Metallomics

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Metallomics*, 2013, **5**, 896

Mercury modulates selenium activity *via* altering its accumulation and speciation in garlic (*Allium sativum*)†

Jiating Zhao,[ab] Yi Hu,[a] Yuxi Gao,*[a] Yufeng Li,[a] Bai Li,[a] Yuanxing Dong[c] and Zhifang Chai[a]

Received 30th December 2012,
Accepted 20th May 2013

DOI: 10.1039/c3mt20273a

www.rsc.org/metallomics

Combined pollution of selenium (Se) and mercury (Hg) has been known in Wanshan district (Guizhou Province, China). A better understanding of how Se and Hg interact in plants and the phytotoxicity thereof will provide clues about how to avoid or mitigate adverse effects of Se/Hg on local agriculture. In this study, the biological activity of Se has been investigated in garlic with or without Hg exposure. Se alone can promote garlic growth at low levels (<0.1 mg L^{-1}), whereas it inhibits garlic growth at high levels (>1 mg L^{-1}). The promotive effect of Se in garlic can be enhanced by low Hg exposure (<0.1 mg L^{-1}). When both Se and Hg are at high levels, there is a general antagonistic effect between these two elements in terms of phytotoxicity. Inductively coupled plasma mass spectrometry (ICP-MS) data suggest that Se is mainly concentrated in garlic roots, compared to the leaves and the bulbs. Se uptake by garlic in low Se medium (<0.1 mg L^{-1}) can be significantly enhanced as Hg exposure levels increase ($P < 0.05$), while it can be inhibited by Hg when Se exposure levels exceed 1 mg L^{-1}. The synchrotron radiation X-ray fluorescence (SRXRF) mapping further shows that Se is mainly concentrated in the stele of the roots, bulbs and the veins of the leaves, and Se accumulation in garlic can be reduced by Hg. The X-ray absorption near edge structure (XANES) study indicates that Se is mainly formed in C–Se–C form in garlic. Hg can decrease the content of inorganic Se mainly in SeO_3^{2-} form in garlic while increasing the content of organic Se mainly in C–Se–C form (MeSeCys and its derivatives). Hg-mediated changes in Se species along with reduced Se accumulation in garlic may account for the protective effect of Hg against Se phytotoxicity.

Introduction

Selenium (Se), as a trace element, has been known to be essential for human health. It serves as a cofactor of several key enzymes in the redox-homeostasis machinery, including glutathione peroxidase and thioredoxin reductase.[1,2] Se deficiency has been reported to be associated with several human diseases, including muscular weakness, cartilage dysfunction, and reproductive impairment, with senility and furthering cognitive decline in the elderly.[3,4] However, high levels of Se exposure may also pose a significant threat to human health. Chronic Se toxicosis resulting from excess dietary Se intake has been reported to cause hair and nail loss, liver enlargement, and gastrointestinal and neurological pathologies.[5] The toxicity of selenite may result from the oxidation of glutathione by selenite produced superoxide,[6] and the toxicity of Se compounds is presumably attributed to their prooxidant activity to generate superoxide, hydrogen peroxide, or other cascading oxyradicals.[7]

Se becomes an ecotoxicological concern when natural processes or human activities such as mining or irrigation mobilize the element and thus produce elevated concentrations of Se in water and biota.[8] Fly ash from coal-fired power plants, oil refineries and metal ores smelting are the main sources of Se entering the ecosystems.[9,10] In Wanshan district (Guizhou Province, China), large scale mining and retorting of high Se-containing cinnabar and ores, together with the usage of SeO_2 as a catalyst in the electrolytic manganese metal industry, have dramatically increased Se levels in the local environment.[11,12] It has been reported that Se concentrations in the soil samples

---

[a] *CAS Key Laboratory of Nuclear Analytical Techniques, Key Lab for Biomedical Effects of Nanomaterial and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China. E-mail: gaoyx@ihep.ac.cn*

[b] *University of Chinese Academy of Sciences (UCAS), Beijing 100049, China*

[c] *Department of Physics, Xinzhou Teachers University, Xinzhou 034000, China*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3mt20273a

from Wanshan district range from 0.16 to 36.6 mg kg^{-1}, where approximately 10% of the Se concentrations are higher than 3.0 mg kg^{-1}.[13] As for Se accumulation in crops, Se concentrations in the rice from Wanshan district range from 0.02 to 0.67 mg kg^{-1}, where a large portion of them are higher than recommended Se levels (0.04–0.07 mg kg^{-1}) in agricultural products by the Chinese Nutrition Society. Other than Se, owing to an abandoned Hg mine in Wanshan district, mercury (Hg) concentrations can be up to 790 mg kg^{-1} in paddy soil and average Hg concentrations in the crops there are as high as 78 μg kg^{-1}.[13,14] As we know, food consumption is one of the main pathways of Se or Hg intake in humans. Therefore, analysis of the absorption, accumulation, and location of these two elements or metalloids in crops will be important for a solution of minimizing daily Se/Hg intake in humans.

High levels of Se or Hg are also harmful to agricultural production. In light of Se/Hg combined pollution in Wanshan district, the study of how Se and Hg interaction in crops to modulate their phytotoxicity is thus needed. Recently, we and other researchers have examined the protective effects of Se against Hg phytotoxicity,[15–18] but little on the effects of Hg to Se in plants has been reported. In this study, we took garlic as a crop model to investigate the effects of Hg on Se phytotoxicity. In addition, the absorption, transportation, distribution, accumulation and speciation of Se in garlic have also been examined using inductively coupled plasma mass spectrometry (ICP-MS), together with synchrotron radiation micro X-ray fluorescence spectroscopy (SRXRF) and X-ray absorption near edge structure (XANES).

Materials and methods

Plant cultivation and sample preparation

In Wanshan district of Guizhou Province, we analyzed Se and Hg levels around a large electrolytic manganese factory which is also close to an abandoned Hg mining spot. We found that Se concentrations were as high as 1.81 μg L^{-1} in the surface water and 16.97 mg kg^{-1} in the soils, while Hg concentrations were up to 19.62 μg L^{-1} and 83.93 mg kg^{-1} in the surface water and soils, respectively (unpublished data). To mimic the natural environment in Wanshan district, Se or Hg exposure levels to garlic were set to 0, 0.01, 0.1, 10, and 50 mg L^{-1} in this study.

Well developed garlic bulbs were selected and thoroughly washed with Milli Q water (18.2 MΩ cm). All the garlic bulbs were grown in a green-house at about 20 °C in daytime (16 h, humidity 50%) and 15 °C at night (8 h, humidity 60%). Following one week of the germination in 25% Hoagland solution,[19] the homogeneous seedlings without apparent phenotypical difference were selected and transplanted to the freshly prepared Hoagland solutions supplemented with different dosages of Na_2SeO_3 and $HgCl_2$. Each group contained 8 seedlings as biological replicates. In total, 36 groups of garlic seedlings were cultured in the Hoagland solution containing 0, 0.01, 0.1, 1, 10, 50 mg L^{-1} analytical grade Na_2SeO_3 with or without $HgCl_2$. After another four weeks of growth, all plants were harvested, washed thoroughly with deionized water, and completely dried at room temperature. The effects of Se/Hg on the garlic growth were estimated as a ratio of the difference in dry weights between Se/Hg treated plants and controls to dry weights of the controls.

Measurement of Se content in garlic tissues using ICP-MS

To further analyze Se accumulation in garlic tissues, the freshly harvested plants were divided into roots, bulbs and leaves, followed by lyophilization at −50 °C. 50 mg of lyophilized powder was transferred into a tube with 5 mL of digestion solution containing HNO_3 (BV-III grade reagent) and H_2O_2 (MOS grade reagent) (8 : 1 (v/v)). After incubation overnight at room temperature for pre-nitrolysis, the samples were digested in a sealed pot at 150 °C for 6 hours. The transparent solution was volatilized to a volume of 0.5 mL at 90 °C, and then diluted with 2% HNO_3 (containing 0.01% β-mercaptoethanol) up to 5 mL for Se analysis. Thermo X7 ICP-MS (Thermo Electron Corp., USA) in collision cell mode was used for Se analysis. The operating conditions of ICP-MS are described in Table S1 (ESI†). The calibration standards of Se were prepared by serially diluting the Se standard stock solution (1000 mg L^{-1}, Quality Control Standard 21, Spex. CLMS-1, USA) with 2% (v/v) HNO_3 (containing 0.01% β-mercaptoethanol). A standard Se-containing reference sample, poplar leaf CRM (GSV-3, China) with a labeled Se value of 0.14 ± 0.02 mg kg^{-1}, was used to validate the ICP-MS method. Poplar leaf CRM was processed in parallel with the garlic samples. Before running ICP-MS, optimization was carried out with a normal tuning solution (1 ng mL^{-1} Be, Co, In, U).

Se distribution examination by SRXRF and Se speciation analysis by XANES

For synchrotron radiation X-ray fluorescence (SRXRF) analysis, the roots and bulbs of garlic exposed to 50 mg L^{-1} Se/Hg were embedded, respectively, in tissue freezing medium (Germany), snap-frozen in liquid nitrogen, and then cut into 40 μm thick sections using a cryomicrotome (Leica CM 1850, Germany). The sections were fixed onto 3 μm thick Mylar films (polycarbonate), then dried and stored at −20 °C until analyzed with SRXRF. The leaf samples were clamped between two layers of cellophane to keep them flat and dried at room temperature. All treated leaves were stored in a vacuum desiccator before being analyzed using SRXRF. The uniform fine powders of lyophilized roots, bulbs or leaves were pressed to 1 mm thick tablets for the XANES analysis.

The spatial distributions of Se or Hg in different garlic tissues were measured at 4W1B beamline in Beijing Synchrotron Radiation Facility (BSRF, China). The storage ring ran at an energy of 2.5 GeV and a current intensity of 200–300 mA, with space resolution of several micrometers and detection limit in the ng g^{-1} range. The incident beam was focused approximately 50 μm × 50 μm using a Pb collimator with two cross-slices. A monochromatic X-ray with a photon energy of 15 keV was used to excite the samples. The sample was mounted on an XYZ translation stage and the sample platform was moved by a 2D stepping motor along the X/Z directions of 50 μm for each step. A light microscope was coupled to a computer for sample observation. The count time was 10 s per pixel. In order to correct the effect of the SR beam flux variation on the signal intensity, the peak areas of the elements were normalized to the current intensity (I_0) in the ionization chamber which was placed in front of the samples.

The Se K-edge XANES of the garlic tissues was collected at beamline 14W (3.5 GeV, 250 mA) in Shanghai Synchrotron Radiation Facility (SSRF, China). The beamline 14W was equipped with a Si (111) double-crystal monochromator that was detuned to minimize the harmonic content of the beam. The XANES data were recorded in fluorescence mode (using a 19-elemental Ge solid detector) for all samples. After background subtraction and normalization, the data for the plant samples were fit to all model compounds using WinXAS 3.1 software.[20,21] In successive fits the components which have the lowest percentage of composition were removed, and the remaining compounds were then refit until a reasonable fit was achieved.[20–22] The percentage of each Hg-containing species in total Hg equals the fractional contribution of a model spectrum to the sum.

Results and discussion

Effects of Hg on Se activity in garlic

To investigate the influence of Hg on Se phytotoxicity, the relative growth rates of the garlic upon exposure to Se with or without Hg were determined. As shown in Fig. 1a, garlic growth is slightly stimulated by SeO_3^{2-} at dosages of less than 0.1 mg L^{-1}, indicating a beneficial effect of Se on garlic growth at low levels. However, the growth of the garlic is inhibited when the Se exposure level is over 1 mg L^{-1}. The biomass of garlic is significantly ($P < 0.05$) reduced when the Se concentration exceeds 10 mg L^{-1} in the culture medium (Fig. 1a). As for the 50 mg L^{-1} Hg and Se co-exposed group, the garlic growth is sharply inhibited ($p < 0.01$), indicating significant phytotoxicity of Se and Hg (Fig. 1b).

A modified Bürgi formula (q = observed value/expected value) (described in Table S2, ESI†) was used to evaluate the effects of Hg on Se activity in garlic.[23] If the expected value represents the "addition of element combination", $q = 1$ means a simple addition; $q > 1$, synergism; $q < 1$, antagonism. However, considering an inherent variation in reality, a tolerance of $q \pm 0.15$ was proposed as an upper and lower limit.[23] As for Se–Hg co-exposed garlic, the stimulation of garlic growth by low levels of Se is also enhanced by $HgCl_2$ at low levels (≤ 0.1 mg L^{-1} Hg) (Fig. 1b, Table S2, ESI†). This may be ascribed to the hormesis of the toxicants such as Se and Hg when both are at lower dosages as previously reported in some other organisms.[24–26] By contrast, an antagonistic effect between low levels of Se and higher Hg levels (1 mg L^{-1} or 10 mg L^{-1}) is observed. When the Se level increases to 1 mg L^{-1} or 10 mg L^{-1}, there is a general antagonistic effect between Se and Hg. Furthermore, the inhibitive effect of 50 mg L^{-1} of Se can hardly be modulated by Hg except for a marginal effect induced by 10 mg L^{-1} of Hg (Fig. 1b, Table S2, ESI†).

Se remains controversial regarding its possible roles as a nutrient or toxic agent. In the previous studies, Se as a fertilizer was applied to investigate the effect of Se on the yield and quality of green tea leaves harvested in early spring.[27] The researchers found that the numbers of tea sprouts and the yield were significantly increased by the application of Se, which is consistent with our results that Se at low concentrations can promote the garlic growth. Thangavel *et al.* reported that Se could expedite the decaying process of the leaves of *Portulaca oleracea*, and the protective effect of Se against Hg toxicity was observed only at very low concentrations.[28] For the interactions between Se and Hg, accumulating evidence suggests that the protection of Se against Hg is a common phenomenon in different organisms. On the other hand, the toxic effects of Se have also been observed to be partially alleviated by mercury chloride in chicks as measured by growth retardation and mortality.[29] However, the effects of Hg on Se still remain largely unexplored in plants and thus further study of the phytotoxicity of Se and Hg is needed.

Se accumulation and transportation in garlic

Fig. 2 shows the contents of Se in various parts of the garlic grown in the culture medium containing various dosages of Se and Hg. When garlic was treated with Se(IV), Se levels increase in garlic tissues along with elevated Se exposure levels. For different garlic tissues, a relatively high level of Se was found in roots when Se exposure levels are higher than 0.1 mg L^{-1}.

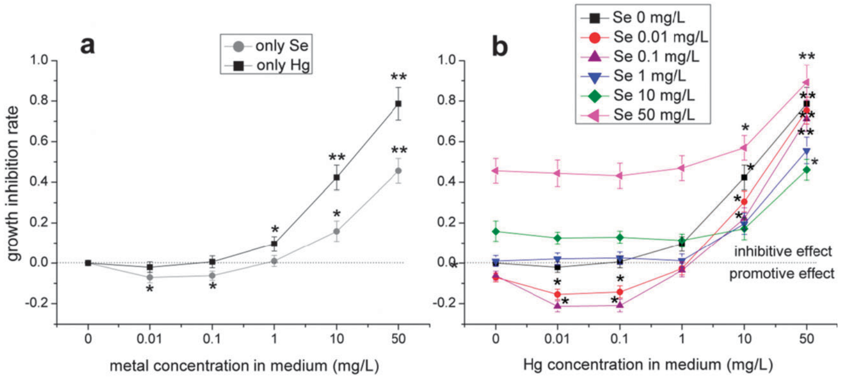

Fig. 1 The growth inhibition rates of garlic exposed to different dosages of Se and Hg. (a) Growth inhibition rates of garlic exposed to different dosages of Se or Hg alone. (b) Growth inhibition rates of garlic co-exposed to different dosages of Se and Hg (*, significant difference at $p < 0.05$; **, significant difference at $p < 0.01$).

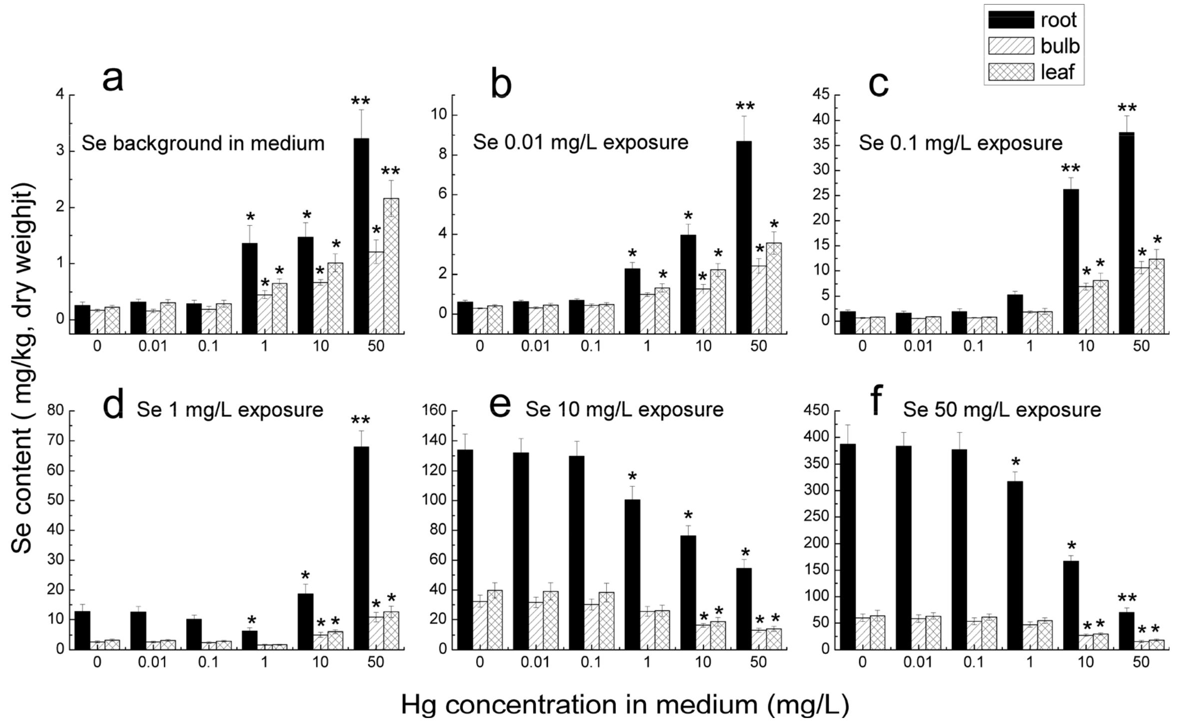

Fig. 2 Se concentration (μg kg^{-1} dry weight) in roots, bulbs and leaves of garlic affected by the Hg level in the culture media. (a) Se content in garlic exposed to Hg alone; (b–f) Se content in garlic exposed to Se at 0.01 mg L^{-1}, 0.1 mg L^{-1}, 1 mg L^{-1}, 10 mg L^{-1} and 50 mg L^{-1} levels, respectively, with Hg co-exposure levels increasing from 0 to 50 mg L^{-1} in medium. Values shown here are mean ±SD of three replicates (*, significant difference at $p < 0.05$; **, significant difference at $p < 0.01$).

For the groups co-exposed to Se and Hg, at the lower level of Se (<1 mg L^{-1}), a significant ($P < 0.05$) increase in Se accumulation in the roots, bulbs and leaves is observed with increasing the Hg exposure level, especially at the higher Hg level (Hg ≥ 1 mg L^{-1}) (Fig. 2a–c). This suggests that excessive Hg exposure can induce Se uptake to balance the Se–Hg stress to garlic growth. A similar phenomenon was previously observed in Singh's study, where the cadmium uptake by oats and rape could be significantly increased by phosphorus at high levels, but not under normal phosphorus exposure conditions.[30] When the concentrations of Se are not less than 10 mg L^{-1} in culture medium, there is no significant differences in Se accumulation in different garlic tissues in the presence of Hg at lower levels (≤1 mg L^{-1}) in the medium. However, for the plant pretreated with higher levels of Se (≥10 mg L^{-1}), the Se accumulation in different garlic tissues is markedly decreased with Hg concentrations (1–50 mg L^{-1}) in the culture medium (Fig. 2e and f), indicating that the Se burden in garlic at a high Se exposure level can be considerably alleviated by Hg. In particular, the content of Se in the roots of the garlic exposed to Se alone is significantly higher than that of the garlic co-exposed to Se and Hg, indicating a possible role of Hg in inhibiting Se uptake by garlic roots.

The bioaccumulation factor (BAF) is used to evaluate the efficiency of Se uptake by garlic. BAF of Se in garlic is defined as: BAF = total Se concentration in garlic tissues/total Se concentration in culture medium.[31,32] The BAFs of Se in garlic are shown in Fig. 3. The BAF is gradually decreased in garlic upon increasing the concentration of Se in the medium for only Se exposure groups (Fig. 3a). For the Se/Hg co-exposure groups, when Se concentrations are less than 1 mg L^{-1} in the medium, the BAFs (Fig. 3b–d) of Se increase with elevated Hg exposure levels in culture medium. By contrast, when Se concentration is more than 10 mg L^{-1} in the medium (Fig. 3e and f), the BAF of Se decreases with elevated Hg exposure levels. The decrease in Se uptake by plants treated with high concentrations of Se and Hg may be attributed to the reduction of Se(IV) forming the Se–Hg complex once reported by Shanker *et al.* in culturing potato with $NaSeO_3$ and $HgCl_2$.[33] The complex has been considered to be stable and insoluble which appears to be unavailable for plant absorption.[33]

The transfer factor (TF) of Se is used to estimate the transportation efficiency of Se from roots to the shoots (bulbs and leaves) of the garlic. TF is defined as total Se concentration in shoots/total Se concentration in roots.[34] As shown in Table S3 (see ESI†), the ratio of Se transported from the roots to the shoots is much smaller for the garlic upon exposure at higher Se levels than that at lower Se levels. This indicates that most of the Se was sequestered in the roots, which may be due to a protective mechanism when the root was stressed by a toxicant. For the Se/Hg co-exposure garlic, the TF is decreased with increasing Hg exposure dosage when Se concentration is less than 1 mg L^{-1} in the medium. However, when Se concentration is more than 1 mg L^{-1} in the culture medium, no significant difference in the TF value was found.

The factors further support the observations that Hg at higher levels can enhance the uptake of Se by garlic upon lower Se level exposure, while inhibiting Se uptake by the garlic grown under higher Se conditions. Moreover, the transportation of Se from roots to the bulbs and leaves of the garlic can be somewhat inhibited by Hg addition in the culture medium. In previous

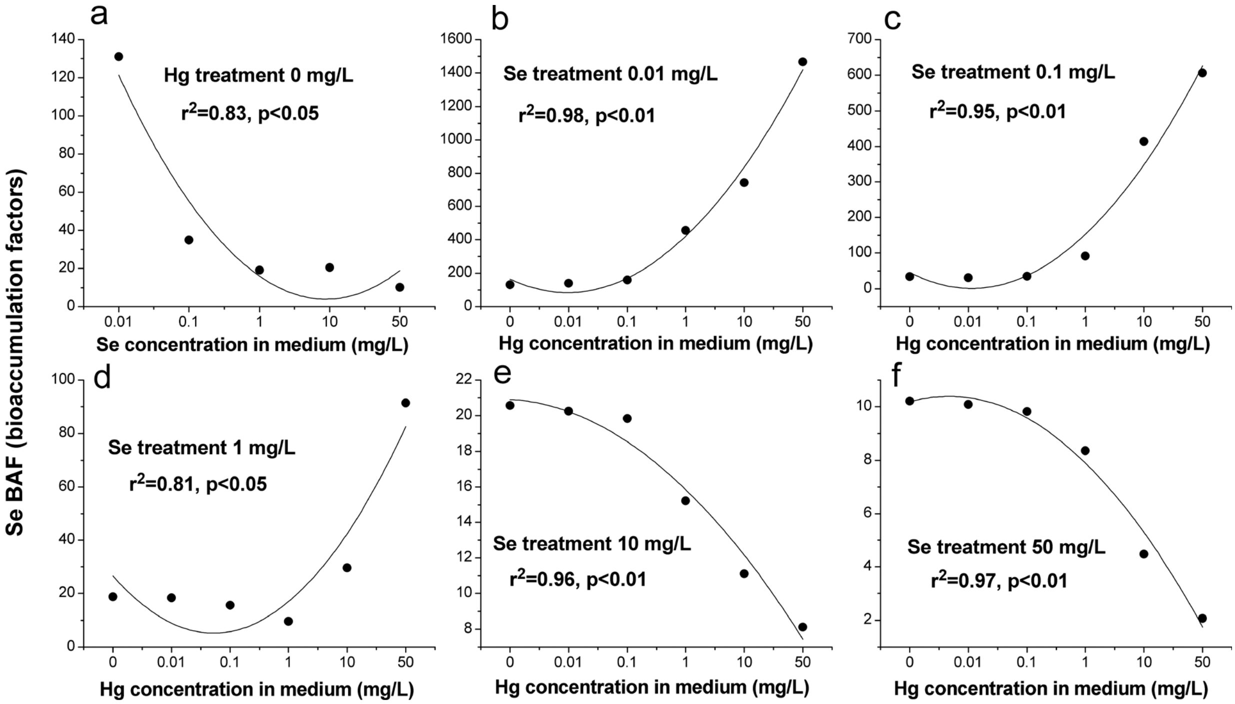

Fig. 3 The bioaccumulation factor (BAF) of Se in garlic cultured in media at different concentrations of Se or Hg. (a) BAF of Se in garlic exposed to Se alone; (b–f) BAF of Se in garlic exposed to Se at 0.01 mg L^{-1}, 0.1 mg L^{-1}, 1 mg L^{-1}, 10 mg L^{-1} and 50 mg L^{-1} levels, respectively, with Hg co-exposure levels increasing from 0 to 50 mg L^{-1} in media.

study, researchers found that the Se distribution pattern was unaffected by the presence of Hg in soybean, but in plants supplemented with 60 mM Se along with Hg, the amount of Se accumulated was almost the same as 120 mM supplemented plants.[35] They concluded that the uptake amount of Se was enhanced in the plants co-exposed to Hg, similar to our results.

Se distribution in garlic by SRXRF analysis

The synchrotron radiation X-ray fluorescence (SRXRF) can identify trace elements in biological samples with high sensitivity (sub-mg kg^{-1}) and high spatial resolution (sub-μm), providing information about 2-D or 3-D distribution of trace elements.[36] SRXRF is used to explore the distribution pattern of Se affected by Hg exposure in garlic. The strong signals of Se and Hg in the XRF images of the roots, bulbs and leaves indicate Se/Hg accumulation in different compartments of the Se/Hg co-exposed garlic.

Fig. 4 shows the SRXRF mapping of Se and Hg in the cross-sections of the roots, bulbs and leaves. In Fig. 4a and b, Se and Hg are mainly concentrated in the epidermis and the stele of the root tip which is the most important site for absorption and translocation of elements to the whole plant. In particular, there are significant amounts of Se and Hg coexisted in the vascular tissues of the root which are the main channels for the transportation of elements. Compared to the group exposed to Se alone, Hg addition can reduce Se accumulation in the stele of the roots, while has little impact on Se accumulation in the epidermis of the roots. These results suggest that both Se and Hg can be sequestered on the surface of the garlic roots; on the other hand, much may be absorbed by the roots and then transferred *via* the vascular tissues to the upper side of the whole plant. Besides, the reduction of the Se content in the stele of the Se–Hg co-exposure garlic roots may be attributed to the interactions between Se and Hg in the culture medium to form a Se–Hg complex that has a low bioavailability for plants. The existence of the casparian strip is believed to be the barrier of the roots for the entry of external substances. This was confirmed by a subsequent analysis of the precipitates from the culture medium at the end of the plant cultivation that Se and Hg exist in the collected precipitates.

The distributions of Se and Hg in the bulbs are shown in Fig. 4c and d. Unlike in the roots, Se is mainly concentrated in the central cylinder of the bulbs, with less in the edge of the bulbs (Fig. $4c_1$ and d_1). Hg, in contrast, is mainly concentrated in the epidermis of the bulbs, with a small amount concentrated in the interface between the storage bulbs and the pericladium, and nearly absent in the stele (Fig. $4d_2$). This may be attributed to different uptake mechanisms and different transportation patterns for Se and Hg. Se is easily absorbed by the roots and then transported upwards, concentrated in the stele of the bulbs, while Hg is mainly sequestered in the epidermis of the roots and bulbs and only a little can be transported upwards from the roots. In addition, comparing with the Se treated group, Hg addition profoundly reduced the Se accumulation in the stele of the bulbs in the Se/Hg co-exposure group. This may be ascribed to the formation of the Se–Hg complex in culture medium, interfering Se uptake by roots and the transportation of Se to the bulbs.

The distributions of Se and Hg in leaves are shown in Fig. 4e–h. Se content decreases slightly from the base to the tips of the leaves,

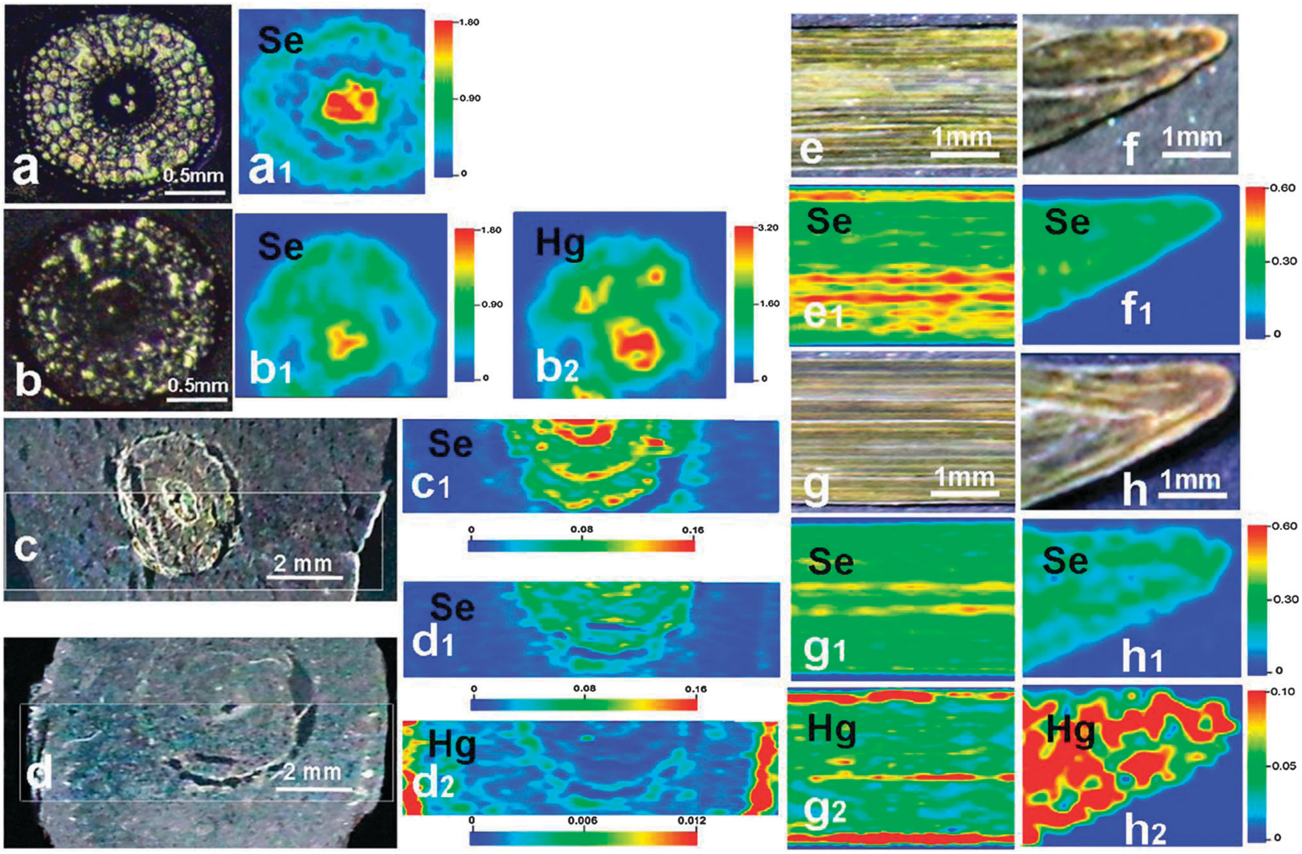

Fig. 4 Se and Hg distributions in cross-sections of garlic roots, bulbs, and leaves taken using the μ-SRXRF elemental imaging technique. (a) Se distributions in root slices of garlic exposed to 50 mg L^{-1} Se alone; (b) Se or Hg distributions in roots of garlic co-exposed to 50 mg L^{-1} Se and Hg. (c) Se distributions in bulbs of garlic exposed to 50 mg L^{-1} Se alone; (d) Se or Hg distributions in bulbs of garlic co-exposed to 50 mg L^{-1} Se and Hg. (e and f) Se distributions in leaves of garlic exposed to 50 mg L^{-1} Se alone; (g and h) Se or Hg distributions in leaves of garlic co-exposed to 50 mg L^{-1} Se and Hg.

and a large amount of Se is concentrated in the leaf veins, with much in the leaf edges (Fig. 4e_1 and f_1). Unlike the distribution patterns of Se, Hg content increases from the base to the tips of leaves. The most abundant of Hg is observed to concentrate in the leaf edge and the leaf tip which are the most vigorous parts of the leaves (Fig. 4g_2 and h_2). The results indicate that Se can diffuse into the mesophyll tissues when it is transferred from the leaf veins, even be transported across the cell wall and membrane. Therefore, the transportation of Se to the leaf tips from the basal part of the leaves is attenuated. However, Hg, as a more serious toxicant, can hardly diffuse through the cell wall and membrane owing to the self-protective mechanism of the plant; it can accumulate in the leaf edges and some may be transported to the leaf tips through the leaf veins with the assistance of the transpiration or accompanied by water transportation. Comparing with the Se exposure group, Se content in the leaves of Se/Hg co-exposure garlic decreases remarkably, especially in the leaf edge of Se/Hg co-exposure garlic (Fig. 4g_1 and h_1).

Se speciation in garlic by XANES analysis

The X-ray absorption near edge structure (XANES) spectrum can provide the chemical forms of an element in samples with minimal sample requirement.[20] It is known that the XANES spectrum reflects the local surroundings of an analyte, including chemical bonds, atomic composition and geometric arrangement of the atoms. To further elucidate the speciation of Se in different garlic tissues and the effects of Hg exposure on Se chemical forms, the Se K-edge XANES spectra were collected. Fig. 5 shows the XANES of Se K-edge of different garlic tissues, along with those of the standard Se-containing compounds. Standards used in the XANES spectra fitting represent a series of Se bonding forms which have been confirmed in plant tissues by other researchers.[37,38] The numerical results of least-square linear combination fitting of Se K-edge XANES spectra in roots, bulbs and leaves are shown in Table 1.

Fig. 5a shows that the spectra of all samples are similar to the spectra of Se in C–Se–C (represented by selenomethionine (SeMet)) and C–Se–H (represented by selenocysteine (SeCys)) forms. The results are further confirmed by least squares fit for the spectra of Se in samples to the spectra of the standard Se-containing compounds (Fig. 5b). For garlic exposed to Se alone, the species of Se in different garlic tissues are mainly in C–Se–C and C–Se–H forms and these two forms represent about 80–90% of the total Se in garlic tissues. The amount of inorganic Se in the SeO_3^{2-} form decreases from roots to leaves while the organic forms of Se in C–Se–C and C–Se–H forms increase (Table 1), indicating a conversion of Se speciation from inorganic to organic forms during absorption and transportation processes.

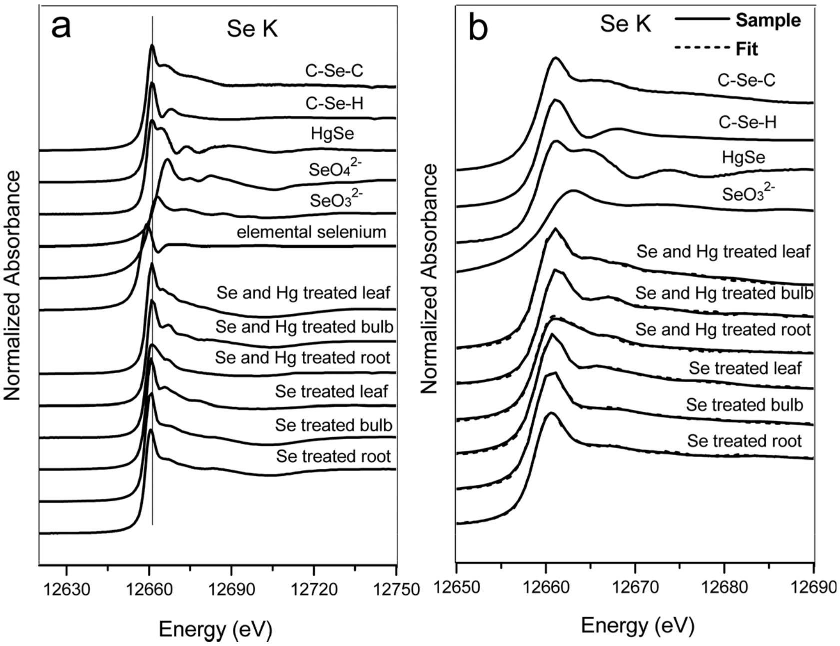

Fig. 5 (a) Normalized Se K edge XANES of the model Se compounds and roots, bulbs, and leaves of garlic exposed to Se alone or co-exposed to Se and Hg. (b) Linear combination analysis of Se near-edge spectra of roots, bulbs and leaves of garlic to the model Se compounds. Se standards spectra are shown as follows: elemental selenium, SeO_3^{2-} (selenite), SeO_4^{2-} (selenate), C–Se–H (selenocysteine) and C–Se–C (selenomethionine).

XANES is sensitive to the local bond geometry and electronic structure of the central Se atom and incompetent to distinguish different Se-containing species which have the same Se-binding motif of C–Se–C, such as Se-methylselenocysteine (MeSeCys), gamma-glutamyl-Se-methylselenocysteine (γGluMeSeCys) and SeMet.[39] In the present study, the main Se-containing species as the C–Se–C form account for above 50% of total Se in different garlic tissues. These compounds may be MeSeCys and its derivatives such as γGluMeSeCys, and they may also be SeMet in Se-containing proteins or in the free amino acid form. The result is comparable to the previous reports that MeSeCys and its derivatives such as γGluMeSeCys are considered to be the major Se metabolites in garlic tissues.[40–42] As the organic forms of Se are of less toxicity than the inorganic forms of Se to organisms, the conversion of the SeO_3^{2-} form to the C–Se–C form in garlic may be one of the important defense mechanisms in garlic to cope with Se toxicity.

Table 1 The numerical results of the least squares fit for the spectra of Se in garlic to that of the standard Se-containing compounds

Se K edge XANES least squares fitting results of garlic tissues to model compounds (%)				
Sample	C–Se–H	C–Se–C	SeO_3^{2-}	HgSe
Sample-1	23.3 ± 1.7	51.9 ± 3.6	25.9 ± 2.3	—
Sample-2	21.5 ± 2.1	56.5 ± 3.2	12.9 ± 1.6	10.6 ± 1.5
Sample-3	29.4 ± 2.7	61.2 ± 4.7	10.3 ± 1.5	—
Sample-4	23.6 ± 3.6	68.9 ± 5.1	4.2 ± 1.3	<1
Sample-5	25.9 ± 3.2	73.6 ± 7.2	—	—
Sample-6	21.7 ± 3.5	76.1 ± 6.9	—	—

Samples 1, 3 and 5 represent garlic roots, bulbs and leaves only exposed to Se; samples 2, 4 and 6 represent garlic roots, bulbs and leaves co-exposed to Se and Hg, respectively. C–Se–H bond containing compounds are represented by SeCys; C–Se–C bond containing compounds are represented by SeMet.

Compared to Se exposure alone, Hg–Se co-exposure decreases SeO_3^{2-} levels in roots and bulbs of the garlic. The inorganic Se in the HgSe form is mainly observed in the roots (about 10% of total Se), with a little in the bulbs (<1% of total Se) and absent in the leaves. The formation of HgSe complex around the rhizosphere may be one of the reasons for the inhibition of Se uptake by roots with Hg co-exposure. In addition, Hg would remarkably promote the transformation of inorganic Se to the organic forms. In the presence of Hg in garlic, the amount of Se species in the C–Se–H form decreases, while the C–Se–C form (most likely MeSeCys and its derivatives such as γGluMeSeCys) increases in all tissues of garlic. As previously reported,[43] some plants like alliums could tolerate the uptake of large amounts of Se from Se rich sites by converting the majority of the toxic inorganic Se into MeSeCys and can be safely accumulated. Furthermore, the Se species in the C–Se–H form such as SeCys could be methylated to the C–Se–C form such as MeSeCys and its derivatives by the action of selenocysteine methyltransferase (SMT).[43] Therefore, the Hg-mediated increase in organic Se in the C–Se–C form may account for Hg–Se antagonism in garlic.

Conclusions

Low-level exposure to Se or Hg is beneficial for garlic growth, whereas Se or Hg levels above certain threshold limits can be harmful. In this study, we show that Hg can attenuate Se phytotoxicity *via* reducing uptake of Se by garlic and inhibiting upward transportation of Se from roots. The XRF results reveal that Hg can play an important role in limiting the accumulation of Se in garlic. The XANES results demonstrate that Hg can modulate Se phytotoxicity *via* promoting the conversion of inorganic forms of Se (mainly in the SeO_3^{2-} form) to organic forms of Se (mainly in the C–Se–C form). The present study reveals some possible pathways by which Hg modifies biological effects of Se in garlic grown under Se/Hg co-contaminated conditions. Further research on the molecular mechanisms of Se and Hg interactions, migration and transformation in plants are still needed.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (Grant No. 20931160430, 20777075 and 11205168), National Basic Research Program of China (No. 2011CB933101), IHEP Innovation program (No. Y2515560U1) and Guizhou Science and Technology Department (No. 2010-3008). Y.-F. Li gratefully acknowledges the support from K.C. Wong Education Foundation, Hong Kong, and the CAS Youth Innovation Association, Chinese Academy of Sciences. SRXRF and XANES analyses were carried out at beamline 4W1B in Beijing Synchrotron Radiation Facility (BSRF) and beamline 14W in Shanghai Synchrotron Radiation Facility (SSRF). We thank Dongliang Chen, Wei Xu, Xiaoli Zhang, Juncai Dong in BSRF and Yuying Huang, Zheng Jiang, Jiong Li, Shuo Zhang in SSRF for their assistance during the SRXRF and XANES measurement.

References

1 G. F. Combs and W. P. Gray, *Pharmacol. Ther.*, 1998, **79**, 179–192.
2 G. H. Lyons, G. J. Judson, I. Ortiz-Monasterio, Y. Genc, J. C. Stangoulis and R. D. Graham, *J. Trace Elem. Med. Biol.*, 2005, **19**, 75–82.
3 M. P. Rayman, *Lancet*, 2000, **356**, 233–241.
4 G. J. Beckett and J. R. Arthur, *J. Endocrinol.*, 2005, **184**, 455–465.
5 M. J. McLaughlin, D. R. Parker and J. M. Clarke, *Field Crop. Res.*, 1999, **60**, 143–163.
6 Y. Seko, Y. K. J. Saito and N. Imura, in *Selenium in biology and medicine*, ed. A. Wendel, Springer-Vedag, Berlin, 1989, pp. 70–73.
7 J. Spallholz, *Free Radical Biol. Med.*, 1994, **17**, 45–64.
8 A. M. H. Debruyn and P. M. Chapman, *Environ. Sci. Technol.*, 2007, **41**, 1766–1770.
9 R. Eisler, *Handbook of chemical risk assessment: health hazards to humans, plants, and animals*, Lewis Publishers, CRC Press, Boca Raton, FL, 2000, pp. 1649–1705.
10 A. D. Lemly, *Selenium assessment in aquatic ecosystems: a guide for hazard evaluation and water quality criteria*, Springer-Verlag, New York, 2002, pp. 3–16.
11 Z. Bao, *Geol. Explor.*, 1975, **11**, 35–37.
12 M. Horvat, N. Nolde, V. Fajon, V. Jereb, M. Logar, S. Lojen, R. Jacimovic, I. Falnoga, L. Y. Qu, J. Faganeli and D. Drobne, *Sci. Total Environ.*, 2003, **304**, 231–256.
13 H. Zhang, X. B. Feng, T. Larssen, L. Shang and P. Li, *Environ. Sci. Technol.*, 2010, **44**, 4499–4504.
14 G. L. Qiu, X. B. Feng, S. Wang and L. Shang, *Appl. Geochem.*, 2005, **20**, 627–638.
15 K. Shanker, S. Mishra, S. Srivastava, R. Srivastava, S. Dass, S. Prakash and M. M. Srivastava, *Food Chem. Toxicol.*, 1996, **34**, 883–886.
16 S. Mounicou, M. Shah, J. Meija, J. A. Caruso, A. P. Vonderheide and J. Shann, *J. Anal. At. Spectrom.*, 2006, **21**, 404–412.
17 P. Thangavel, A. S. Sulthana and V. Subburam, *Sci. Total Environ.*, 1999, **244**, 1–8.
18 J. T. Zhao, Y. X. Gao, Y. F. Li, Y. Hu, X. M. Peng, Y. X. Dong, B. Li, C. Y. Chen and Z. F. Chai, *Environ. Res.*, 2013, DOI: 10.1016/j.envres.2013.01.010.
19 D. R. Hoagland, *Circ. - Calif. Agric. Exp. Stn.*, 1950, 347.
20 Y. F. Li, X. Y. Wang, L. M. Wang, B. Li, Y. X. Gao and C. Y. Chen, *J. Anal. At. Spectrom.*, 2010, **25**, 426–430.
21 T. Ressler, *J. Synchrotron Radiat.*, 1998, **5**, 118–122.
22 T. Ressler, J. Wong, J. Roos and I. Smith, *Environ. Sci. Technol.*, 2000, **34**, 950–958.
23 Z. J. Jin, *Acta Pharmacol. Sin.*, 1980, **1**, 70–76.
24 A. R. D. Stebbing, *Sci. Total Environ.*, 1982, **22**, 213–234.
25 D. Cargnelutti, L. A. Tabaldi, R. M. Spanevello, G. O. Jucoski, V. Battisti, M. Redin, C. E. B Linares, V. L. Dressler, E. M. M. Flores, F. T. Nicoloso, V. M. Morsch and M. R. C. Schetinger, *Chemosphere*, 2006, **65**, 999–1006.
26 M. P. Mattson, E. J. Calabrese, *Hormesis*, 2010, pp. 1–13.
27 Q. H. Hu, J. Xu and G. X. Pang, *J. Agric. Food Chem.*, 2003, **51**, 3379–3381.
28 P. Thangavel, A. S. Sulthana and V. Subburam, *Sci. Total Environ.*, 1999, **244**, 1–8.
29 C. H. Hill, *J. Nutr.*, 1974, **104**, 593–598.
30 B. R. Singh, *Norw. J. Agric. Sci.*, 1990, **4**, 239–250.
31 E. Moreno-Jimenez, R. Gamarra, R. O. Carpena-Ruiz, R. Millan, J. M. Penalosa and E. Esteban, *Chemosphere*, 2006, **63**, 1969–1973.
32 J. X. Wang, X. B. Feng, C. W. N. Anderson, G. L. Qiu, L. Ping and Z. D. Bao, *J. Hazard. Mater.*, 2011, **186**, 119–127.
33 K. Shanker, S. Mishra, S. Srivastava, R. Srivastava, S. Daas, S. Prakash and M. M. Srivastava, *Plant Soil*, 1996, **183**, 233–238.
34 J. X. Wang, X. B. Feng, C. W. N. Anderson, H. Wang, L. R. Zheng and T. D. Hu, *Environ. Sci. Technol.*, 2012, **46**, 5361–5368.
35 S. K. V. Yathavakilla and J. A. Caruso, *Anal. Bioanal. Chem.*, 2007, **389**, 715–723.
36 Y. X. Gao, C. Y. Chen and Z. F. Chai, *J. Anal. At. Spectrom.*, 2007, **22**, 856–866.
37 F. Aureli, L. Ouerdane, K. Bierla, J. Szpunar, N. Prakash and F. Cubadda, *Metallomics*, 2012, **4**, 968–978.
38 G. X. Sun, X. Liu, P. N. Williams and Y. G. Zhu, *Environ. Sci. Technol.*, 2010, **44**, 6706–6711.
39 G. S. Bañuelos, S. S. Walse, S. I. Yang, I. J. Pickering, S. C. Fakra, M. A. Marcus and J. L. Freeman, *Anal. Chem.*, 2012, **84**, 6024–6030.
40 H. H. Ge, X. J. Cai, J. F. Tyson, P. C. Uden, E. R. Denoyer and E. Block, *Anal. Commun.*, 1996, **33**, 279–281.
41 E. Dumont, Y. Ogra, F. Vanhaecke, K. T. Suzuki and R. Cornelis, *Anal. Bioanal. Chem.*, 2006, **384**, 1196–1206.
42 I. Arnault and J. Auger, *J. Chromatogr., A*, 2006, **1112**, 23–30.
43 B. Neuhierl and A. Böck, *Eur. J. Biochem.*, 1996, **239**, 235–238.