Selenium fractions in organic matter from Se-rich soils and weathered stone coal in selenosis areas of China

Hai-bo Qin [a,b], Jian-ming Zhu [a,*], Hui Su [c]

[a] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, China
[c] Center for Disease Control and Prevention of Enshi Prefecture, Enshi 445000, China

ARTICLE INFO

ABSTRACT

A high degree of association between Selenium (Se) and organic matter has been demonstrated in natural environments, but Se fractions and speciation in organic matter is unclear. In this study, a method for quantifying organic matter associated with Se (OM-Se) was developed to investigate Se fractions in organic matter in Se-rich soils and weathered stone coal from Enshi, China, where Se poisoning of humans and livestock has been documented. Initially, Se was extracted using water and a phosphate buffer. Subsequently, OM-Se was extracted using NaOH, and then speciated into Se associated with fulvic acids (FA-Se) and humic acids (HA-Se). Both FA-Se and HA-Se were further speciated into the weakly bound and strongly bound fractions using a customized hydride generation reactor. The results show that FA-Se (1.91–479 mg kg^{-1}) is the predominant form of Se in all Se-rich soils and the weathered stone coal samples, accounting for more than 62% of OM-Se (3.07–484 mg kg^{-1}). Weakly bound FA-Se (1.33–450 mg kg^{-1}) was prevalent in the total FA-Se, while weakly bound HA-Se (0.62–26.2 mg kg^{-1}) was variable in the total HA-Se (1.15–32.5 mg kg^{-1}). These data indicate that OM-Se could play a significant source and sink role in the biogeochemical cycling of Se in the supergene environment. Weakly bound FA-Se seems to act as a potential source for bioavailable Se, whereas strongly bound HA-Se is a possible OM-Se sink which is not readily transformed into bioavailable Se.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Selenium (Se) is an essential trace element for humans and animals, and it is of interest as both a nutrient and an environmental toxin, owing to a narrow range between deficient and toxic concentrations. Selenium deficiency in humans is regarded as one of the causes for two endemic diseases: "keshan disease" and "white muscle disease" (Wang and Gao, 2001; Lenz and Lens, 2009). Selenium toxicity results in waterfowl death, nervous system disorders, nails and hair loss, and other symptoms in humans, livestock and sea animals (Yang et al., 1983; Zheng et al., 1999; Dörr et al., 2008; Hoang and Klaine, 2008; Lenz and Lens, 2009). In addition, the long-lived ^{79}Se (half-life $\sim$1.1 million years), a fission product of ^{235}U, is considered to be one of the most hazardous radionuclides; corresponding environmental effects have been observed (Bienvenu et al., 2007; Kamei-Ishikawa et al., 2008; Jordan et al., 2009).

In the natural environment, Selenium occurs in several oxidation states (-II, 0, IV, and VI) and organic forms. Its solubility, mobility, bioavailability, and toxicity are strongly controlled by its chemical speciation (Frankanberger and Benson, 1994). Selenate (Se(VI)), the most oxidized form of Se, is highly water soluble, bioavailable and highly mobile. Selenite and hydroselenite species (Se(IV)) generally tend to adsorb to the surfaces of organic matter, clay minerals, and metal-oxides such as Fe-, Mn- or Al-oxides. Elemental Se (Se(0)), which can be produced by microbial and abiotic reduction of both oxidized forms (Chen et al., 2009; Ranjan Mishra et al., 2011), is poorly soluble, and thus less mobile and bioavailable. The lowest oxidation state (Se(-II)) is found in insoluble selenide minerals and organic Se compounds formed by biotic reduction (Frankanberger and Benson, 1994; Kulp and Pratt, 2004; Lenz et al., 2008; Lenz and Lens, 2009). It is, therefore, not sufficient to assess Se bioavailability and geochemical behavior by using total Se content. The analysis of Se speciation is crucial and has received considerable attention (Zhang and Moor, 1996; Zawislanski et al., 2003).

Previous studies have demonstrated a high degree of association between Se and organic matter (generally humic substances) in aquatic and terrestrial environments (Kang et al., 1991a, 1993; Gustafsson and Johnsson, 1994; Martens and Suarez, 1997a; Séby

* Corresponding author. Address: Institute of Geochemistry, Chinese Academy of Sciences, No. 46, Guanshui Road, Guiyang 550002, China. Tel.: +86 851 5895787; fax: +86 851 5891609.
E-mail address: zhujianming@vip.gyig.ac.cn (J. Zhu).

et al., 1997; Dhillon and Dhillon, 1999; Wright et al., 2003; Kulp and Pratt, 2004; Dhillon et al., 2010; Oram et al., 2008; Coppin et al., 2009), but the interaction between Se and organic matter is not clearly understood and is still in debate. One possibility is that Se is directly complexed with organic matter (Dhillon and Dhillon, 1999; Bruggeman et al., 2007; Dhillon et al., 2007; Darcheville et al., 2008). The second hypothesis is that Se is indirectly absorbed by OM-metal (Fe, Al etc.) complexes to form a "Se-OM-metal" ternary system (Gustafsson and Johnsson, 1994; Coppin et al., 2009; Jordan et al., 2009). The third explanation centers around that Se(-II) production by microbes or plants can be incorporated into amino-, carboxyl- or hydroxyl-groups present in organic matter (Van Dorst and Peterson, 1984; Abrams et al., 1990; Gustafsson and Johnsson, 1994; Darcheville et al., 2008; Dhillon et al., 2010). However, it is not possible to adequately interpret the interaction between Se and organic matter in nature with the above explanations alone. To better understand the association between Se and organic matter, analysis of the Se fraction and speciation in organic matter is necessary. Moreover, organic matter associated Se (OM-Se) is of particular interest in evaluating Se bioavailability owing to a part being directly or indirectly taken up by plants (Abrams and Burau, 1989; Kang et al., 1993; Gao et al., 2007; Dhillon et al., 2007). Some studies have asserted that selenomethionine, a form of Se absorbed by plants, occurs in the fulvic acids fraction of soils (Abrams and Burau, 1989), whereas others showed that selenocystine could be oxidized to inorganic Se and thus released in some conditions (Martens and Suarez, 1997b).

In general, operationally defined OM-Se was usually extracted using NaOH solution, yet only a few studies are available on Se speciation in NaOH extract (Kang et al., 1991a, 1993; Martens and Suarez, 1997a; Kulp and Pratt, 2004; Gao et al., 2007; Pumure et al., 2010). Measurement of Se(IV) is the most important step to determine Se speciation in NaOH extract, and other Se species can be calculated based on the Se(IV) determination (Séby et al., 1997; Kulp and Pratt, 2004; Gao et al., 2007). However, determination of Se(IV) content is hindered by the high organic matter content present in NaOH extract when Hydride Generation-Atomic Fluorescence Spectrometry/Atomic Absorption Spectrometry (HG-AFS/AAS) is used to quantify concentration. HG-AFS/AAS has been widely employed for Se estimation due to its low detection limits (Zhang et al., 1999a; Cai, 2000; Irizarry et al., 2001; Capelo et al., 2006; Zhu et al., 2008). However, organic matter can react with boron hydride (BH_4^-) to produce effervescence and/or foam which perturbs Se determination (Zhang et al., 1999a). Although polymeric adsorbent resins (XAD) can be used to remove organic matter, it cannot be neglected that a fraction of Se(IV) was also removed due to its association with organic matter (Gustafsson and Johnsson, 1994; Zhang et al., 1999a). Conventionally, it is assumed that only Se(IV) can react with BH_4^- and produce H_2Se; however, some organic Se compounds (selenomethionine, selenoethionine, dimethylselenone, etc.) have been proven to react with BH_4^- to form volatile Se compounds by recent studies (Chatterjee and Irgolic, 1998; Zhang et al., 1999a; Zhang and Frankanberger, 1999b; Chatterjee et al., 2001). These volatile Se compounds would increase H_2Se signal intensity and thus cause an overestimation of Se(IV) content using HG-AFS/AAS. For these reasons, it is necessary and urgent to develop a more reliable method for quantifying Se fraction and speciation in organic matter.

Enshi Prefecture of Hubei Province, is one of the high-Se areas in China, where a sudden incidence of human Se poisoning occurred. In this area, previous studies (Yang et al., 1983; Fordyce et al., 2000; Zhu and Zheng, 2001; Zhu et al., 2004, 2008) have mostly focused on the total and water-soluble Se level, but reports about Se fractions in organic matter from soils and rocks are lacking. The objectives of this study are to develop an effective method for quantifying OM-Se fractions, and investigate the distribution of Se fractions in OM-Se in Se-rich soils and weathered stone coal samples from Enshi, China, in order to better understand OM-Se biogeochemistry in the Se-rich surface environment. Moreover, the results of this study will provide strong evidence for the local government to take measures to prevent misuse of stone coal as a crop fertilizer in order to avoid possible Se poisoning in Enshi, China.

2. Materials and methods

2.1. Sample collection and preparation

The Yutangba study area is located in the southeast part of Enshi District, Hubei Province, China (Fig. 1). Its geology has been well described in previous studies (Fordyce et al., 2000; Zhu and Zheng, 2001; Zhu et al., 2004, 2008). In this area, soils are thin with poorly developed horizons, and the A, B, and C soil horizons cannot be distinguished. Thus, near-surface (0–30 cm) soil samples were collected. Stone coal, namely Se-rich carbonaceous chert and carbonaceous shale, was collected from discarded spoils in the field. The spoils result from the use of Se-rich coal as fuel for making lime or baking soil, or as a fertilizer after being ground into powder by local villagers. Based on the extent of weathering and impact of human activities, representative samples were selected and categorized into three groups: (1) stone coal (SCS-1 to SCS-3), representing the weathered samples, (2) uncultivated soils (US-1 to US-3), representing samples with less human activity, and (3) cropland soils (CS-1 to CS-2), representing samples with greater human activity. In the laboratory, soil samples, free of plant roots and detritus, along with the weathered stone coal samples were freeze-dried, and then ground to pass through a 150 mesh sieve (106 μm) and stored in clean polyethylene bags for chemical analysis.

2.2. Water-soluble and ligand-exchangeable Se extraction

Water-soluble and ligand-exchangeable Se were sequentially extracted using Milli-Q water (18.2 MΩ cm) and 0.1 M KH_2PO_4+ K_2HPO_4 buffer (P-buffer, pH 7.0), respectively (Martens and Suarez, 1997a; Kulp and Pratt, 2004; Pumure et al., 2010). Samples were first weighed (0.5–1 g) into a 50 mL in-house blown glass centrifuge tube (to avoid organic matter adsorption onto the inner wall of the tube). Milli-Q water (10 mL) was added and mixed using a vortex2 mixer, then the tube was shaken on a reciprocating shaker (175 oscillations min^{-1} at room temperature) for 2 h. After centrifugation (3500g; 20 min), the supernatant was decanted into a polyethylene vessel. The residue was washed with 10 mL Milli-Q water and the above procedures were repeated. Ten mL of 0.1 M P-buffer (pH 7.0) was added to the residue from the water extraction, repeating the procedures mentioned above, and then 10 mL of Milli-Q water was added for rinsing. The supernatant water was combined with the previous P-buffer extract.

2.3. OM-Se extraction and separation

To get OM-Se extraction, the remaining residue from the P-buffer treatment was extracted with 10 mL 0.1 M NaOH under N_2 atmosphere according to the method described by IHSS (Swift, 1996; Janos, 2003). In our study, OM-Se was initially speciated into Se associated with fulvic acids (FA-Se) and humic acids Se (HA-Se). Both FA-Se and HA-Se were further speciated into weakly bound (reducible by hydride) and strongly bound (hydride inert) fractions using the customized hydride generation reactor. The protocol for Se extraction is illustrated in Fig. 2 and the detailed procedures are described below.
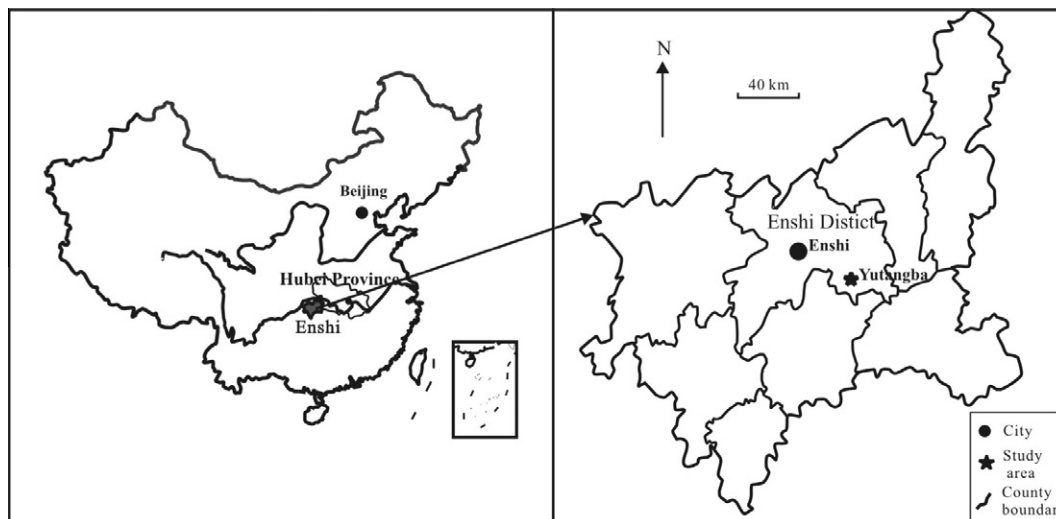
Fig. 1. Sketch map showing the location of study area in Enshi prefecture, China.
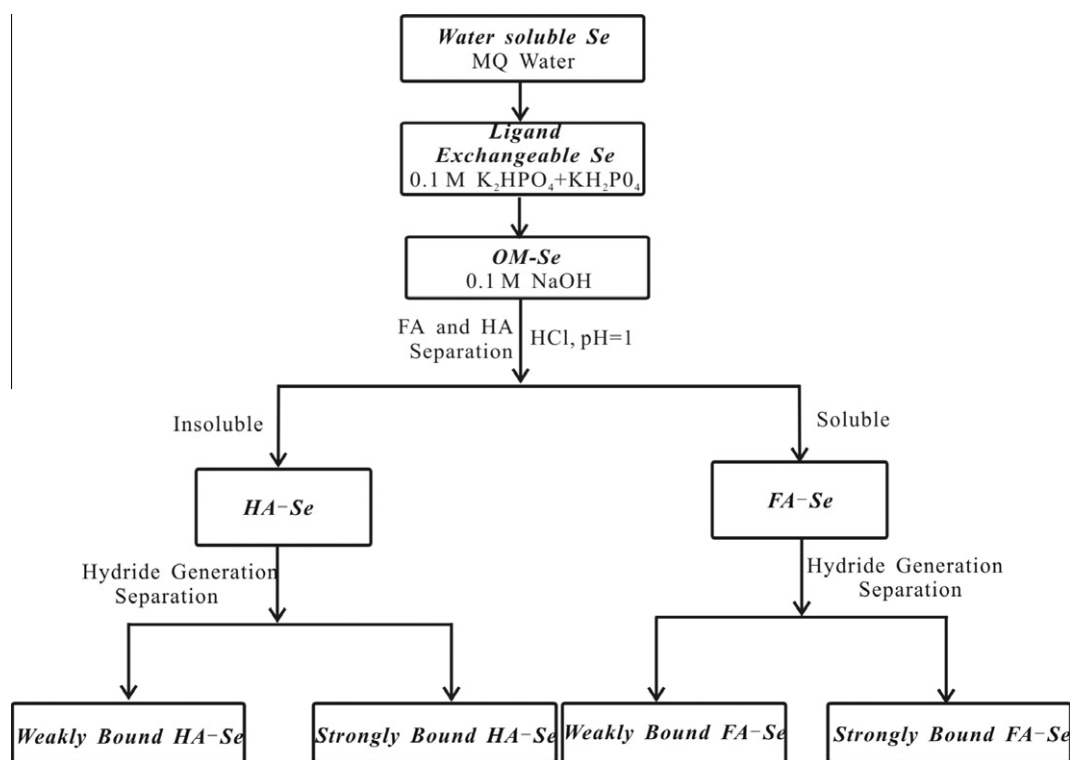

Fig. 2. Flowchart describing Se extraction procedures.

2.3.1. FA-Se and HA-Se separation

The NaOH extract was acidified to pH 1 with HCl. After standing for 12 h to 16 h, the supernatant (FA-Se) was separated from the residue by centrifugation (10 000g; 30 min). The residue was re-dissolved by adding 0.1 M NaOH to obtain HA-Se.

2.3.2. Weakly and strongly bound FA/HA-Se separation

Initially, 6 M HCl was added into a customized hydride genera-tion reactor (Fig. 3), from which oxygen was removed by purging nitrogen gas (N_2). A mixture of 1% (wt.%) KBH_4, 0.2% (wt.%) NaOH and FA/HA-Se aliquots were slowly syringed through PFA (Teflon) tubing into the reactor. The volatile Se compounds were generated and carried by a N_2 carrier gas at a flow rate of 0.6 L min^{-1} into a series of two glass tubes filled with an alkaline H_2O_2 solution.

Based on previous studies (Chatterjee and Irgolic, 1998; Zhang et al., 1999a; Zhang and Frankanberger, 1999b; Chatterjee et al., 2001), Se(IV) and some organic Se compounds can react with BH_4^- and produce volatile Se compounds. In our study, weakly bound FA/HA-Se is considered to be the part of the FA/HA-Se that readily reacts with BH_4^-, and is consequently captured by alkaline H_2O_2 solution, while strongly bound FA/HA-Se does not react with BH_4^-, and therefore remains after Se hydride is generated.

2.4. Selenium analysis

For total Se analysis, samples were digested by placing 0.05–0.10 g samples in a 30 mL PTFE (Teflon) bomb with 2.7 mL concen-trated HNO_3 and 0.5 mL HF, standing for 2 h to degas, and then was
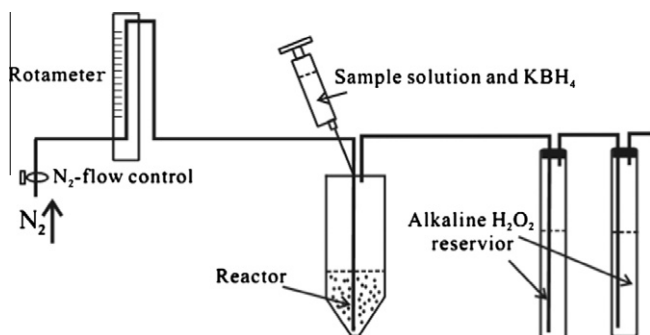
Fig. 3. Schematic diagram of the custom made hydride generation system.

sealed and heated at 150 °C for 16 h. Total Se concentration was determined by HG-AFS (Zhu et al., 2008). The Se concentrations in samples and their basic geochemical properties are given in Table 1.

In order to determine the total Se concentration in each extract, an aliquot of extracted Se was treated with 3 mL concentrated HNO_3 and kept in a closed PTFE bomb at 150 °C for 6 h. After cooling, 2 mL H_2O_2 was added and the bomb was once again heated at 75 °C for 1 h. The solution was transferred into a 15 mL PFA beaker and evaporated to dryness at 75 °C. Selenium was reduced to Se(IV) by adding 3 mL 5 M HCl in borosilicate glass tube with PTFE-Faced rubber-lined screw cap and kept at 95–100 °C for 45 min in aluminum block. The final solution was diluted to 20 mL with Milli-Q water for HG-AFS analysis.

The determination of weakly and strongly bound FA/HA-Se is as follows: The alkaline H_2O_2 solution containing Se was heated in a hot water bath at 90 °C to decompose all H_2O_2 (Zhang and Frankanberger, 1999b), and then an aliquot of the solution was first treated with concentrated HCl, diluted to 5 M HCl and kept at 95–100 °C for 45 min to transform Se to Se(IV). The solution was diluted again with Milli-Q water to attain 0.75 M HCl to determine weakly bound FA/HA-Se by HG-AFS. For measurement of strongly bound FA/HA-Se, an aliquot of the remaining liquid in the reactor was processed using the procedures given above for the total Se in extract.

2.5. Quality control

Quality assurance and quality control of Se analysis were assessed using method blanks, spikes, certified reference materials and duplicates. The measured mean Se concentration of soil standards GBW07405 (GSS-5) and GBW07406 (GSS-6) was $1.60 ± 0.07$ mg kg^{-1} ($n = 6$) and $1.26 ± 0.06$ mg kg^{-1} ($n = 6$), respectively, which is consistent with the certified values (GSS-5: $1.60 ± 0.20$ mg kg^{-1}, GSS-6: $1.34 ± 0.17$ mg kg^{-1}). The relative

percentage difference of sample duplicates was <10%. The recoveries on spikes of Se in samples were in the range of 95.9–103.0%, with an average of 99.1%.

3. Results

3.1. Selenium recovery in hydride generation reactor

In our study, in contrast with conventional hydride generation techniques, each solution containing Se was first mixed with KBH$_4$ on line, and then was slowly syringed into the customized hydride generation reactor containing 6 M HCl (Fig. 3), the gaseous Se produced in the reactor was carried into glass tubes pre-filled with alkaline H_2O_2 solution by N_2 carrier gas at a flow rate of 0.6 L min^{-1}. Under optimized experiment conditions, the recovery of spiked Se(IV) standard in the customized hydride generation system is in the range of 97.8–101.6%, with an average of 99.2%.

For OM-Se in natural Se-rich samples, the sum of weakly and strongly bound FA-Se or HA-Se is consistent with the total FA-Se or HA-Se, which corresponds to recoveries that range from 96.9% to 101.9% with a mean of 99.4% and from 95.9% to 100.7% with a mean of 98.8%, respectively (Table 3). This is further confirmed by their significant linear relationship ($r = 0.999$ at $p < 0.001$ for FA-Se and HA-Se) between the sum of weakly and strongly bound FA/HA-Se and the total FA/HA-Se. All observations indicate that the speciation method of weakly and strongly bound FA/HA-Se using our customized hydride generation system is adequate for our studies.

3.2. Se fractions in stone coal and soils

Results of water-soluble, ligand-exchangeable Se and OM-Se fractions occurring in stone coal and soils from Enshi are presented in Table 2. In all samples, a small (generally <3%) but variable (1.05–15.3%) proportion of total Se is extracted by water, and the percentage of ligand-exchangeable Se is higher and more variable (0.87–28.1%, with a mean of 12.4%). SCS-1 and SCS-3 contain higher water-soluble Se and ligand-exchangeable Se than SCS-2, uncultivated (US-1 to US-3) and cropland soils (CS-1, CS-2), which is partially explained by the degree of samples exposure and weathering. It is expected that more soluble Se is present in samples with less leaching and exposure to weathering (Martens and Suarez, 1997a; Kulp and Pratt, 2004; Bujdoš et al., 2005). In addition, Selenium extracted using water and the P-buffer represents the major Se forms that are available for plant uptake (Chao and Sanzolone, 1989; Sharmasarkar and Vance, 1995; Séby et al., 1997; Kulp and Pratt, 2004). A large variation in water-soluble and ligand-exchangeable Se is observed in soils, which probably indicates different level of bioavailable Se.

Table 1
The Geochemical properties of the studied samples.

Samples	pH[a]	Organic C[b] (%)	Total N[b] (%)	Total Se[c] (mg kg^{-1})
SCS-1	4.42	22.3	1.00	943
SCS-2	4.98	16.4	0.69	572
SCS-3	4.63	6.45	0.46	573
US-1	5.92	1.69	0.33	61.0
US-2	6.12	3.97	0.34	122
US-3	7.10	5.43	0.37	36.4
CS-1	6.93	3.98	0.38	24.3
CS-2	6.64	1.51	0.27	5.90

[a] Sample pH was determined by a pH electrode with a 2.5:1 water to solid ratio.
[b] Organic C and total N content of samples were measured by means of an element analyzer PE2400-II.
[c] Total Se content was determined by HG-AFS.

Table 2
Results of water soluble, ligand exchangeable Se and OM-Se fractions in the Se-rich weathered stone coal and soils.

Samples	Water soluble Se	Ligand exchangeable Se	OM-Se
SCS-1	21.6(2.29)	237(25.1)	484(51.3)
SCS-2	14.7(2.57)	13.5(2.36)	272(47.6)
SCS-3	87.5(15.3)	161(28.1)	182(31.8)
US-1	0.64(1.05)	0.53(0.87)	23.9(39.2)
US-2	2.43(1.99)	18.5(15.2)	40.4(33.1)
US-3	0.90(2.47)	2.19(6.02)	16.2(44.5)
CS-1	2.35(9.67)	2.13(8.77)	12.1(49.8)
CS-2	0.21(3.56)	0.74(12.5)	3.07(52.0)

The value not in parentheses indicates the concentration of each Se fraction (mg kg^{-1}), the value in parentheses indicates the percentage of each Se fraction in the total Se (%).

Table 3
Selenium fractions of OM-Se in the Se-rich weathered stone coal and soils.

Samples	FA-Se (mg kg⁻¹)		$\sum$FA-Se (mg kg⁻¹)	Total FA-Se (mg kg⁻¹)	Recovery of FA-Se (%)	HA-Se (mg kg⁻¹)		$\sum$HA-Se (mg kg⁻¹)	Total HA-Se (mg kg⁻¹)	Recovery of HA-Se (%)[a]	$\sum$OM-Se (mg kg⁻¹)	Total OM-Se (mg kg⁻¹)	Recovery of OM-Se (%)
	Weakly bound FA-Se	Strongly bound FA-Se				Weakly bound HA-Se	Strongly bound HA-Se						
SCS-1	450	35.4	485.4	479	101.3	11.7	1.82	13.52	14.1	95.9	493.1	484	101.9
SCS-2	209	40.6	249.6	245	101.9	12.3	5.02	17.32	17.6	98.4	262.6	272	96.5
SCS-3	127	24.2	151.2	153	98.8	26.2	6.03	32.23	32.5	99.2	185.5	182	101.9
US-1	16.8	2.02	18.82	19.0	99.1	2.26	2.94	5.20	5.19	100.2	24.19	23.9	101.2
US-2	24.4	4.06	28.46	29.0	98.1	8.90	3.79	12.69	12.6	100.7	41.6	40.4	103.0
US-3	7.92	2.06	9.98	10.3	96.9	3.41	2.19	5.60	5.73	97.7	16.03	16.2	99.0
CS-1	6.25	3.16	9.41	9.37	100.4	1.01	1.63	2.64	2.68	98.5	12.05	12.1	99.6
CS-2	1.33	0.56	1.89	1.91	99.0	0.62	0.53	1.15	1.15	100.0	3.06	3.07	99.7

[a] The $\sum$OM-Se was calculated as the sum of total FA-Se and HA-Se.

In all samples, OM-Se is the dominant Se fraction, accounting for an average 43.7% (from 31.8% to 51.3%) of total Se, which is in good agreement with previous studies (Kang et al., 1991a, 1993; Gustafsson and Johnsson, 1994; Séby et al., 1997; Coppin et al., 2006; Oram et al., 2008). This suggests that there is a significant association between Se and organic matter.

3.3. Distribution of FA/HA-Se fractions in OM-Se

The distribution of FA-Se and HA-Se in OM-Se extracted from the weathered stone coals, uncultivated and cropland soils are shown in Table 3 and Fig. 4. In all samples, FA-Se (1.91–479 mg kg⁻¹) is the predominant form of OM-Se (3.07–484 mg kg⁻¹), accounting for more than 62% (62.4–97.1%) of OM-Se. This suggests Se is preferentially incorporated into the fulvic acids fraction rather than humic acids fraction. The results are in agreement with previous studies on the distribution of FA/HA-Se that showed FA-Se accounts for 54–65%, 70% and 44.6–77.2% in the NaOH extract in soils from the UK (Coppin et al., 2006), Ireland (Séby et al., 1997) and Japan (Kang et al., 1991a), respectively.

As shown in Table 3, weakly bound FA-Se (1.33–450 mg kg⁻¹) is prevalent in the FA-Se fraction, accounting for 66.4–92.7% of total FA-Se, and strongly bound FA-Se is only a minor component. Weakly bound HA-Se is highly variable (0.62–26.2 mg kg⁻¹), accounting for 38.3–86.5% of total HA-Se (1.15–32.5 mg kg⁻¹), whereas strongly bound HA-Se (0.53–6.03 mg kg⁻¹) accounts for 13.5–61.7% of total HA-Se.
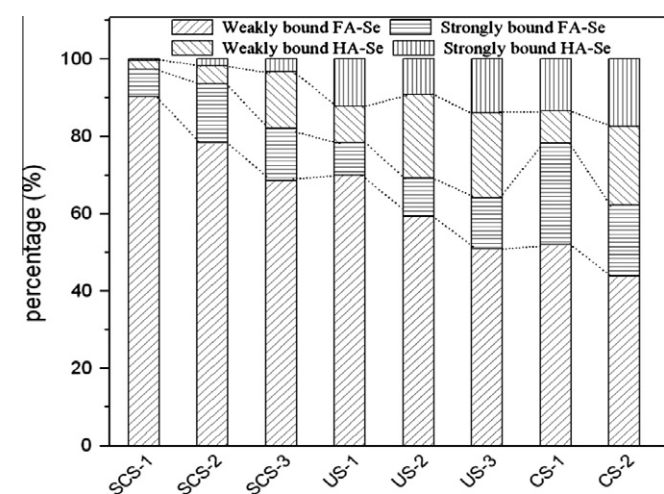

Fig. 4. Percent of Se fractions in OM-Se from the Se-rich weathered stone coal and soils.

4. Discussion

4.1. Efficiency of hydride generation system

The common problems for a hydride generation system include: (1) the presence of various anions and cations in solutions that may interfere with the formation of H_2Se and (2) a very rapid reaction that results in an incomplete trapping of the formed gaseous Se (Zhang et al., 2002). Hershey and Keliher (1986) and Zhang et al. (2002) showed that nearly all ion interferences were reduced, or eliminated, in highly acidic solutions. In order to completely collect volatile Se compounds, the trapping method and other factors that affect trapping efficiency need to be optimized. The alkaline H_2O_2 solution (a 12:1 solution of 0.2 M NaOH and 30% H_2O_2) is preferentially selected to capture volatile Se due to its high trapping efficiency and high compatibility with subsequent HG-AFS determination (Hansen et al., 1998; Zhang and Frankanberger, 1999b). Liquid nitrogen and active carbon are not used because they are incompatible with HG-AFS (Olivas et al., 1994; Chatterjee et al., 2001).

In order to evaluate the impact of organic matter on Se recovery in the customized hydride generation reactor, humic acids standard from Aldrich Chemical Co. were added to Se(IV) standard solutions. The results show that organic matter has hardly any effect on Se recovery, even at levels as high as 780 mg L⁻¹. Three reasons could explain these good results. The first is that the complexes between Se(IV) and HA may be very weak so that Se(IV) can react with BH_4^- to produce H_2Se in the hydride generation system (Zhang et al., 1999a). The second is that excess BH_4^- react with HCl and produces excessive H_2, which inhibit the interference from organic matter in samples and ensure that Se(IV) in solution is completely transformed into volatile Se. The third could be that the produced volatile Se is completely trapped by alkaline H_2O_2 solution and then determined by HG-AFS, which would eliminate the effervescence interference produced during the reaction of organic matter and BH_4^-.

4.2. FA-Se and HA-Se fractions in organic matter

A significant positive correlation ($R^2 = 0.924$; $P < 0.001$) is observed between OM-Se content and organic C in samples from Enshi. However, the organic matter content is not a good proxy for OM-Se levels unless the samples have similar inputs (Martens and Suarez, 1997a). Coppin et al. (2006) found approximately 60% of OM-Se was extracted in soils with different quantities of organic matter, and concluded that OM-Se retention was controlled by both quantity and quality of organic matter. Thus, besides organic matter content, further studies about distribution

of FA/HA-Se in OM-Se could be helpful for understanding the relationships between organic matter and Se.

A significant difference in the proportion of FA-Se in OM-Se is observed between the weathered stone coal (82.5–97.1%, with a mean of 90.9%), uncultivated (64.3–78.5%, with a mean of 70.8%) and cropland soils (62.4–70.8%, with a mean of 70.1%), while HA-Se exhibits the opposite trend (Fig. 4). Particularly, FA-Se in stone coal is much higher than in soils, which is probably because FA-Se may be transformed and released during the weathering process of stone coal. Previous studies (Abrams and Burau, 1989; Abrams et al., 1990; Kang et al., 1991a,b; Gustafsson and Johnsson, 1994) showed that FA-Se contained inorganic Se associated with FA and organic Se with low and high molecular weight, whereas HA-Se was usually assumed to be in the form of seleno-amino acids in proteins or peptides. Since selenomethionine and selenocystine with low molecular weight could be mineralized to inorganic Se in soils (Martens and Suarez, 1997b), FA-Se could be decomposed and utilized by plants under appropriate environmental conditions, while HA-Se is stable and difficult to decompose. Although a minor variation was found in the proportion of FA/HA-Se in uncultivated and cropland soils, it could be partially explained by the difference between the cultivated history of two soils. Martens and Suarez (1997b) suggested that greater and faster organic Se mineralization occurred in the heavily cultivated soils compared with uncultivated soils.

However, it is noted that even the same Se species, when incorporated into different solid phases, could behave differently with respect of the mobilization, transformation, and bioavailability of Se. Thus, to better understand the geochemical behavior of FA/HA-Se in the surface environment, further classification of FA/HA-Se fractions is discussed in detail below.

4.3. Weakly and strongly bound FA/HA-Se fractions

Based on the facts that weakly bound Se(IV) and some organic Se compounds can react with BH_4^- to produce gaseous Se in the hydride generation system (Chatterjee and Irgolic, 1998; Zhang et al., 1999a; Zhang and Frankanberger, 1999b; Chatterjee et al., 2001), weakly bound FA-Se was expected to include inorganic Se(IV) weakly associated with FA, organic Se with low molecular weight and/or cleavable Se-C bonds, while strongly bound FA-Se was assumed to contain organic Se compounds with higher molecular weight or more stable structure. Therefore, weakly bound FA-Se is predominant in the FA-Se fraction. That is, the inorganic Se and organic Se with low molecular weight are the dominant species in FA-Se fraction. It is not certain that Se(VI) exists in strongly bound FA-Se. However, it is even less likely that Se(VI) still remains following the water extraction due to the high solubility of Se(VI) (Kulp and Pratt, 2004).

Several studies have been conducted to investigate fractions of FA-Se in NaOH extract (Kang et al., 1991a, 1993; Séby et al., 1997). Séby et al. (1997) observed that Se(IV), Se(VI) and organic Se species accounted for 44%, 15% and 41%, respectively of total FA-Se in a Se-rich soil from Ireland. Kang et al. (1993) found 28.6%, 39% and 32.4% of total FA-Se in the form of Se(IV and VI), organic Se with low, and high molecular weight in five soils, respectively. However, the NaOH-extracted Se characterized by previous researchers likely contained mixtures of Se fractions due to the lack of extraction by water and P-buffer before NaOH. It is known that water-soluble Se is comprised of more Se(VI), Se(IV) and organic Se with low molecular weight, while ligand-exchangeable Se includes more Se(IV) adsorbed on the surface of oxide minerals (Fe, Mn oxide etc.) and clay minerals, as well as a few organic Se (Martens and Suarez, 1997a; Kulp and Pratt, 2004; Wright et al., 2003). Indeed, if Se speciation in water-soluble and ligand exchangeable Se was excluded when characterizing FA-Se

speciation, these previous results would be in good agreement with ours. Therefore, Se extracted by NaOH after the sequential extraction of water and P-buffer better characterizes the Se fractions in OM-Se.

An evident decrease was observed in weakly bound FA-Se in the samples as follows: stone coal > uncultivated soils > cropland soils. As discussed above, weakly bound FA-Se was considered to include weakly bound Se(IV) and organic Se with low molecular weight and/or cleavable Se-C bonds. In this fraction, organic Se with low molecular weight may contain seleno-amino acids, which can be readily taken up by plants (Abrams et al., 1990; Kang et al., 1991a, 1993). Other organic Se that is not directly used by plants is likely to be decomposed and released as inorganic Se and seleno-amino acids under appropriate pH and redox potential (Abrams et al., 1990; Masscheleyn et al., 1990; Kang et al., 1991a, 1993; Peters et al., 1999). In comparison with stone coal, uncultivated and cropland soils were subject to more exposure, oxidation and human activities, so that more weakly bound FA-Se was released. The discrepancy of weakly bound FA-Se in uncultivated and cropland soils was partially explained by the cultivation history and impact of human activities as discussed in the Section 4.2. More human activities and heavy cultivation in soils lead to more release and plant uptake of weakly bound FA-Se. Therefore, we would conclude that weakly bound FA-Se may be the potential source of bioavailable Se in the OM-Se.

HA-Se was usually assumed to be essentially present in the form of seleno-amino acids since the direct analysis of HA-Se speciation was never performed (Kang et al., 1991b, 1993). However, Séby et al. (1997) showed that Se(IV) also occurred in the HA-Se fraction of NaOH extracts, and the existence of Se(IV) does not totally result from an oxidation of organic Se. In our study, Se(IV) and/or organic Se with low molecular weight may be the predominant form in the weakly bound HA-Se, while strongly bound HA-Se is markedly in the form of organic Se with high molecular weight or stable structure, and is related to proteins or peptides.

Elevated strongly bound HA-Se in OM-Se in uncultivated and cropland soils compared to the stone coal can be observed in Fig. 4. This suggests Se has been accumulated by HA in the form of strongly bound HA-Se. It has been proven that microorganisms control Se incorporation into organic matter, which can reduce Se to Se(-II) combined with amino-, carboxyl- or hydroxyl- functional groups (Van Dorst and Peterson, 1984; Gustafsson and Johnsson, 1994; Darcheville et al., 2008). Cropland soils with more human activities including irrigation, tilling, fertilization and plant growth would lead to more microbially mediated processes, and thus result in higher accumulation of strongly bound HA-Se. Martens and Suarez (1997a) reported that plant growth and plant residues would be favorable to the formation of Se(-II), which supports the accumulation trend of strongly bound HA-Se. Since strongly bound HA-Se is present in the form of organic Se with high molecular weight and stable structure, which is not easy to decompose, we conclude that strongly bound HA-Se may be a sink of Se in the OM-Se.

4.4. Environmental implications

It is suggested that stone coals were responsible for the sudden prevalence of human Se poisoning in Enshi in the early 1960s (Yang et al., 1983; Zhu et al., 2004). Yang et al. (1983) reported that leaching, weathering, and possibly biological action would mobilize Se from the stone coal to the soils, and thus make it available to the crops. Besides these natural factors, Zhu et al. (2008) suggested that human activities were another main reason for human Se poisoning. The stone coal powder was dispersed into croplands as a fertilizer, and then agricultural management (e.g. tillage and reclamation) affected the transformation of Se and its availability to plants, which

was demonstrated by the fact that plants with higher Se content were always distributed in the croplands containing stone coals or close to abandoned stone coal spoils (Zhu et al., 2008). As we know, water soluble and ligand exchangeable Se is readily taken up by plant, but this fraction is very small in this study and other similar studies (Kang et al., 1993; Séby et al., 1997).

The observation that weakly bound FA-Se acts as the potential source for bioavailable Se has significant implications for Se mobility and uptake by plants in Enshi and other Se-rich areas. The more weakly bound FA-Se found in Se-rich soils and weathered stone coals, the more Se is prone to transform into bioavailable Se. This provides a further explanation for the accident of human selenosis in Enshi. The dry weather, microbial activities, and human activities such as application of lime to croplands and tillage could accelerate the release and transformation of weakly bound FA-Se into inorganic Se and lower molecular organic Se, which is easily taken up by plants. This will result in the enrichment of Se in crops and plants. Once the foods with high Se content were consumed; Selenium toxicity of humans and livestock was made possible. In order to avoid the possible Se poisoning in Enshi and similar areas in China, local residents should avoid misusing stone coal as a crop fertilizer, and dispersing lime to improve soils quality. Consumption of crops planted in the Se-rich soils should be avoided. If there are no other good measures, local people should mix their diet with exotic food to decrease the Se intake.

5. Conclusions

An effective method for quantifying Se fractions in organic matter was developed in this study. The method was used to study the distribution of OM-Se and its fractions in Se-rich stone coal and soils from Enshi. In all samples, FA-Se was the predominant form of OM-Se. Weakly bound FA-Se was dominant in the total FA-Se while weakly bound HA-Se was variable in the HA-Se fraction. Based on the differences between FA-Se and HA-Se fractions in different samples, we conclude that weakly bound FA-Se may be the potential source of bioavailable Se in the OM-Se, while strongly bound HA-Se is a possible OM-Se sink. Thus, OM-Se is implied to play the dual roles of source and sink in the biogeochemical cycling of Se in supergene environments.

The occurrence of strongly bound FA/HA-Se suggests that Se can form stable complexes with organic matter; however, its molecular structure and speciation remain unclear. The separation of strongly bound FA/HA-Se will provide an opportunity to further study the molecular characteristics of OM-Se complexes using X-ray absorption spectroscopy (XAS); a widely used technique to determine element speciation and binding mechanisms at a molecular level (Pickering et al., 1995; Oram et al., 2008; Wiramanaden et al., 2010).

Acknowledgments

References

Abrams, M.M., Burau, R.G., 1989. Fractionation of selenium and detection of selenomethionine in a soil extract. Commun. Soil. Sci. Plan. 20, 221–237.

Abrams, M.M., Burau, R.G., Zasoski, R.J., 1990. Organic selenium distribution in selected california soils. Soil. Sci. Soc. Am. J. 54, 979–982.

Bienvenu, P., Cassette, P., Andreoletti, G., Bé, M., Comte, J., Lépy, M., 2007. A new determination of [79]Se half-life. Appl. Radiat. Isotopes. 65, 355–364.

Bruggeman, C., Maes, A., Vancluysen, J., 2007. The interaction of dissolved Boom Clay and Gorleben humic substances with selenium oxyanions(selenite and selenate). Appl. Geochem. 22, 1371–1379.

Bujdoš, M., Mulová, A., Kubová, J., Medve, J., 2005. Selenium fractionation and speciation in rocks, soils, waters and plants in polluted surface mine environment. Environ. Geol. 47, 353–360.

Cai, Y., 2000. Speciation and analysis of mercury, arsenic, and selenium by atomic fluorescence spectrometry. TrAC, Trends Anal. Chem. 19, 62–66.

Capelo, J.L., Fernandez, C., Pedras, B., Santos, P., Gonzalez, P., Vaz, C., 2006. Trends in selenium determination speciation by hyphenated techniques based on AAS or AFS. Talanta 68, 1442–1447.

Chao, T.T., Sanzolone, R.F., 1989. Fractionation of soil selenium by sequential partial dissolution. Soil. Sci. Soc. Am. J. 53, 385–392.

Chatterjee, A., Irgolic, K.J., 1998. Behaviour of selenium compounds in FI-HG-AAS. Anal. Commun. 35, 337–340.

Chatterjee, A., Shibata, Y., Yoneda, M., Banerjee, R., Uchida, M., Kon, H., Morita, M., 2001. Identification of volatile selenium compounds produced in the hydride generation system form organoselenium compounds. Anal. Chem. 73, 3181–3186.

Chen, Y.W., Thi Truong, H.Y., Belzile, N., 2009. Abiotic formation of elemental selenium and role of iron oxide surfaces. Chemosphere 74, 1079–1084.

Coppin, F., Chabroullet, C., Martin-Garin, A., Balesdent, J., Gaudet, J.P., 2006. Methodological approach to assess the effect of soil ageing on selenium behaviour: first results concerning mobility and solid fractionation of selenium. Biol. Fert. Soils. 42, 379–386.

Coppin, F., Chabroullet, C., Martin-Garin, A., 2009. Selenite interactions with some particulate organic and mineral fractions isolated from a natural grassland soil. Eur. J. Soil. Sci. 60, 369–376.

Darcheville, O., Février, L., Haichar, F.Z., Berge, O., Martin-Garin, A., Renault, P., 2008. Aqueous, solid and gaseous partitioning of selenium in an oxic sandy soil under different microbiological states. J. Environ. Radioactiv. 99, 981–992.

Dhillon, K.S., Dhillon, S.K., 1999. Adsorption-desorption reactions of selenium in some soils of India. Geoderma 93, 19–31.

Dhillon, S.K., Hundal, B.K., Dhillon, K.S., 2007. Bioavailability of selenium to forage crops in a sandy loam soil amended with Se-rich plant materials. Chemosphere 66, 1734–1743.

Dhillon, K.S., Dhillon, S.K., Dogra, R., 2010. Selenium accumulation by forage and grain crops and volatilization from seleniferous soils amended with different organic materials. Chemosphere 78, 548–556.

Dörr, A.J.M., Pacini, N., Abete, M.C., Prearo, M., Elia, A.C., 2008. Effects of a selenium-enriched diet on antioxidant response in adult crayfish (Procambarus clarkii). Chemosphere 73, 1090–1095.

Fordyce, F.M., Zhang, G., Green, K., Liu, X., 2000. Soil, grain and water chemistry in relation to human selenium-responsive disease in Enshi District, China. Appl. Geochem 15, 117–132.

Frankanberger Jr., W.T., Benson, S., 1994. Selenium in the Environment. Marcel Dekker. Inc, New York. pp. 1–416.

Gao, S., Tanji, K.K., Dahlgren, R.A., Ryu, J., Herbel, M.J., Higashi, R.M., 2007. Chemical status of selenium in evaporation basins for disposal of agricultural drainage. Chemosphere 69, 585–594.

Gustafsson, J.P., Johnsson, L., 1994. The association between selenium and humic substances in forested ecosystems-laboratory evidence. Appl. Organomet. Chem. 8, 141–147.

Hansen, D., Duda, P.J., Zayed, A., Terry, N., 1998. Selenium removal by constructed wetlands: role of biological volatilization. Environ. Sci. Technol. 32, 591–597.

Hershey, J.W., Keliher, P.N., 1986. Some hydride generation inter-element interference studies utilizing atomic absorption and inductively coupled plasma emission spectrometry. Spectrochim. Acta B. 41, 713–723.

Hoang, T.C., Klaine, S.J., 2008. Characterizing the toxicity of pulsed selenium exposure to Daphnia magna. Chemosphere 71, 429–438.

Irizarry, R., Moore, J., Cai, Y., 2001. Atomic fluorescence determination of selenium using hydride generation technique. Intern. J. Environ. Anal. Chem. 79, 97–109.

Janos, P., 2003. Separation methods in the chemistry of humic substances. J. Chromatogr. A 983, 1–18.

Jordan, N., Marmier, N., Lomenech, C., Giffaut, E., Ehrhardt, J.J., 2009. Competition between selenium (IV) and silicic acid on the hematite surface. Chemosphere 75, 129–134.

Kamei-Ishikawa, N., Yasuo, N., Tagami, K., Uchida, S., 2008. Sorption behavior of selenium on humic acid under increasing selenium concentration or increasing solid/liquid ratio. J. Environ. Radioactiv. 99, 993–1002.

Kang, Y., Nozato, N., Kyuma, K., Yamada, H., 1991a. Distribution and forms of selenium in paddy soil. Soil. Sci. Plant. Nutr. 37, 477–485.

Kang, Y., Yamada, H., Kyuma, K., Hattori, T., Kigasawa, S., 1991b. Selenium in soil humic acid. Soil. Sci. Plant. Nutr. 37, 241–248.

Kang, Y., Yamada, H., Kyuma, K., Hattori, T., 1993. Speciation of selenium in soil. Soil. Sci. Plant. Nutr. 39, 331–337.

Kulp, T.R., Pratt, L.M., 2004. Speciation and weathering of selenium in Upper Cretaceous chalk and shale from South Dakota and Wyoming, USA. Geochim. Cosmochim. Acta 68, 3687–3701.

Lenz, M., Janzen, N., Lens, P.N.L., 2008. Selenium oxyanion inhibition of hydrogenotrophic and acetoclastic methanogenesis. Chemosphere 73, 383–388.

Lenz, M., Lens, P.N.L., 2009. The essential toxin: the changing perception of selenium in environmental sciences. Sci. Total. Environ. 407, 3620–3633.

Martens, D.A., Suarez, D.L., 1997a. Selenium speciation of marine shales, alluvial soils, and evaporation basin soils of California. J. Environ. Qual. 26, 424–432.

Martens, D.A., Suarez, D.L., 1997b. Mineralization of selenium-containing amino acids in two California soils. Soil. Sci. Soc. Am. J. 61, 1685–1694.

Masscheleyn, P.H., DeLaune, R.D., Patrick Jr., W.H., 1990. Transformations of selenium as affected by sediment oxidation-reduction potential and pH. Environ. Sci. Technol. 24, 91–96.

Olivas, R.M., Donard, O.F.X., Camara, C., Quevauviller, P., 1994. Analytical techniques applied to the speciation of selenium in environmental matrices. Anal. Chim. Acta 286, 357–370.

Oram, L.L., Strawn, D.G., Marcus, M.A., Fakra, S.C., Moller, G., 2008. Macro- and microscale investigation of selenium speciation in blackfoot river, Idaho sediments. Environ. Sci. Technol. 42, 6830–6836.

Peters, M., Maher, W.A., Jolley, D., Carroll, B.I., Gomes, V.G., Jenkinson, A.V., Mcorist, G.D., 1999. Selenium contamination, redistribution and remobilisation in sediments of Lake Macquarie. NSW. Org. Geochem. 30, 1287–1300.

Pickering, I.J., Brown, G.E., Tokunaga, T.K., 1995. Quantitative speciation of selenium in soil using x-ray absorption spectroscopy. Environ. Sci. Technol. 29, 2456–2459.

Pumure, I., Renton, J.J., Smart, R.B., 2010. Ultrasonic extraction of arsenic and selenium from rocks associated with mountaintop removal/valley fills coal mining: estimation of bioaccessible concentrations. Chemosphere 78, 1295–1300.

Ranjan Mishra, R., Prajapati, S., Das, J., Kanti Dangar, T., Das, N., Thatoi, H., 2011. Reduction of selenite to red elemental selenium by moderately halotolerant Bacillus megaterium strains isolated from Bhitarkanika mangrove soil and characterization of reduced product. Chemosphere 84, 1231–1237.

Séby, F., Potin-Gautier, M., Lespés, G., Astruc, M., 1997. Selenium speciation in soils after alkaline extraction. Sci. Total. Environ. 207, 81–90.

Sharmasarkar, S., Vance, G.F., 1995. Fractional partitioning for assessing solid-phase speciation and geochemical transformations of soil selenium. Soil. Sci. 160, 43–55.

Swift, R.S., 1996. Organic matter characterization. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpura, P.N., Tabatai, M.A., Johnston, C.T., Summer, M.E. (Eds.), Methods of Soil Analysis. Part 3. Chemical Methods. Soil. Sci. Soc. Am. J. Madison, WI, USA, pp. 1018–1020.

Van Dorst, S.H., Peterson, P.J., 1984. Selenium speciation in the soil solution and its relevance to plant uptake. J. Sci. Food. Agr. 35, 601–605.

Wang, Z.J., Gao, Y.X., 2001. Biogeochemical cycling of selenium in Chinese environments. Appl. Geochem. 16, 1345–1351.

Wiramanaden, C.I.E., Liber, K., Pickering, I.J., 2010. Selenium speciation in whole sediment using X-ray absorption spectroscopy and micro X-ray fluorescence imaging. Environ. Sci. Technol. 44, 5389–5394.

Wright, M.T., Parker, D.R., Amrhein, C., 2003. Critical evaluation of the ability of sequential extraction procedures to quantify discrete forms of selenium in sediments and soils. Environ. Sci. Technol. 37, 4709–4716.

Yang, G.Q., Wang, S.Z., Zhou, R.H., Sun, S.Z., 1983. Endemic Se intoxication of humans in China. Am. J. Clin. Nutr. 37, 872–881.

Zawislanski, P.T., Benson, S.M., Terberg, R., Borglin, S.E., 2003. Selenium speciation, solubility, and mobility in land-disposed dredged sediments. Environ. Sci. Technol. 37, 2415–2420.

Zhang, B., Xu, H., Yu, J.C., 2002. Determination of total gaseous selenium in atmosphere by honeycomb denuder/differential pulse cathodic stripping voltammetry. Talanta 57, 323–331.

Zhang, Y.Q., Moore, J.N., 1996. Selenium fractionation and speciation in a wetland system. Environ. Sci. Technol. 30, 2613–2619.

Zhang, Y.Q., Frankenberger Jr., W.T., Moore, J.N., 1999a. Measurement of selenite in sediment extracts by using hydride generation atomic absorption spectrometry. Sci. Total. Environ. 229, 183–193.

Zhang, Y.Q., Frankanberger Jr., W.T., 1999b. Effect of soil moisture on dimethylselenide transport and transformation to nonvolatile selenium. Environ. Sci. Technol. 33, 3415–3420.

Zheng, B.S., Ding, Z.H., Huang, R.G., Zhu, J.M., Yu, X.Q., Wang, A.M., Zhou, D.X., Mao, D.J., Su, H.C., 1999. Issues of health and disease relating to coal use in southwestern China. Int. J. Coal. Geol. 40, 119–132.

Zhu, J.M., Zheng, B.S., 2001. Distribution of selenium in mini-landscape of Yutangba, Enshi, Hubei Province China. Appl. Geochem. 16, 1333–1344.

Zhu, J.M., Zuo, W., Liang, X.B., Li, S.H., Zheng, B.S., 2004. Occurrence of native selenium in Yutangba and its environmental implications. Appl. Geochem. 19, 461–467.

Zhu, J.M., Wang, N., Li, S.H., Li, L., Su, H.C., 2008. Distribution and transport of selenium in Yutangba, China: impact of human activities. Sci. Total. Environ. 392, 252–261.