

# Implications of Mercury Speciation in Thiosulfate Treated Plants

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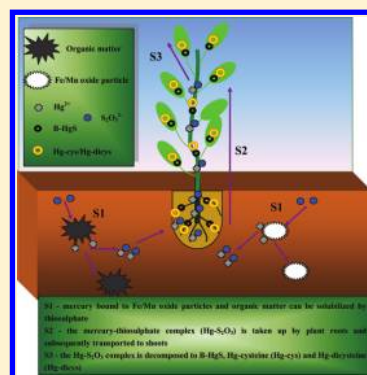
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**ABSTRACT:** Mercury uptake was induced in two cultivars of *Brassica juncea* under field conditions using thiosulfate. Analysis was conducted to better understand the mechanism of uptake, speciation of mercury in plants, and redistribution of mercury in the soil. Plant mercury and sulfur concentrations were increased after thiosulfate treatment, and a linear correlation between mercury and sulfur was observed. Mercury may be absorbed and transported in plants as the Hg–thiosulfate complex. The majority of mercury in treated plant tissues (two cultivars) was bound to sulfur in a form similar to  $\beta$ -HgS (66–94%). Remaining mercury was present in forms similar to Hg-cysteine (1–10%) and Hg-dicysteine (8–28%). The formation of  $\beta$ -HgS may relate to the transport and assimilation of sulfate in plant tissues. Mercury–thiosulfate complex could decompose to mercuric and sulfide ions in the presence of free protons inside the plasma membrane, while sulfide ions would be produced by the assimilation of sulfate. The concomitant presence of mercuric ions and  $S^{2-}$  would precipitate  $\beta$ -HgS. The mercury concentration in the rhizosphere decreased in the treated relative to the nontreated soil. The iron/manganese oxide and organic-bound fractions of soil mercury were transformed to more bioavailable forms (soluble and exchangeable and specifically sorbed) and taken up by plants.



## INTRODUCTION

Mercury (Hg) is ubiquitous in the environment and is present as a result of both natural and anthropogenic processes. In recent years, public concern regarding mercury has increased because of its toxicity, mobility, volatility, and the ease with which this element can become methylated under anaerobic environmental conditions.

Large areas of land around the world have become contaminated with mercury due to extensive mining<sup>1,2</sup> and industrial activities.<sup>3,4</sup> Recently, phytoextraction has been proposed as an option for the remediation of contaminated soils due to the low cost, low impact, aesthetic value, and environmental sustainability of this technology.<sup>5</sup> Many efforts have been conducted on the phytoextraction of heavy metals such as gold (Au), arsenic (As), and nickel (Ni).<sup>6–8</sup> However, very few studies have been focused on the phytoextraction of mercury. This is because no mercury hyperaccumulator plant species have been identified to date. The natural efficiency for the phytoextraction of mercury from polluted soil is limited due to the low bioavailability of this metal in soil. Low bioavailability can be overcome through the use of chemicals (ligands) to promote soil solution solubility. Thiosulfate has been demonstrated to be an effective ligand for increasing mercury bioavailability in soil and thereby enhancing mercury uptake by plants.<sup>9,10</sup> Moreno et al.<sup>11</sup> reported that the mercury concentration in the roots and shoots of thiosulfate-treated *Vicia villosa* (winter vetch) could reach 131 and 15 mg·kg<sup>−1</sup>,

respectively, values which were significantly higher than for the control plants. According to the hard and soft acid–base principle, thiosulfate is a soft base and will preferentially form complexes with soft acid. Thiosulfate can therefore complex with a variety of metals from the IB and IIB classes of the periodical table (e.g., Au and Hg) and will dissolve many insoluble forms of mercury under alkaline conditions.<sup>12–14</sup> Thiosulfate is also a naturally occurring chemical, unlike purely synthetic chemicals used to complex metals in soil, such as ethylenediaminetetraacetic acid (EDTA). Free thiosulfate ions ( $S_2O_3^{2-}$ ) will form through the oxidation of mineral sulfides such as pyrite in the pH range 6–9.<sup>15</sup> However,  $S_2O_3^{2-}$  is unstable under acidic conditions, and thus during chemically assisted phytoextraction the pH of the soil environment must be considered so that any formed mercury–thiosulfate complex has sufficient stability to allow uptake.

Mercury is highly toxic to plants. Generally, plant resistance to environmental mercury has been shown to involve an increased level of thio-containing biomolecules such as phytochelatins (PCs) and total thiols (–SH).<sup>16–18</sup> But thiosulfate-treated plants possess a remarkable ability to accumulate high concentrations of mercury in their roots and

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shoots. However, the mechanisms by which this ligand enhances metal accumulation have not been well elucidated.

Understanding metal speciation is essential to clarify the mechanisms of heavy metal tolerance and accumulation in plants. Lombi et al.<sup>19</sup> analyzed As speciation in the fronds of *Pteris vittata* (Chinese brake fern) using X-ray absorption near-edge spectroscopy (XANES). Their data indicated that the majority of As (75%) was present in the As(III) oxidation state, which might be detoxified through complexation with PCs. In the Zn hyperaccumulator *Arabidopsis halleri* (thale cress), Zn was found to be complexed to malate and carboxyl/hydroxyl functional groups in leaf tissues and to malate and citrate in roots.<sup>20</sup>

The plant species *Brassica juncea* has been demonstrated to have great capacity to accumulate heavy metals and has therefore been widely used as a potential candidate plant for phytoextraction purposes.<sup>21</sup> In this study, two cultivars (*Brassica juncea* Czern. et Coss var. DPDH and *Brassica juncea* Czern. et Coss var. CHBD), which have a native distribution throughout Guizhou province of China and which may have high capacity to accumulate mercury, were used to extract mercury from a contaminated soil.

This study specifically aimed to investigate the speciation of mercury in shoots and roots of the two *B. juncea* cultivars via XANES. The information gained from using this technique is fundamental to a better understanding of the mechanisms of thiosulfate-induced mercury accumulation by plants. In addition, the study also sought to investigate the redistribution (fractionation) of mercury in rhizosphere soil that results from the use of thiosulfate.

## MATERIALS AND METHODS

**Experimental Design.** A field trial for thiosulfate-assisted mercury accumulation in plants was conducted at the Wanshan mercury mine, the largest mercury mine in China. The local environment including soil, water, air, and crop plants has been seriously contaminated with mercury due to extensive mining and refining activities conducted over the past 3000 years.<sup>22</sup> A field experiment covering an area of 20 m<sup>2</sup> constituted the field trial. The area was divided into two plots (5 m × 2 m), with one planted with *B. juncea* Czern. et Coss var. DPDH and the other with *B. juncea* Czern. et Coss var. CHBD. Each plot was divided into two equal-sized subplots (5 m × 1 m), which were designated for control or thiosulfate treatment. Each subplot contained three equal-sized grids of 1.5 m<sup>2</sup> with 50–60 plants per grid. Seeds of the two cultivars were sown into the appropriate plots. Throughout the experiment, weeding, watering, fertilization, and tilling of the soil was done manually as needed. The plants were maintained for 75 days. On day 70, a thiosulfate solution was added to the relevant subplot at a treatment rate of 8 g of thiosulfate/kg of soil, the experimentally derived optimum rate for mercury extraction from Wanshan soil (unpublished data). A target remediation depth of 15 cm was assumed, and the total mass of soil for thiosulfate treatment was calculated as soil density × soil depth × experimental area. The total soil mass in each grid of CHBD and DPDH was calculated to be 0.25 and 0.26 t, respectively; thus 2–2.1 kg of thiosulfate was applied per grid. Five days after treatment with the thiosulfate solution, three individual plant samples were randomly collected from each of the three thiosulfate-treated grids and the three control grids. A soil core with a diameter of 4 cm and height of 10 cm was collected from the root zone of the collected plants (rhizosphere soil). After

harvest, the root and shoot fractions were separated and washed in running tap and then deionized water. All plant samples were freeze-dried and ground to powder. Soil samples were air-dried, ground in a ceramic disk mill, and sieved to 200 mesh.

**Sample Analysis.** The following soil sample properties were measured. The pH of the soil was measured with deionized water (1:2.5 w/w) using a pH meter (Hanna HI3M, Hanna Instruments). Soil texture was determined by use of a Malvern Mastersizer 2000 (Malvern Ltd.), and organic matter (OM) was determined according to the potassium dichromate volumetric method.<sup>24</sup> Total carbon, total nitrogen, and total sulfur were directly measured on an elemental analyzer (PE2400-II). The total mercury concentration in all plant samples was directly measured (solid sample) on a Lumex RA915+ mercury analyzer equipped with a Pyro 915+ pyrolysis attachment by way of thermal decomposition to Hg<sup>0</sup>. The total mercury concentration in the soil was determined by cold vapor atomic absorption spectrometry (CVAAS) after sample digestion as described by Wang et al.<sup>14</sup> The total sulfur concentration in the plant biomass was directly measured with an elemental analyzer (PE2400-II). The geochemical fractionation of mercury in the soil was determined via the sequential extraction procedure described by Wang et al.<sup>14</sup> The recovery rate for soil [GBW (E) 070009] and plant (GBW 10020) reference materials for mercury were in the range of 92–96% and 98–101%, respectively. The recovery rate for plant (GBW 10020) reference materials for sulfur was in the range of 105–111%.

**X-ray Absorption Near-Edge Structure Analyses.** The freeze-dried plant samples were pressed into thin tablets for X-ray absorption spectroscopic (XAS) analysis. Reference compounds (solid) to investigate different mercury oxidation states and chemical structures included cinnabar ( $\alpha$ -HgS), metacinnabar ( $\beta$ -HgS), yellow mercuric oxide (yellow HgO), red mercuric oxide (red HgO), mercuric chloride (HgCl<sub>2</sub>), mercury sulfate (HgSO<sub>4</sub>), mercuric acetate [(CH<sub>3</sub>COO)<sub>2</sub>Hg], Hg-cysteine, Hg-dicysteine, and mercuric selenide (HgSe). Of these compounds, Hg-cysteine and Hg-dicysteine were synthesized by the methods of Andrews and Wyman<sup>25</sup> and Neville and Drakenberg.<sup>26</sup> The other solid compounds were purchased from Dongxin Chemical Reagent Corp., China. All mercury reference compounds were laid on Kapton tape for XAS analysis.

Mercury L<sub>III</sub>-edge X-ray absorption spectra were collected by use of EXAFS beamline 1W1B of the Beijing Synchrotron Radiation Facility (BSRF). An energy range of –200 to 1000 eV from the L<sub>III</sub> edge of mercury (12.28 KeV) was used to acquire the spectra. Data for all plant samples were collected in fluorescence mode under ambient conditions, and data for the mercury reference compounds were collected in transmission mode. Data normalization (baseline and background corrections) together with cubic spline interpolation, Fourier transformation, and EXAFS fitting were performed with the IFEFFIT XAS analysis package.<sup>27</sup>

**Data Analysis.** Statistical analysis was carried out with SPSS 17.0 for Windows. The differences among the treatments was tested by LSD test (equal variance assumed) or Tamhane's T2 test (equal variance not assumed) of one-way analysis of variance (ANOVA).

## RESULTS

**Physicochemical Properties of the Wanshan Soils.** The physicochemical properties of the Wanshan soil are presented

in Table 1. The soil was a sandy loam with an average total mercury concentration across the plot area of  $499 \text{ mg}\cdot\text{kg}^{-1}$ ,

**Table 1. Physicochemical Properties of the Field-Study Soil**

	mean $\pm$ SD, $n = 3$	
	DPDH	CHBD
Soil Parameters		
soil density, $\text{g}\cdot\text{cm}^{-3}$	$1.17 \pm 0.01$	$1.09 \pm 0.02$
pH, 1:2.5	$7.52 \pm 0.01$	$7.67 \pm 0.01$
OM, $\text{g}\cdot\text{kg}^{-1}$	$86.8 \pm 5.9$	$64.2 \pm 4.2$
total C, $\text{g}\cdot\text{kg}^{-1}$	$42.55 \pm 0.8$	$40.55 \pm 0.3$
total N, $\text{g}\cdot\text{kg}^{-1}$	$5.23 \pm 0.53$	$5.54 \pm 0.4$
total S, $\text{g}\cdot\text{kg}^{-1}$	$1.31 \pm 0.18$	$0.81 \pm 0.06$
total mercury, $\text{mg}\cdot\text{kg}^{-1}$	$509.93 \pm 19.21$	$487.50 \pm 58.88$
Particle Size Distribution		
sand ( $>0.05 \text{ mm}$ ), %	58.39	59.45
silt ( $0.002\text{--}0.05 \text{ mm}$ ), %	38.91	38.01
clay ( $<0.002 \text{ mm}$ ), %	2.70	2.54

which is 2 orders of magnitude above the maximum concentration of  $1.5 \text{ mg}\cdot\text{kg}^{-1}$  recommended by the China Environmental Protection Agency for farmland.<sup>28</sup> The soil density was  $1.17 \text{ g}\cdot\text{cm}^{-3}$  for DPDH soil and  $1.09 \text{ g}\cdot\text{cm}^{-3}$  for CHBD soil. The soil pH was slightly alkaline, with consistent total carbon and nitrogen across the plot area but a higher total sulfur concentration for the DPDH plot relative to that for CHBD.

**Mercury Concentration in Plant Tissues.** The mercury concentration in plant shoots and roots is shown in Figure 1. The application of thiosulfate significantly increased mercury accumulation relative to the control plants. The root and shoot mercury concentration of the control plants (both varieties) was below  $0.4 \text{ mg}\cdot\text{kg}^{-1}$ , but this increased to approximately  $56.1 \text{ mg}\cdot\text{kg}^{-1}$  for roots and  $97.3 \text{ mg}\cdot\text{kg}^{-1}$  for shoots after thiosulfate treatment (Figure 1). There was no difference in mercury concentration between the two cultivars. The mercury concentration in the shoots of the thiosulfate-treated plants was significantly higher than that in the roots ( $p < 0.05$ ), indicating that soil mercury mobilized by thiosulfate could be taken up into the roots and subsequently translocated to the shoots of the plants.

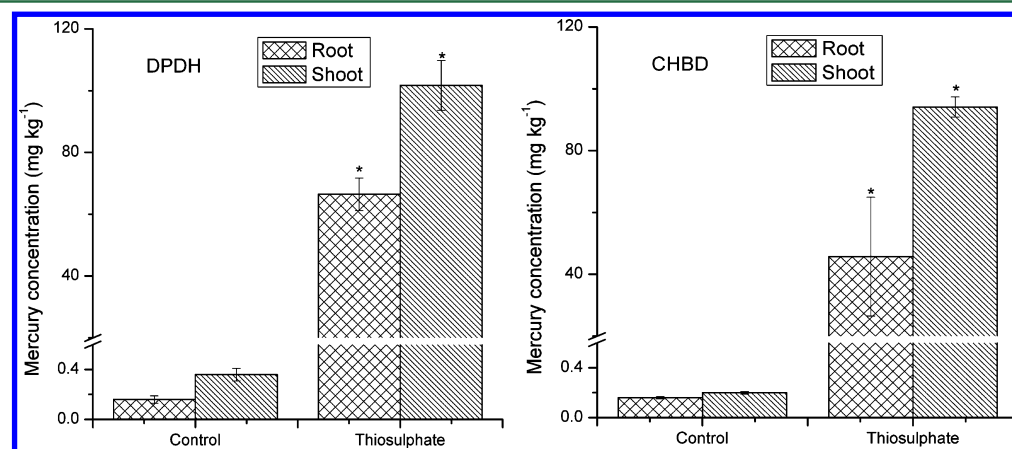
The biomass (dry weight, DW) and calculated amount of mercury extracted by *B. juncea* is presented in Table 2. Cultivar

DPDH shows higher shoot biomass than CHBD for both the control and thiosulfate treatments. However, there was no difference in root biomass (Table 2). There was no reduction in shoot biomass affected by thiosulfate treatment. The application of thiosulfate significantly increased the mass of mercury accumulated by the two cultivars, with the shoots of DPDH accumulating the highest amount of mercury ( $2808 \mu\text{g}/\text{plant}$ ).

**Sulfur Concentration in Plant Tissues.** The sulfur concentration in shoots and roots is shown in Figure 2. The average sulfur concentration in the roots and shoots of the control plants (both cultivars) was  $7.3 \text{ mg}\cdot\text{g}^{-1}$  and  $9.9 \text{ mg}\cdot\text{g}^{-1}$ , respectively. This increased after thiosulfate treatment ( $p < 0.05$ ) to approximately  $21.47 \text{ mg}\cdot\text{g}^{-1}$  for roots and  $43.69 \text{ mg}\cdot\text{g}^{-1}$  for shoots. There was no difference in sulfur concentration between the two cultivars. The concentration of sulfur in roots and shoots was positively correlated with the concentration of mercury (Figure 3), suggesting that a sulfur–mercury complex may be absorbed by the plant roots and translocated to shoots.

**X-ray Absorption Near-Edge Spectroscopy.** The  $L_{III}$ -edge XANES spectra for the different mercury standards and plant samples are shown in Figure 4. The spectrum for each of the mercury standards [yellow  $\text{HgO}$ ,  $\text{HgCl}_2$ ,  $\text{Hg}(\text{CH}_3\text{COOH})_2$ ,  $\text{HgSO}_4$ , and red  $\text{HgO}$ ] contained a peak at an energy around  $12.28 \text{ KeV}$ . However, no distinct peaks were observed in the spectra of Hg-cysteine, Hg-dicysteine, HgSe,  $\alpha$ -HgS, and  $\beta$ -HgS. Generally, Hg-cysteine and Hg-dicysteine type bonds are associated with covalent bonds to sulfhydryl groups in proteins or phytochelatin.<sup>29</sup> No distinct peaks were observed in the spectra of the plant samples. Least-squares linear combination fitting of mercury  $L_{III}$ -edge XANES (Table 3) showed that mercury speciation in the root and shoot samples fits to a combination of inorganic and organic Hg–S coordination, with  $\beta$ -HgS (66–94%), Hg-cysteine (1–10%), and Hg-dicysteine (8–28%) accounting for mercury in the plant biomass.

**Mercury Concentration and Fractionation in Soils.** The total mercury concentration in the rhizosphere soil for both cultivars was significantly ( $p < 0.01$ ) decreased at the end of the experiment for the treated plots relative to the initial soil (Table 4). The concentration of mercury in the soluble and exchangeable, and specifically sorbed fractions was increased at the end of the experiment for the treated plots, relative to the



**Figure 1.** Total mercury concentration in the roots and shoots of *B. juncea* cultivars DPDH and CHBD. Bars denote standard deviation from the mean of three replicates. Significant differences among the control and thiosulfate treatments are indicated by asterisks ( $p < 0.05$ ).



Table 2. Shoot and Root Dry Weight and Total Mercury Mass of Field-Harvested *B. juncea* Biomass

treatment	mean $\pm$ SD, $n = 3$			
	shoot dry weight (g)	root dry weight (g)	shoot Hg mass ( $\mu\text{g}$ )	root Hg mass ( $\mu\text{g}$ )
<i>B. juncea</i> Cultivar DPDH				
control	21.55 $\pm$ 1.46 <sup>a</sup>	1.21 $\pm$ 0.33	7.79 $\pm$ 1.02 <sup>a</sup>	0.19 $\pm$ 0.04
thiosulfate	27.5 $\pm$ 2.99 <sup>a</sup>	1.48 $\pm$ 0.41	2807.69 $\pm$ 485.67 <sup>a</sup>	99.61 $\pm$ 33.93
<i>B. juncea</i> Cultivar CHBD				
control	14.21 $\pm$ 2.48	1.24 $\pm$ 0.36	2.84 $\pm$ 0.63	0.2 $\pm$ 0.06
thiosulfate	16.94 $\pm$ 2.18	1.41 $\pm$ 0.28	1568.22 $\pm$ 158.11	64.46 $\pm$ 32.84

<sup>a</sup>Significant differences between the two cultivars ( $p < 0.05$ ).

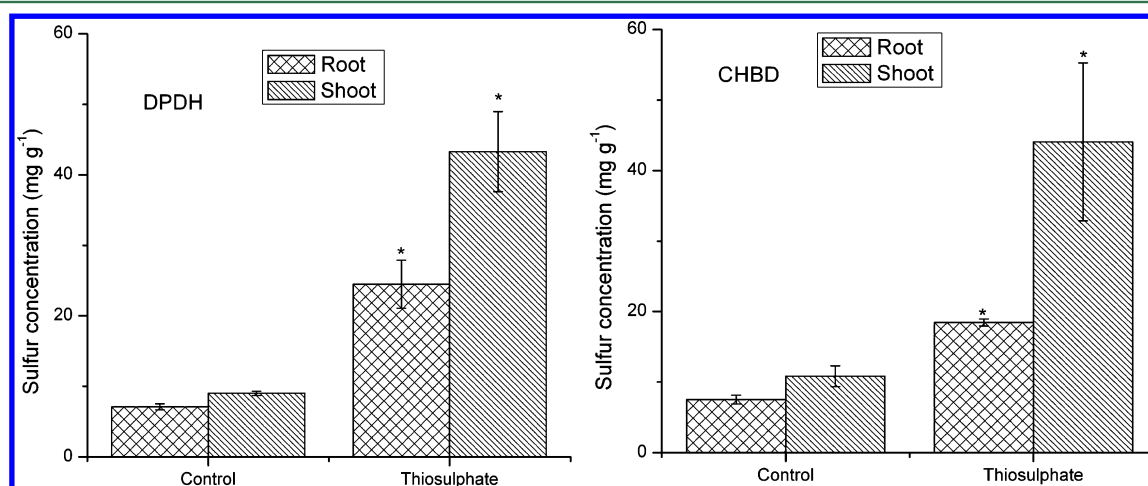


Figure 2. Total sulfur concentration in roots and shoots of *B. juncea* cultivars DPDH and CHBD. Bars denote standard deviation from the mean of three replicates. Significant differences among the control and thiosulfate treatments are indicated by asterisks ( $p < 0.05$ ).

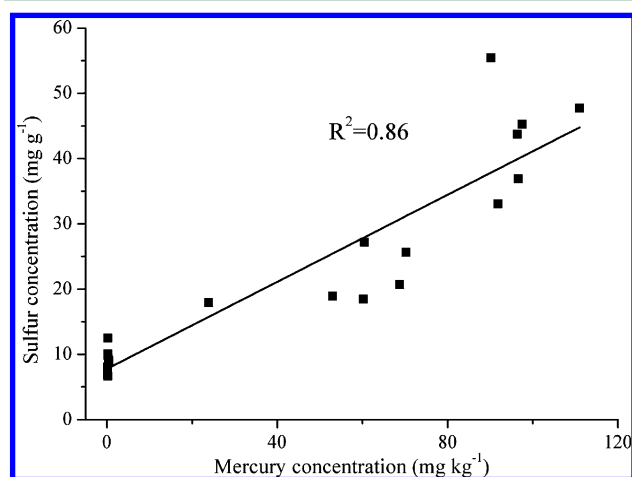


Figure 3. Linear correlation between mercury and sulfur in two cultivars of *B. juncea* (DPDH and CHBD). Data are individual replicates.

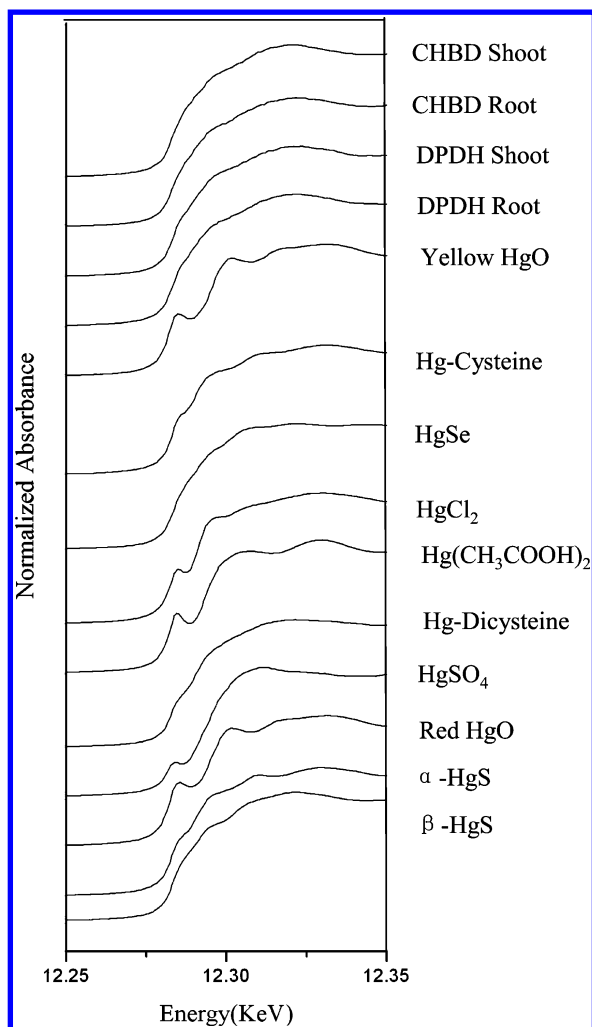
initial soils; while that associated with the iron/manganese oxide and organic bound fractions, decreased. The extent of this decrease for the organic bound mercury was dramatic. With the exception of nontreated CHBD, which shows a lower concentration of mercury bound to iron/manganese oxide than the initial soil, there was no difference in the total mercury concentration or the geochemical fractionation of mercury for the nontreated plots.

Our data indicate that the addition of thiosulfate to soil significantly decreased the total mercury concentration in the rhizosphere soil of the treated plants and that this effect was not

dependent on the cultivar used. Mercury associated with the organic and iron/manganese oxide fractions of the soil appears to have been transformed to a more bioavailable form (soluble and exchangeable and specifically sorbed fractions) and subsequently taken up by plants. The concentration of mercury associated with the residual fraction was relatively stable and not significantly affected by thiosulfate. This indicates that the potentially plant-available pool of mercury in the Wanshan soil is predominantly associated with the iron/manganese oxide fraction and the organic bound fraction.

## DISCUSSION

The two cultivars of *B. juncea* used in this research show similar patterns of ecological distribution and have been used as the parent species in Chinese rape breeding programs.<sup>30</sup> However, the present study is the first application of these species to the field extraction of mercury at a contaminated site. A prerequisite for viable mercury phytoextraction is efficient transport of mercury from soil into the harvestable above-ground biomass. In the present study, the natural uptake of the two cultivars was expected to be limited, based on previous experience and the low bioavailability of mercury in the soil.<sup>14</sup> A chemical (thiosulfate) was therefore added to the soil to induce the accumulation of mercury by the two cultivars, thereby enhancing phytoextraction efficiency.<sup>9,14,31</sup> The principle behind induced or assisted phytoextraction is to apply the chemical to the soil at the point where the plant reaches the greatest biomass, and for *B. juncea*, this occurs about 70 days after germination. Accumulation of the soluble metal complex likely occurs as a function of mass flow driven by evapotranspiration. However, application of the chemical to soil



**Figure 4.** Comparison of normalized Hg L<sub>III</sub> XANES of plant samples (roots and shoots of cultivars DPDH and CHBD) with 10 selected solid-phase model compounds: red HgS (cinnabar), black HgS (metacinnabar), yellow mercuric oxide (yellow HgO), red mercuric oxide (red HgO), mercuric chloride (HgCl<sub>2</sub>), mercury sulfate (HgSO<sub>4</sub>), mercuric acetate [(CH<sub>3</sub>COO)<sub>2</sub>Hg], Hg-cysteine, Hg-dicysteine, and mercuric selenide (HgSe).

**Table 3.** Hg L<sub>III</sub> XANES Least-Squares Fitting of Roots and Shoots to Model Compounds

	$\beta$ -HgS, %	Hg-cysteine, %	Hg-dicysteine, %
DPDH root	74 ± 3	7 ± 3	21 ± 4
DPDH shoot	66 ± 2	7 ± 2	28 ± 2
CHBD root	74 ± 2	10 ± 2	16 ± 2
CHBD shoot	94 ± 1	1 ± nd <sup>a</sup>	8 ± 1

<sup>a</sup>Not detected.

can induce plant stress through water loss and wilting as a function of an increased salt concentration in the soil. This may retard biomass production, and for this reason the chemical is applied only during the later stages of growth. Accumulation occurs rapidly and biomass is harvested within a week of treatment. An alternative treatment option not used in the current study is the more regular application of a low rate of chemical throughout the growth season to promote a constantly high level of bioavailable mercury in the soil.<sup>32</sup> This option could alleviate physiological stress that may be

associated with the application of a concentrated amount of salt to soil.

In the present study, the two cultivars treated with thiosulfate showed significant accumulation of mercury, and shoots accumulated a significantly greater concentration of mercury than roots. Our results are consistent with previous studies that have shown that thiosulfate can increase the concentration of bioavailable mercury in the soil.<sup>14</sup> This soluble mercury can then be quickly taken up by roots and subsequently translocated to the above-ground tissues.<sup>14</sup> The concentration of sulfur in the thiosulfate-treated plants was significantly higher than that in the nontreated control, and it was highly correlated with the mercury concentration in plant tissues. We propose that this provides direct evidence for the complexation of thiosulfate with mercury to form Hg-S<sub>2</sub>O<sub>3</sub>, which is then transported through the root, via the xylem, and will accumulate in the aerial parts of the plants.

The physiological mechanism for this induced mercury uptake and accumulation is unclear. The plasma membrane surrounding root cells is thought to play a major role in forming a barrier to control the uptake and translocation of elements.<sup>33</sup> We speculate that the Hg-S<sub>2</sub>O<sub>3</sub> complex may be transported through some special transporters that are embedded in this barrier. An investigation into the mechanism of transport of the Ag-S<sub>2</sub>O<sub>3</sub> complex in the alga *Chlamydomonas reinhardtii* showed that Ag-S<sub>2</sub>O<sub>3</sub> uptake was mediated by one or more sulfate transporters.<sup>34</sup> A similar result has been observed for arsenate, which is an analogue of the macronutrient phosphate and is transported through phosphate transporters.<sup>35</sup> We assume that mercury may behave similarly to silver and be taken up as an Hg-S<sub>2</sub>O<sub>3</sub> complex by the roots and translocated to the shoots. The shoots of both cultivars recorded a significantly higher total mercury and sulfur concentration than roots. This demonstrates that the Hg-thiosulfate complex may be preferentially transported from roots to shoots, showing similarity to SO<sub>4</sub><sup>2-</sup>, which is preferentially transported from roots to above-ground tissues through stem vessels, a process mediated by the metabolism of sulfur.<sup>36</sup>

The percent abundance of the three dominant forms of mercury in shoots and roots of the two cultivars as determined by XANES follows the trend  $\beta$ -HgS > Hg-dicysteine > Hg-cysteine. The majority of mercury in shoots and roots of the two cultivars was in a form similar to  $\beta$ -HgS (>66%). Both  $\alpha$ -HgS (trigonal type, hexagonal unit cell) and  $\beta$ -HgS (zincblende type, cubic unit cell) are present in the natural environment, but they have very low solubility in the soil, and it is therefore highly unlikely that a plant can directly accumulate these species of mercury from soil. Instead, we propose that  $\beta$ -HgS was formed inside plant tissues. The physiological basis for the formation of  $\beta$ -HgS is unknown. It is known that sulfate is transported across the plasma membrane through the SO<sub>4</sub><sup>2-</sup> transporter, and is symported with protons at a ratio of 1 SO<sub>4</sub><sup>2-</sup>:3 H<sup>+</sup>, a mechanism driven by a proton gradient maintained by a proton ATPase.<sup>37</sup> Literature indicates that S<sub>2</sub>O<sub>3</sub><sup>2-</sup> may also be transported into the cell membrane through the SO<sub>4</sub><sup>2-</sup> transporter.<sup>34</sup> Therefore, the transportation of sulfate may enhance the accumulation of H<sup>+</sup> inside of the membrane, and a relatively acidic condition may become established. Olivares and Aguiar<sup>38</sup> found a positive correlation between free sulfate concentrations and titratable protons in leaf extracts of *Fourcroya humboldtiana* (agavaceae). As discussed above, we speculate that the Hg-S<sub>2</sub>O<sub>3</sub> complex was transported into the plasma membrane through some special

Table 4. Mercury Concentrations, Total and Each Fraction, in the Rhizosphere Soil before and after Experiments

	mercury concentration (mg·kg <sup>-1</sup> ), mean ± SD, n = 3						
	soluble and exchangeable	specifically sorbed	iron/manganese oxide bound	organic bound	residual	summation of each fractions	total mercury by single digestion
	<i>B. juncea</i> Cultivar DPDH						
initial soil	0.01 ± 0.01	0.01 ± 0.001	0.14 ± 0.02	88.53 ± 4.64	420.16 ± 12.41	508.84 ± 9.06	509.93 ± 31.49
planted + treated	0.75 ± 0.03 <sup>a</sup>	0.11 ± 0.07	0.07 ± 0.01 <sup>b</sup>	12.39 ± 1.58 <sup>a</sup>	412.03 ± 14.22	425.36 ± 13.42 <sup>a</sup>	396.46 ± 19.21 <sup>a</sup>
planted	0.04 ± 0.01	0.01 ± 0.003	0.13 ± 0.04	84.48 ± 4.38	425.85 ± 23.15	510.51 ± 24.77	497.25 ± 29.37
	<i>B. juncea</i> Cultivar CHBD						
initial soil	0.01 ± 0.001	0.01 ± 0.005	0.34 ± 0.03	90.04 ± 6.13	408.78 ± 23.15	499.18 ± 24.38	487.50 ± 58.88
planted + treated	0.83 ± 0.06 <sup>a</sup>	0.13 ± 0.01 <sup>a</sup>	0.06 ± 0.01 <sup>a</sup>	6.58 ± 3.16 <sup>a</sup>	361.95 ± 35.49	369.55 ± 38.60 <sup>a</sup>	379.37 ± 1.89 <sup>a</sup>
planted	0.01 ± 0.002	0.01 ± 0.001	0.13 ± 0.01 <sup>a</sup>	85.49 ± 2.32	403.09 ± 12.41	488.73 ± 10.34	465.05 ± 6.99

<sup>a</sup>Significant differences among initial soil, control, and thiosulfate ( $p < 0.01$ ). <sup>b</sup>Significant differences among initial soil, control, and thiosulfate ( $p < 0.05$ ).

transporters. But once across the plasma membrane, it may be decomposed into mercuric ions and sulfate due to the low pH inside the membrane. During the assimilation of sulfur in plant cells, sulfur is transformed to reduced forms such as APS (adenosine 5'-phosphosulfate),  $\text{SO}_3^{2-}$ ,  $\text{S}^{2-}$ , and cysteine, which are the main products of organic sulfur biosynthesis.<sup>39</sup> The occurrence of  $\text{Hg}^{2+}$  and  $\text{S}^{2-}$  may lead to the precipitation of HgS inside plant cells. Additionally,  $\text{S}^{2-}$  is a substrate for synthesizing cysteine. Mercury may therefore preferentially combine with  $\text{S}^{2-}$  rather than cysteine, a hypothesis that agrees with our observation that the majority of mercury is present inside the plant in a form similar to  $\beta$ -HgS. In general, in the presence of sulfide, mercury should form soluble mercury-sulfur complexes or insoluble mercuric sulfide (HgS) solids, depending on the pH and concentration of sulfide and mercury. Insoluble HgS would form at low pH and low sulfide concentrations.<sup>40</sup> The precipitation of  $\beta$ -HgS has been observed in/on the root of *Spartina foliosa* (cordgrass), which was collected from the San Francisco Bay area of the United States. However, in this case the precipitation of  $\beta$ -HgS was attributed to the reduction of sulfate to sulfide by sulfate-reducing bacteria (SRB).<sup>41</sup>

Besides  $\beta$ -HgS, mercury has been found in association with organic sulfur compounds such as cysteine in plant tissues. In the present study, a mercury signal identified as Hg-dicysteine and Hg-cysteine accounted for less than 35% of the total mercury in the plant tissues of the two cultivars. Thiol-containing proteins (for example, cysteine) are thought to play a primary role in the detoxification of trace elements in plants.<sup>39</sup> Rajan et al.<sup>29</sup> used Hg L<sub>III</sub> XANES to study mercury speciation in *Eichhornia crassipes* (water hyacinth). Use of least-squares fitting of the XANES data to model mercury compounds revealed that root mercury was dominated by the two similar forms Hg-dicysteine (68–69%) and Hg-cysteine (10–11%) and that shoot mercury was dominated by the two forms Hg-dicysteine (54–77%) and Hg-methionine (20–25%). Patty et al.<sup>41</sup> reported the use of X-ray microscopy and Hg L<sub>III</sub> XANES to study mercury binding in the roots of *S. foliosa*. Their results indicated that three main types of mercury speciation found in plant tissues were Hg(II)-cysteine (40–72%),  $\beta$ -HgS (3–19%), and methylmercury acetate (9–18%). Carrasco-Gil et al.<sup>18</sup> exposed seedlings of *Medicago sativa* (alfalfa) to 30  $\mu\text{M}$   $\text{HgCl}_2$  for 7 days and analyzed the mercury speciation in the root by extended X-ray absorption fine structure (EXAFS). Their results revealed that mercury was bound in vivo to organic S compounds, such as biomolecules containing cysteine. It is

reported that Hg-cysteine (HgCys), Hg-glutathione (HgGSH), and Hg-phytochelatins (HgPCs) show very similar spectra because mercury in each of these compounds is bound via a sulfhydryl cysteine group of the biothiol and/or protein.<sup>18</sup> Mercury speciation in thiosulfate-treated plant tissues of the present research may therefore not be limited to Hg-cysteine and Hg-dicysteine. It is reasonable to expect that mercury will have also complexed with other thio-containing biomolecules.

Cultivar DPDH exhibits a similar content of  $\beta$ -HgS, Hg-cysteine, and Hg-dicysteine in root and shoot biomass. However, the contents of  $\beta$ -HgS, Hg-cysteine, and Hg-dicysteine in the roots and shoots of cultivar CHBD were different. Specifically, the content of  $\beta$ -HgS was elevated in shoots over roots for cultivar CHBD (Table 3). The TF (transfer factor, defined as the ratio between the shoot Hg or S concentration and root Hg or S concentration) of mercury and sulfur for thiosulfate-treated DPDH was 1.54 and 1.77, respectively, while that for CHBD was 2.42 and 2.4, respectively (Table 5). The higher TF for CHBD indicates

Table 5. Transfer Factors of Mercury and Sulfur<sup>a</sup>

	TF, mean ± SD, n = 3	
	Hg	S
<i>B. juncea</i> Cultivar DPDH		
control	2.34 ± 0.38	1.27 ± 0.04
thiosulfate	1.54 ± 0.25	1.77 ± 0.02
<i>B. juncea</i> Cultivar CHBD		
control	1.24 ± 0.11	1.46 ± 0.32
thiosulfate	2.42 ± 1.40	2.40 ± 0.63

<sup>a</sup>TF = element concentration in shoot/element concentration in root (Hg or S).

that this cultivar transports more sulfur and mercury from roots to shoots than DPDH. The transport of sulfur would enhance the accumulation of  $\text{H}^+$  in plant tissues. Thus increased transfer of sulfur into shoots of CHBD may lead to increased shoot accumulation of  $\text{H}^+$  relative to DPDH, and therefore the pH in the shoots of CHBD may be lower than in DPDH, which may favor the precipitation of HgS.

It is believed that heavy metal-resistant plants either compartmentalize and/or transform metal to other less phototoxic species in order to withstand a high metal burden.<sup>35</sup> The thiosulfate-treated plants in the present study had a higher mercury concentration than the control plants. However, the

majority of this mercury was present in a form similar to  $\beta$ -HgS, a chemical species that is known to have low bioavailability in soil and low toxicity to plants. This indicates that reduction may be a key route for mercury detoxification inside plant tissues. Besides the transport of mercury from soil to the above-ground tissues, another important trait for successful phytoextraction of mercury-contaminated soils is low emission of mercury from leaves to the atmosphere during the phytoextraction process. In a previous study, Moreno et al.<sup>23</sup> reported a reduction of mercury emission from the aerial parts of plants after thiosulfate treatment of soil. The storage of mercury as  $\beta$ -HgS may explain this inhibition of mercury volatilization from thiosulfate-treated plants.

The geochemical fractionation of mercury in the rhizosphere soil changed as a function of the phytoextraction protocol used in this study. The total mercury concentration in the rhizosphere soil of the thiosulfate-treated plot was significantly decreased at the end of the experiment relative to the initial soil, while no significant difference was observed for the control plot. Analysis of the mercury fractionation analysis shows that mercury bound to iron/manganese oxides and organic matter was transformed to more bioavailable forms and subsequently taken up by plants. The results were comparable with a previous study where Wang et al.<sup>14</sup> found a decrease in the concentration of mercury bound to iron/manganese oxides and organic matter after thiosulfate treatment under greenhouse conditions. However, Wang et al.<sup>14</sup> found no increase in the more bioavailable forms of mercury after soil treatment and proposed that all transformed mercury was accumulated by plants. This discrepancy with the present study may be attributed to a greater magnitude of the bioavailable pool of mercury in the field soil relative to pot soil after thiosulfate treatment. The iron/manganese oxide-bound mercury concentration in the soil of the nontreated CHBD was lower than that in the initial soil, while no difference was observed for DPDH. The result may indicate that the roots of CHBD may have an ability to solubilize mercury in soil. It is known that root metabolism can release organic compounds such as low molecular weight organic acids, which are widely reported to have a capacity to increase the metal bioavailability.<sup>42</sup> In general, residual mercury constitutes primary or secondary minerals that may hold mercury within their crystal structure. In the present study, the concentration of residual mercury was relatively stable and was not affected by thiosulfate treatment.

During the trial, solubilized mercury may not have been fully absorbed by plants before harvest due to the continuous migration of soluble mercury from the bulk soil into the rhizosphere. The high concentration of bioavailable mercury in the soil after treatment may pose a risk to the environment. Although outside the scope of the present research, the risk for migration of solubilized mercury away from the rhizosphere and potentially into underlying water resources should be explored.

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### Notes

The authors declare no competing financial interest.

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