

**Research Article**

**Thiosulphate assisted phytoextraction of mercury contaminated soils at the Wanshan Mercury Mining District, Southwest China**

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**Abstract:** Wanshan, known as the “Mercury Capital” of China, is located in the Southwest of China. Due to the extensive mining and smelting works in the Wanshan area, the local ecosystem has been seriously contaminated with mercury. In the present study, a number of soil samples were taken from the Wanshan mercury mining area and the mercury fractionations in soils were analyzed using sequential extraction procedure technique. The obtained results showed that the dominant mercury fractions (represent 95% of total mercury) were residual and organic bound mercury. A field trial was conducted in a mercury polluted farmland at the Wanshan mercury mine. Four plant species *Brassica juncea* Czern. et Coss.var. ASKYC (ASKYC), *Brassica juncea* Czern. et Coss.var.DPDH (DPDH), *Brassica juncea* Czern. et Coss.var.CHBD(CHBD), *Brassica juncea* Czern. et Coss.var.LDZY (LDZY) were tested their ability to extract mercury from soil with thiosulphate amendment. The results indicated that the mercury concentration in the roots and shoots of the four plants were significantly increased with thiosulphate treatment. The mercury phytoextraction yield of ASKYC, DPDH, CHBD and LDZY were 92, 526, 294 and 129 g/ha, respectively.

**Keywords:** Hg mine, soil, Hg fractionation, phytoextraction, thiosulphate

**Introduction**

The soils contaminated with mercury have posed a major environmental and human health problem around the world due to coal combustion, mercury and gold mining activities, as well as industry activities (Li et al., 2009). The remediation of soils polluted with mercury is particularly important because mercury does not degrade and thus persists almost indefinitely in the environment. Therefore, there is great interest in developing methods for mercury removal from contaminated soils.

Traditional remediation methods involve excavation and disposal, stabilization/solidification, electro-remediation, soil washing, thermal desorption and so on. However, they have been losing public acceptance and economic favor, and phytoextraction as an alternative technology has emerged. Phytoextraction is a new cleanup technology that involves the use of plants to clean contaminated soils. The characteristics such as low-cost, low-impact, visually benign, and environmentally sound are

attracting more people involving in this field. For phytoextraction to be worthwhile, the dry biomass of a phytoremediator crop should contain substantially higher concentrations of the mercury than the polluted soil. Unfortunately, several bottleneck processes limiting mercury accumulation in plants. On the one hand, no plant species have been identified as mercury hyper-accumulator plants.

In general, plants accumulate a small amount of mercury in its aboveground tissues. On the other hand, the bioavailability of mercury in soil is limited. Most of mercury in soil is firmly bound to organic matter or sulphides. Only trace concentrations of this element are found in soil solution. As an alternative for hyperaccumulator plants, the large biomass crops have been used for phytoextraction of heavy metal contaminated soil.

The strategy of using crop plants for phytoremediation is a promising approach, since the crop plants are easily cultivated, relatively fast growing and the biomass production is much greater compared to most of the hyper-accumulators. For

mercury, a few studies were conducted to use crops for phytoremediation purpose. The capability of the *Hordeum spp.*, *Lens culinaris*, *Cicer arietinum*, *Lupinus spp.* and *Triticum aestivum* for shoot accumulation mercury were tested in pot experiments. The soil with mercury content ranged between 18.03 and 32.4 mg/kg. The results showed that phytoextraction yield of 4.7g/ha for *Hordeum spp.*, 2.8 g/ha for *Lens culinaris*, 0.4 g/ha for *Cicer arietinum* and *Lupinus spp.*, 0.28 g/ha for *Triticum aestivum*, respectively (Rodriguez et al., 2007). However, such amounts of mercury extracted yearly are negligible in comparison to the magnitude of mercury contamination in the soils (more than 100 kg/ha of total mercury in the 0-25cm).

Recently, many efforts have been done to find some compounds namely chelates which could be used to increase the bioavailability of mercury in soil. Mercury (II) is a soft Lewis acid and complexes readily with soft Lewis bases such as reduced-S ligands. Thus, thio-ligands such as ammonium thiosulphate, sodium thiosulphate were frequently used in phytoextraction of mercury contaminated soil (Moreno et al., 2004; Moreno et al., 2005). In addition, KI, EDTA and urease have been demonstrated to increase the mercury solubility in soil and subsequently enhance the plant uptake mercury from soil (Wang and Greger, 2006; Smolinska and Cedzynska, 2007). Among these chelates, thiosulphate may be a good chelate due to its high capacity to increase mercury concentration in root and transport to aerial parts of the plant. However, the majority of these studies were conducted in the laboratory, rarely studies, were tested in a field condition.

The objectives of this study were (1) to investigate the distribution of mercury fractionations in Wanshan soils, and (2) to study the capacity of the four plant species namely *Brassica juncea* var.LDZY (LDZY), *Brassica juncea* var.CHBD (CHBD), *Brassica juncea* var.ASKYC (ASKYC) and *Brassica juncea* var.DPDH (DPDH) in conjunction with the thiosulphate to phytoextract mercury under field condition.

## Materials and Methods

### Site Description

Wanshan mercury mine, is located at about 360 km east of Guiyang city in the northeastern of Guizhou province, Southwestern China, has a sub-tropical humid climate with an annual average temperature and rainfall of 13.4 °C and 1300mm (Figure 1). The primary ore mineral in the Wanshan deposits is cinnabar, with minor meta-cinnabar. Elemental Hg and Se-rich minerals occurring either as HgSe or Hg

(Se,S) is found locally (Qiu et al., 2005). Between 1949 and early 1990s in Wanshan mercury mining areas, approximately 125.8 million tons of calcines and 20.2 billion cubic meters of Hg-contained exhaust gas had been dispersed into the adjacent ecosystems. The gangue and calcine piles are continuing releasing Hg to the environment, causing serious Hg contamination (Feng and Qiu, 2008). Local environment including soils, water, air, crops has been seriously contaminated with mercury (Feng and Qiu, 2008).

### Soil Sample Collection

Eight soil samples were collected from Wanshan Hg mine and used for mercury fractionation analysis. These soil samples were collected near the Hg mine tailing or residuals. At each sampling site, the final sample was composed of 3–5 sub-samples collected from several localities within an area of 2 m<sup>2</sup> (approximately 1 kg soil). All samples were collected and stored in sealed polyethylene bags to avoid cross contamination. Then in the lab, they were frozen dried, ground in a ceramic disc mill, and sieved to 200 mesh.

### Field trial

The experimental farmland near abandoned mercury mine tailings (Figure 1), covering an area of 100 m<sup>2</sup>, was selected for conducting field experiment of phytoextraction. The area was divided into four plots (5m×2m), and each was planted with LDZY, CHBD, DPDH and ASKYC, respectively. Each plot was further equally divided into two subplots, which were designed as control and thiosulphate treatment. Seeds of the five plants were sown at the experiment field. Each plant species was planted with the space of 5cm ×5cm. After ten days, the five plant seedlings, which were grown in greenhouse, was transplanted to the plots according to the germination of the seeds. During the experiment period, weeding, watering, fertilizing and loosening of the soil were done manually as needed. The plants were maintained for 75 days. At day 70, the (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution was added to the plot at a treatment rate of 8g of thiosulphate per kg of soil. Five days after the addition of (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, all plants were harvested and carefully washed with tap water, then rinsed with deionized water, and finally dried in an oven at 36°C for 48h. Once dry, the plants were separated into roots and shoots using stainless steel scissors, and subsequently homogenized by grinding in preparation for analysis. Associated soil samples from the study sites were also collected from root zone of plants. They were air dried, ground in a ceramic disc mill, and sieved to 200 mesh.

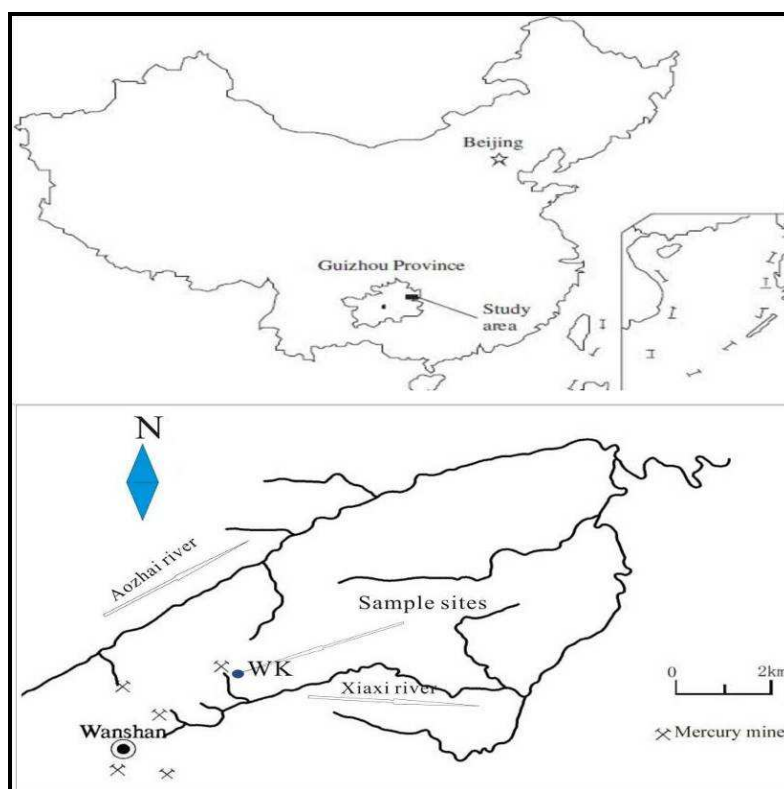


Figure 1. The location of the experimental site

### Sequential Extraction Procedure

The sequential extraction procedure (SEP) which modified from Tessier et al.(1979) and Jeyakumar et al.(2008) was selected because it is well documented, widely used and it has been adapted to the study of soils and sediments(Issaro et al., 2009). Following the sequential extraction procedure, the chemical partitioning of heavy metals allows to distinguish five fractions representing the following chemical phases: exchangeable and soluble (F1), specifically sorbed (F2), oxides bound (F3), organic bound (F4) and residual fraction(F5).

The procedure was carried out with an initial weight of 1 g of the sieved dry soil sample. Deionized water was used in preparing stock solutions that was obtained from a Milli-Q plus system. To check the results of the sequential extraction, the summation of the five fractions for Hg was compared with the results obtained from the total digestion. The sequential extraction procedure is described as:

- a. Fraction1 (F1) The exchangeable and soluble phase: the samples were extracted at room temperature for 1 h with 8 ml of 1 M  $\text{Mg}(\text{NO}_3)_2$  (pH 7) with continuous stirring.

- b. Fraction 2 (F2) The specifically sorbed phase: the washed residue of fraction 1 was leached at room temperature with 8 ml of 1 M NaAc (adjusted to pH 5 with HAc) for 5 h with continuous stirring.
- c. Fraction 3 (F3) The oxide phase: the residue of fraction 2 was extracted with 20 ml of 0.4 M  $\text{NH}_2\text{OH}\cdot\text{HCl}$  in 25% HAc (v/v) for 6 h at 96 °C in a water bath.
- d. Fraction 4 (F4) The organic phase: the residue of fraction 3 was added 8 ml of 30%  $\text{H}_2\text{O}_2$  (adjusted to pH 2 with  $\text{HNO}_3$ ) for 2h at 85°C in a water bath. After 2 h, 3 ml of 30%  $\text{H}_2\text{O}_2$  was added once more (adjusted to pH 2 with  $\text{HNO}_3$ ). The temperature was maintained another 3 h at 85°C in a water bath.
- e. Fraction 5 (F5) The residual phase: the residue of fraction 4 was digested with 10 ml of fresh *aqua regia* for half an hour at 95°C in a water bath.

After each step, the extracts were centrifuged at 3500 rpm per minute and the supernatant was separated after passing through a 0.45  $\mu\text{m}$  micro-filter and the residue was washed two times with 8 ml of DDW before extracting.

### Sample Analysis

The following soil sample properties were measured. The pH of the soil was measured with de-ionized water 1:2 (w/w) using a pH meter. Soil texture was determined using a Malvern Mastersizer 2000 (Malvern Ltd., UK) and organic matter (OM) was determined according to the potassium dichromate volumetric method. Total carbon, total nitrogen, and total sulfur were directly measured using an Elemental Analyzer (PE2400-II, MA, USA).

For THg analysis, soil samples were digested in a water bath (95°C) using a fresh mixture of concentrated HCl and HNO<sub>3</sub> (3:1, v/v) and measured by cold vapor atomic absorption spectrometry (CVAAS) using a F732-S spectrophotometer. The leachate of F3, F4 and F5 were measured by cold vapor atomic absorption spectrometry (CVAAS) using a F732-S spectrophotometer, while F1 and F2 was determined by the dual-stage gold amalgamation method and cold vapor atomic fluorescence spectrometry (CVAFS) using a Tekran 2500.

The plant samples were directly measured (solid sample) using a Lumex RA915+ mercury analyzer equipped with a PYRO 915+ pyrolysis attachment by way of thermal decomposition to Hg<sup>0</sup>. The detection limit of the instrument is 0.2-5 ng/g.

### Statistical analysis

Data were examined by one-way ANOVA followed by LSD (Equal Variance Assumed) or Tamhane's T2 (Equal Variance not Assumed) test as available in the SPSS 17.0 statistical package.

### Quality Control and Assurance

The standard reference materials GBW (E) 070009 and GBW10020 (Manufactured by the Institute of Geophysical and Geochemical Exploration, China) were used for soil and plant analytical QC, respectively. The average total mercury concentration of the geological standard GBW (E) 070009 was  $2.41 \pm 0.3$  mg/kg ( $n = 3$ ), which is comparable with the certified value of  $2.20 \pm 0.40$  mg/kg.

The average total mercury concentration of the orange' foliage standard GBW10020 was  $0.14 \pm 0.03$  mg/kg ( $n = 3$ ), which is comparable with the certified value of  $0.15 \pm 0.02$  mg/kg. The relative percentage difference of sample replicates for soil and plant were < 10% and <6% respectively.

## Results and Discussion

### Hg Fractionations

The validation of SEP method was evaluated as the correlation between the independently determined total Hg concentration and the sum of extracted Hg fractions. There was a good agreement between the independently determined total Hg concentration and the sum of extracted Hg fractions ( $R^2=0.99$ ) (Figure 2), this demonstrated that the sequential extraction technique was able to account for Hg speciation in this geochemical system. The contributions of soluble and exchangeable fraction in the total Hg concentration were quite low (3 ng/g) in Wanshan soil samples (Table 1).

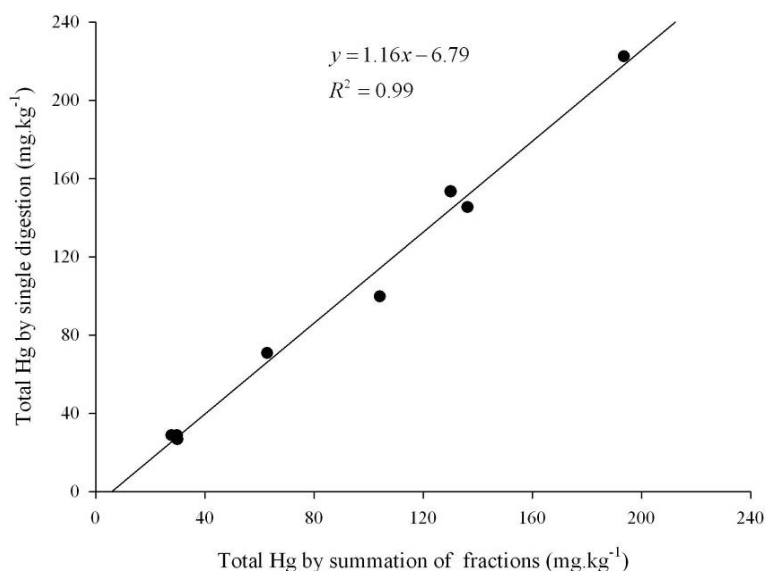


Figure 2. The correlation between Hg concentration obtained from single digestion and summation of each fractions ( $n=8$ )

Table 1 Summary statistical data for concentration of each Hg fraction in Wanshan soil ( $\mu\text{g/g}$ ) ( $n=8$ )

	F1	F2	F3	F4	F5
Wanshan Soil	$0.003 \pm 0.004$	$0.004 \pm 0.01$	$6.5 \pm 11.9$	$29.79 \pm 22.58$	$53.05 \pm 42.41$

The specifically sorbed Hg or carbonate associated Hg means the Hg precipitated or co-precipitated with carbonate constituent. The average concentration of F2 in soil samples were 4 ng/g. The proportion of F1 and F2 represented less than 0.01% of the total Hg in Wanshan soil samples (Figure 3).

The result was comparable with the previous studies. For example, in the soil samples from Badajoz province in Spain, the contribution of exchangeable fractions (extracted by  $1 \text{ mol L}^{-1} \text{NH}_4\text{NO}_3$ ) varied from 0.002% to 0.2 % (García-Sánchez et al., 2009). However, the study performed in relation to the Hg contaminated soil near the Chlor-Alkali plant have found much higher exchangeable Hg levels (represent 39.6% of THg, extracted by 0.5M  $\text{NH}_4\text{Ac-EDTA}$ ) (Neculita et al., 2005).

The oxide bound Hg is mainly bound to metal oxides such as Fe, Al, and Mn oxide and has limited bioavailability. Hg in this fraction was significantly

higher than the F1 and F2. The proportion of F3 represented 0.31%- 13.4% of the total Hg in soil. The organic bound Hg concentration ranged from 4 to 60 mg/kg, which represented 12.3–59% of the total Hg (Figure 3). The reduced sulfur functional groups such as thiol (R-SH) in organic matter have been found to play an important role in binding with Hg (Xia et al., 1999). For instance, Neculita et al. (2005) reported that the organic bound Hg was affected by the soil organic carbon content. Generally, Residual Hg was combined with primary or secondary mineral which may hold trace metals within their crystal structure and Hg in this fraction usually presents less bioavailability and is not available for methylation. Cinnabar ( $\text{HgS}$ ) is the main compound of the Residual Hg. The largest Hg proportion was found within the residual fraction, which represented 20–87% of total Hg. In all soil samples, the organic bound Hg and residual Hg represent nearly 95% of total Hg.

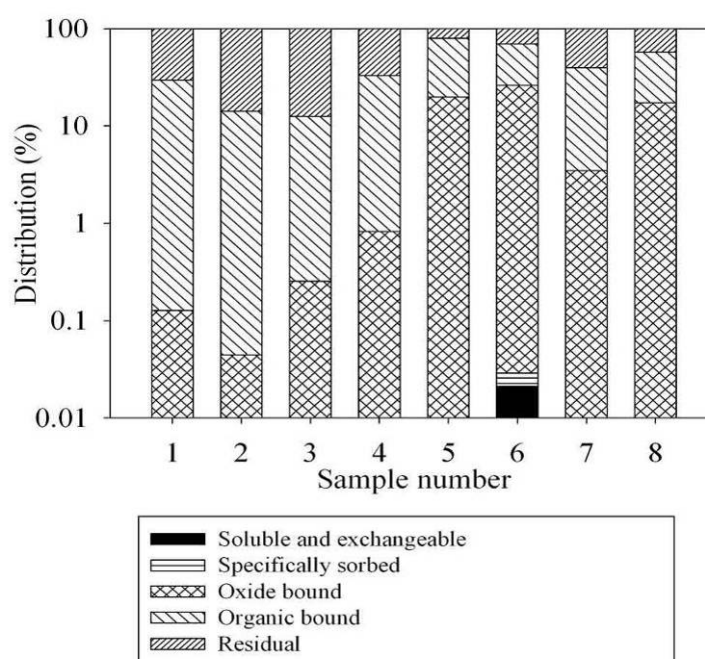


Figure 3. The distribution Hg fractions in Wanshan soils

### Field trial

#### The physico-chemical properties of the soil

The physico-chemical properties of the Wanshan soil are presented in Table 2. The pH of the five tested soils was slightly alkaline. In general, organic matter and total carbon concentrations in the five soils were relatively consistent, while the total nitrogen concentration in the soils of ASKYC was higher than the LDZY, DPDH and CHBD. With the single exception of DPDH, the total sulfur concentration in the other three soils showed similar concentration. The sand loam was the main soil texture for all samples. The total mercury concentration in the soil samples ranged from 404.17 to 515.51 mg/kg.

#### The mercury concentration in the four plant varieties

The majority of mercury in Wanshan soil presented as non-available forms, and thus the thiosulphate was

added to soil to increase the bioavailability of mercury in soil. The shoots and roots mercury concentration of both control and thiosulphate treated plants are showed in Table 3. In the control plots, the concentration of mercury in the roots and shoots of the four plants were in the range of 0.12-1.02 mg/kg and 0.19-0.36 mg/kg, respectively. The highest shoots and roots mercury concentration were recorded in the DPDH and LDZY respectively. The application of thiosulphate significantly increased both the roots and shoots mercury concentration ( $p<0.05$ ). The DPDH and CHBD exhibited the highest shoot mercury concentration with an average value at 101.7 and 92.8 mg/kg respectively. Similarly, the highest root mercury concentration was recorded in DPDH (66.5 mg/kg), however, no significant differences were observed among the DPDH, CHBD and LDZY ( $0.05<p$ ).

Table 2. Physico-chemical properties of the Wanshan soil (mean $\pm$ sd, n=3).

Soil parameters		LDZY	DPDH	CHBD	ASKYC
pH (1:2.5)		7.82 $\pm$ 0.02	7.52 $\pm$ 0.01	7.67 $\pm$ 0.01	7.86 $\pm$ 0.02
OM (g/kg)		84.1 $\pm$ 4.3	86.8 $\pm$ 5.9	64.2 $\pm$ 4.2	61.8 $\pm$ 0.7
Total C (g/kg)		40.64 $\pm$ 0.13	42.55 $\pm$ 0.80	40.55 $\pm$ 0.30	41.39 $\pm$ 0.14
Total N (g/kg)		5.45 $\pm$ 0.46	5.23 $\pm$ 0.53	5.54 $\pm$ 0.40	6.69 $\pm$ 0.60
Total S (g/kg)		0.63 $\pm$ 0.05	1.31 $\pm$ 0.18	0.81 $\pm$ 0.06	0.53 $\pm$ 0.04
Particle size distribution	Sand %>0.05mm	57.52	58.39	59.45	56.34
	Silt %0.002-0.05mm	39.85	38.91	38.01	40.22
	Clay %<0.002mm	2.62	2.70	2.54	3.44
Total mercury (mg/kg)		495.57 $\pm$ 26.20	515.51 $\pm$ 14.47	505.85 $\pm$ 19.78	404.17 $\pm$ 5.80

Table 3 Mercury concentration in the root and shoot of the four plant varieties (mean $\pm$ sd, n=3).

THg mg/kg	Control		Thiosulphate	
	Root	Shoot	Root	Shoot
LDZY	1.02 $\pm$ 0.16 a	0.35 $\pm$ 0.04 a	39.57 $\pm$ 13.73 ab	26.97 $\pm$ 14.46 b
DPDH	0.16 $\pm$ 0.03 a	0.36 $\pm$ 0.05 a	66.5 $\pm$ 5.26 a	101.7 $\pm$ 8.07 a
CHBD	0.16 $\pm$ 0.01 a	0.20 $\pm$ 0.01a	45.7 $\pm$ 19.22 ab	92.8 $\pm$ 3.22 a
ASKYC	0.12 $\pm$ 0.02 a	0.19 $\pm$ 0.03 a	15.37 $\pm$ 2.00 b	34 $\pm$ 10.75 b

a,b  $p<0.05$

#### The biomass yield of the five plant varieties

Plant biomass was one of important parameters of phytoextraction. The total dry weight yield of the root and shoot's biomass of the four plant species are showed in Table 4. In the control plots, the DPDH produced the largest shoot biomass with the value at 4.04 t d.w. /ha/yr, however, the shoot yield of the four plant species were statistically insignificant ( $0.5<p$ ). Similarly, the root biomass of the four plant species

were statistically insignificant ( $0.5<p$ ). The application of thiosulphate did not have significant effect on the shoot and root biomass of the plants. In the thiosulphate treated plots, the DPDH and LDZY produced the largest shoot biomass with the value around 5 t d.w./ha/yr, while the LDZY produced the largest root biomass with the value at 0.53 t d.w./ha/yr.

Table 4. The biomass and phytoextraction yield of the four varieties of *Brassica juncea* L (mean±sd, n=3).

Treatment	Variety	BP <sub>shoot</sub> (t d.w./ha/yr)	BP <sub>root</sub> (t d.w./ha/yr)	PP <sub>shoot</sub> (g/ha/yr)	PP <sub>root</sub> (g/ha/yr)
Control	LDZY	3.96±0.20 a	0.33±0.10 a	1.41±0.22 a	0.32±0.09 a
Control	DPDH	4.04±0.27 a	0.23±0.06 a	1.46±0.19 a	0.04±0.01 a
Control	CHBD	2.67±0.46 a	0.23±0.07 a	0.53±0.12 b	0.04±0.01 a
Control	ASKYC	3.14±0.50 a	0.36±0.05 a	0.62±0.19 b	0.04±0.002 a
8g/kg Thiosulphate	LDZY	4.96±0.56 a	0.53±0.11 a	129.28±59.23 c	19.88±3.23 a
8g/kg Thiosulphate	DPDH	5.16±0.56 a	0.28±0.08 b	526.70±91.11 a	18.69±6.36 a
8g/kg Thiosulphate	CHBD	3.18±0.41 b	0.26±0.05 b	294.19±29.66 b	12.09±6.16 ab
8g/kg Thiosulphate	ASKYC	2.72±0.02 b	0.23±0.001 b	92.60±29.88 c	3.60±0.45 b

a,b  $p < 0.05$ 

### The phytoextraction yield

The phytoextraction yield of the four plant species are showed in Table 4. In the control plots, the shoot and root of the four plant species had the similar phytoextraction yield. Obviously, the application of thiosulphate significantly enhanced the phytoextraction yield of the four plant species, indicating that the thiosulphate-assisted phytoextraction was more effective than natural phytoextraction. With thiosulphate treatment, the highest shoot's phytoextraction yield was showed by DPDH which had an average value of 526.7 g/ha/yr, followed by CHBD which had an average value of 294.19 g/ha/yr. The shoot's phytoextraction yield of the LDZY and ASKYC showed a similar level under the thiosulphate treatment. The root accumulated less amount of mercury than the shoot. The root of the LDZY, DPDH and CHBD showed a similar level of phytoextraction yield. Among the four plant species, the DPDH showed a great potential for phytoextraction of Hg-contaminated soil with thiosulphate treatment.

### Conclusion

The results from the present study indicate that the dominate mercury fractions in Wanshan soils were residual and organic bound mercury. The bioavailability of mercury in Wanshan soils was limited. With the thiosulphate treatment, the *Brassica juncea* Czern. et Coss.var.DPDH (DPDH) showed a great potential for phytoextraction of Hg contaminated soil under field condition.

### Acknowledgements

This research was financed by the National High-tech Research and Development Program of China (2008AA06Z335). Many thanks for the Institute of Cole, Guizhou Academy of agricultural sciences for supporting the plant seeds.

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