0.01a	2.22 ± 0.08d	1.60 ± 0.02c	1.79 ± 0.08b

Initial soil means the soil was collected before thiosulfate amendment. Different lowercase letters mean the difference in bioavailable Hg content in the soils at same sampling time between different dosage treatments are significant at *p* < 0.05.

2012). To support this statement, we analyzed the pH of soils (Table S1), and found that pH of the thiosulfate-treated soils (days 191, pH = 7.26; days 276, pH = 7.21; days 365, pH = 7.18) were lower relative to the initial soil (pH = 7.45). This decrease in pH in thiosulfate treated soils might be caused by the acidification process as we proposed above (Eqs. (1)–(2)).

$$Hg^{2+} + 2(NH_4)_2S_2O_3 = 4NH_4^+ + Hg(S_2O_3)_2^{2-} \qquad (1)$$

$$Hg(S_2O_3)_2^{2-} + H_2O = HgS + SO_4^{2-} + S_2O_3^{2-} + 2H^+ \qquad (2)$$

We observed a significant increase in the bioavailable Hg contents in the control soil as compared to the initial soil, indicating that the mobilization of Hg by the plant growth, perhaps through the root exudates promoted Hg mobilization (e.g., organic compounds)(Chen and Yang, 2012). The result is consistent with Wang et al. (2011a), who found an increase of mobile Hg content in the soils by *Chenopodium glaucum* L., and attributed this mobilization to the root activities. A similar phenomenon for other trace elements had been reported (Fresno et al., 2017; Kim et al., 2010). Root exudates included amino acids, organic acids, and other low-molecular-weight organic acid anions (OAAs), which might promote Hg mobility by complexing with Hg to form soluble complexes (Ko et al., 2008; Tao et al., 2004). Also, it is reported that root activities could cause the soil acidification, and this process might contribute to the elevated bioavailable Hg contents in our control soils (Zhao et al., 2018; Rothenberg and Feng, 2012).

3.3. Mercury speciation in the rhizosphere soils

We characterized the speciation of Hg in the solid phase of the soils using Hg L_3-edge XANES spectroscopy to investigate the effect of thiosulfate on Hg speciation fractionation. Bulk XANES spectra were analyzed for four reference compounds α-HgS, β-HgS, nano particulated HgS (nano HgS), Hg(SR)₂, and four soil samples which were collected after the addition of thiosulfate on days 0, 191, 276 and 356, respectively. The results from linear combination fitting (LCF) are shown in Fig. 2. The spectra of four soil samples (day 0, 191, 276 and 356) could be constructed by the spectra of α-HgS, nano particulated HgS, and Hg

(SR)₂, and the calculated amount of three reference compounds in each soil sample is shown in Table 3. The α-HgS, nano particulated HgS and Hg(SR)₂ accounted for approximately 9% to 15%, 26% to 73%, and 16% to 56% of total Hg in the soils, respectively.

We synthesized Hg(SR)₂ using cysteine and Hg(II) to mimic the Hg coordination with sulfur-enriched organic matter in the soils (Neville and Drakenberg, 1974). As shown in Table 3, approximately 56% of the total Hg in the initial soil was in a form similar to Hg(SR)₂, suggesting a close association of Hg and organic matter in our soils. This might be explained by the fact that our experimental site was polluted via the irrigation of Hg-polluted river water, in which Hg presented as soluble forms and it might be complexed with thiol-enriched organic matter in the soil to form Hg(SR)₂ complexes (Nagy et al., 2011). Our result was inconsistent with a prior study, which reported that over 90% of the total Hg in the soil samples collected from Wanshan Hg mine in China was dominated by Hg sulfides (α-HgS and β-HgS) (Yin et al., 2016). The dominance of Hg sulfides in the soils in the study by Yin et al. (2016) was due to their soils were polluted with Hg wastes from a Hg calcine pile, where Hg presented as Hg sulfides, nearby their soil sampling sites.

We identified nano particulated HgS in our soils, which is less crystallized and has fewer average neighbor Hg atoms than the well-crystallized β-HgS (Manceau et al., 2015). This Hg species has been observed in sediments, and is thought to be formed through the reaction of dissolved organic matter (DOM), Hg(II), and sulfides (Aiken et al., 2011). Thus the presence of nano particulated HgS in our soils might be partially sourced from the polluted sediments eroded upstream of river (Deonarine and Hsu-Kim, 2009; Ravichandran et al., 1998; Wen et al., 1997). Further, the endogenous of nano particulated HgS in the soils is possible. Enescu et al. (2016) reported a nucleation process of Hg-thiolates by the dealkylation of thiolate groups in Hg-thiolates to form RS-(HgS)$_n$-R chains, which could be transformed to nanoparticles meta-HgS by self-assembly in parallel arrays. This reaction has been found to occur in solutions with and without microorganism under both oxic and reducing conditions (Enescu et al., 2016). Our soils contained high levels of Hg(SR)₂ (accounts for 56% of total Hg, Table 3), and its aggregation might lead to the production of nano particulated HgS.
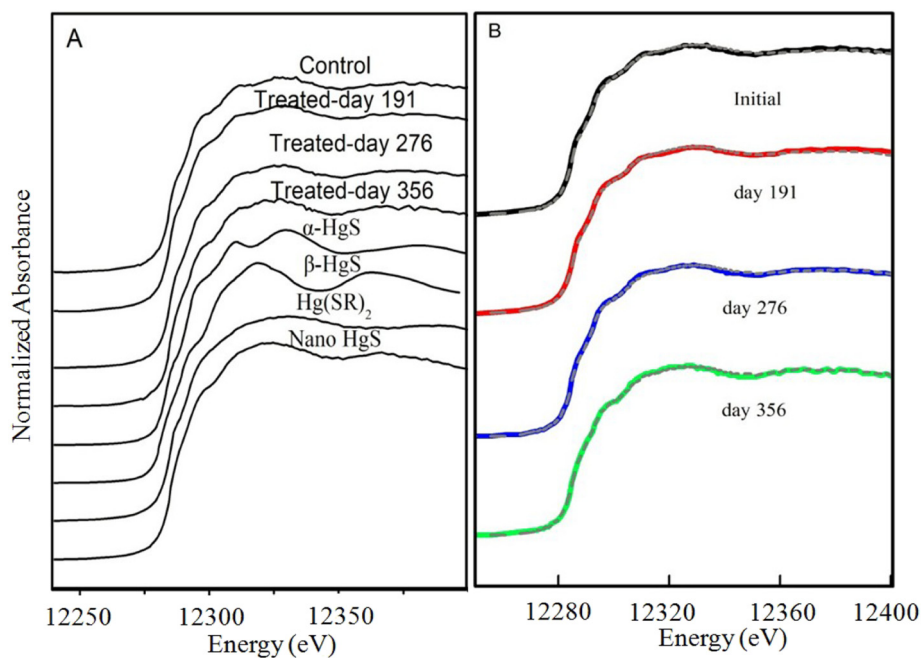

Fig. 2. A: Mercury L_3-edge XANES spectra for different reference Hg compounds (α-HgS, β-HgS, Hg(SR)₂ and nano particulated HgS (Nano HgS)) and soil samples. B: The black, red, blue and green solid lines represents the Hg L_3-edge XANES spectra for the soils collected on the day 0, 191, 276 and 356 respectively, and the gray dashed line represents the best fitted result to respective spectra by linear combination fit (LCF).

Table 3

Hg L$_3$ XANES Least-Squares Fitting of one control and three treated soils to three reference compounds.

Sample	α-HgS (%)	nano particulated HgS (%)	Hg(SR)$_2$ (%)	R factor
Initial	15 ± 6	26 ± 6	56 ± 6	1.1×10^{-3}
Treated day-191	12 ± 5	42 ± 4	43 ± 5	6.8×10^{-4}
Treated day-276	11 ± 4	42 ± 4	46 ± 5	6.1×10^{-4}
Treated day-356	9 ± 5	73 ± 5	16 ± 5	6.8×10^{-4}

We observed that the proportion of nano particulated HgS increased with the time in the thiosulfate-treated soils. The initial soil contained approximately 26% nano particulated HgS, while in the soil samples collected on days 191, 276 and 356 after thiosulfate amendment its proportion was 42%, 42% and 73%, respectively. In contrast to nano particulated HgS, the proportion of Hg(SR)$_2$ in the treated soils almost decreased with the time after thiosulfate amendment, and was 56%, 43%, 46%, 16%, respectively, for the soils of initial, day 191, day 276 and day 356. The oscillation in proportion of nano-β-HgS and Hg(SR)$_2$ indicated a potential transformation of Hg(SR)$_2$ to nano particulated HgS in the thiosulfate-treated soils.

Thiosulfate might preferentially bind with organic matter in the soils through ligand-promoted dissolution processes, and resulted in the mobilization of Hg in this fraction (Wang et al., 2012b; Wang et al., 2014). The thiosulfate-mobilized Hg might either be accumulated by the plants or eventually redistributed into the residual fractions of soils, as documented by previous studies (Wang et al., 2011a). A mechanistic study from Ullah (2012) showed that Hg-thiosulfate complexes could be transformed to HgS upon aging in solutions. It is likely that, in our soils the Hg(SR)$_2$ was mobilized by thiosulfate, and thereafter transformed to nano particulated HgS. The results are also in agreement with our previous discussion (Section 3.2) that the soluble Hg-thiosulfate complexes were transformed into Hg sulfides. In addition, our results showed that the content of nano particulated HgS increased with time, which might indicate a time-dependent transformation of Hg by thiosulfate in the soils. This result is consistent with the previous studies reporting that the extent of Hg-thiolate transformation to nano-β-HgS partially depended on the aging of reaction (Enescu et al., 2016).This may be the scenario by which the immobilization of Hg-thiosulfate complexes in our soils. The proportion of α-HgS (Table 3) remained relatively constant due to its high stability in the environment (Kim et al., 2000; Lowry et al., 2004; Slowey et al., 2005).

3.4. Bioavailable Hg content in the bulk soil profiles

We analyzed the bioavailable Hg contents in the bulk soil profiles sampled after harvesting sweet potatoes (day 356) to investigate the potential leaching of Hg by thiosulfate addition to soils. As shown in Table 4, the content of bioavailable Hg in the bulk soil profiles at 0–5 cm, 5–10 cm, 10–15 cm, and 15–20 cm depths ranged from 0.15 to 0.23 ng g^{-1}, 0.12 to 0.16 ng g^{-1}, 0.15 to 0.19 ng g^{-1}, and 0.17 to 0.19 ng g^{-1}, respectively, in both the control and thiosulfate treatments. The differences in the bioavailable Hg contents in different soil horizons

between control and thiosulfate treatments were not significant, indicating that the application of thiosulfate to soil did not cause a risk of Hg leaching down layer soil profiles after 356 days of incubation.

The bioavailable Hg contents in the bulk soil profiles (0.12 to 0.19 ng g^{-1}) in the thiosulfate treatments were one order of magnitude lower than those in the rhizosphere soils (1.6 to 2.7 ng g^{-1}). It appears that the mobilized Hg was concentrated in the rhizosphere soils (Wang et al., 2012b). We attributed this to the mitigation of the Hg from bulk soil to the rhizosphere driven by mass flow caused by plant transpiration (Wang et al., 2011a), which is comparable with a previous study which reported that *Brassica juncea* was able to drive the Hg movement from bulk soil to its rhizosphere soil by transpiration (Moreno et al., 2005a).

4. Conclusions

Under field conditions, the addition of thiosulfate to the soil resulted in a decrease in the biomass of oilseed rape and corn, which were harvested on 191 days and 276 days, respectively, after the thiosulfate amendment, but did not affect the sweet potatoes which were harvested on day 365. The application of thiosulfate slightly affected the Hg contents in the corn and sweet potato tissues, but increased those in the oilseed rape. Our XANES results indicated that the addition of thiosulfate to soil led to the re-distribution of Hg among its geochemical fractions in the soil, and the proportion of nano particulated HgS was increased, while that of the Hg(SR)$_2$ (organic matter bound Hg) was decreased in the soil with the time extending, and this process appears to be time-dependent. The addition of thiosulfate to soil did not enhance the movement of bioavailable Hg in the soil profiles compared to the control. In summary, application of thiosulfate to the soil led to the immobilization of Hg in the soils, and did not cause the leaching of bioavailable Hg to the downlayer soil profiles, as well as the Hg accumulation in the crops such as sweet potato, in a longer time (>276 days).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.12.010.

Table 4

Bioavailable Hg content in the bulk soil profiles after one year (ng g^{-1}, mean $\pm$ sd, n = 3).

Depth	Control	Ts0.5	Ts2	Ts5
0–5 cm	$0.15 \pm 0.02a(a)$	$0.15 \pm 0.01a(a)$	$0.20 \pm 0.02a(b)$	$0.23 \pm 0.03a(b)$
5–10 cm	$0.14 \pm 0.02a(a)$	$0.16 \pm 0.04a(a)$	$0.16 \pm 0.05a(a)$	$0.12 \pm 0.05b(a)$
10–15 cm	$0.19 \pm 0.04a(a)$	$0.17 \pm 0.06a(a)$	$0.15 \pm 0.03a(a)$	$0.16 \pm 0.04b(a)$
15–20 cm	$0.18 \pm 0.03a(a)$	$0.19 \pm 0.01a(a)$	$0.17 \pm 0.12a(a)$	$0.19 \pm 0.05b(a)$

The different lowercase letters outside brackets mean that the difference in bioavailable Hg content in the soils in each treatment between different soil horizons are significant at $p < 0.05$. Different lowercase letters in the brackets indicate that the difference in bioavailable Hg content in each soil horizon between different treatments is significant at $p < 0.05$.

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