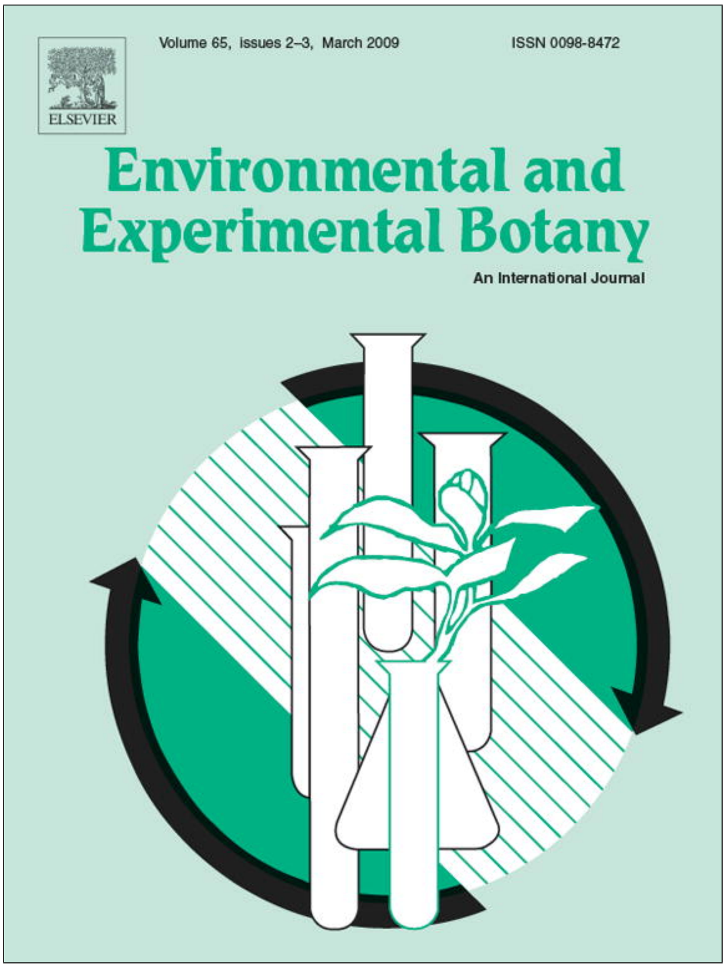
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Interactive effects of selenium and arsenic on their uptake by *Pteris vittata* L. under hydroponic conditions

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ABSTRACT

The interactive effects of selenium (Se) and arsenic (As) on plant uptake of Se and As have rarely been documented. In this study, the interactive effects of As and Se on their uptake by Chinese brake fern (*Pteris vittata*), an As-hyperaccumulator and Se-accumulator, were explored in two hydroponic experiments based on a two-factor, five-level central composite design. At Se levels of less than 2.5 mg L^{-1}, increasing amounts of As stimulated the uptake of Se in Chinese brake fern roots, possibly because of the beneficial effects of Se. In contrast, at Se concentrations greater than 2.5 mg L^{-1}, As suppressed the uptake of Se in Chinese brake fern roots. Uptake of As by both fronds and roots of Chinese brake fern was suppressed by the addition of Se, indicating the antagonistic effects of Se on As. In addition, at Se concentrations of less than 2.5 mg L^{-1}, As stimulated the translocation of Se from roots to fronds; meanwhile, the addition of Se resulted in reduced translocation of As from roots to fronds. These findings demonstrate the interactive effects of As and Se on their uptake by Chinese brake fern.

1. Introduction

Selenium (Se), a non-essential element in plants, is ubiquitous in the environment (Fournier et al., 2005). Over a narrow range of concentrations, the effect of Se on plants changes from beneficial to toxic, and Se has been found to exert dual influences on the metabolism and growth of ryegrass (*Lolium perenne*) (Hartikainen et al., 2000). Generally, in non-accumulating plants, Se levels lower than 1 mg kg^{-1} in soils can enhance plant growth because Se acts as an antioxidant to decrease lipid peroxidation and increase enzyme GSH-Px activity (Hartikainen et al., 2000; Xue et al., 2001; Seppänen et al., 2003; Cartes et al., 2005; Djanaguiraman et al., 2005). More recently, enhanced antioxidative ability was observed in white clover (*Trifolium repens* L.) shoots with Se concentration lower than 200 μg kg^{-1} (Mora et al., 2008). However, excess Se can produce oxidative stress and cause damage to plants (Hartikainen et al., 2000; Xue et al., 2001; Mora et al., 2008) in a manner similar to arsenic (As) (Mascher et al., 2002).

Arsenic and Se play similar roles in many metabolic functions in prokaryotes, such as in assimilation, methylation and detoxification (Stolz et al., 2006). The interaction of As and Se has been documented in humans (Yang et al., 2002) and in mice (Biswas et al., 1999). In general, the two elements exert antagonistic effects on one another in both humans and animals (Zeng et al., 2005), and Se is regarded as an antidote to heavy metal toxicities (such as with Cd^{2+}, Hg^{2+} and possibly Pb^{2+}) (Frost and Lish, 1975). In particular, it has been found that supplementation of Se can counterbalance the negative effects of Pb and Cd on Chinese cabbage (*Brassica rapa*) and lettuce (*Lactuca sativa* L.), resulting in less accumulation of Pb and Cd in these two plants (He et al., 2004). However, the interaction of As and Se in other plants has received little attention.

Chinese brake fern (*Pteris vittata*) has been identified as an As-hyperaccumulator. It has been reported to accumulate more than 1000 mg As kg^{-1} in controlled As-spiked soils (Ma et al., 2001) and in As mines (Chen et al., 2002; Wei and Zhang, 2007). Subsequently, Chinese brake fern was reported to also be a Se-accumulator (Srivastava et al., 2005). Since Chinese brake fern possesses high As and Se tolerance and accumulation properties, this species provides an ideal platform to investigate the interactive effects of As and Se in plants. Furthermore, previous studies of accumulation of As or Se by Chinese brake fern have focused on either As or Se alone; as such, an understanding of the interactive effects of As and Se is highly desired. Consequently, the main objective of this study was to investigate interactive effects of As and Se on their uptake by Chinese brake fern.

2. Materials and methods

2.1. Experimental design

In this study, two sets of experiments were conducted under hydroponic conditions. The treatment levels of both experiments

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Table 1
Treatment levels of Se and As for Experiment I to study the simple effects of As and Se on Chinese brake fern.

Treatment levels	Codes	As (mg L^{-1})	Se (mg L^{-1})
1	−1.414	0	0
2	−1	0.73	0.73
3	0	2.50	2.50
4	1	4.27	4.27
5	1.414	5.00	5.00

The treatment levels were designed according to the codes of a two-factor, five-level orthogonal rotation regression design. Each treatment was replicated three times, the experiment was conducted by imposing the central values of each factor on the other. The central factor is 2.5 mg L^{-1} for both As and Se.

were determined according to the code levels of a two-factor, five-level central composite design (Box et al., 1978). Previous reports have shown that even such low As dosage as 5 mg kg^{-1} might induce oxidative stress in Chinese brake fern under soil cultivation conditions (Cao et al., 2004). Selenium concentration of 1 mg kg^{-1} in soils is considered to be marginally toxic to ryegrass (Hartikainen et al., 2000). Our preliminary hydroponic study using Chinese brake fern with Se levels ranging from 0 to 20 mg L^{-1} also found that Se concentrations lower than 2 mg L^{-1} could significantly decrease the lipid peroxidation (unpublished data). Considering these factors, the central treatment level of 2.5 mg L^{-1} was chosen for both As and Se for the experiments in this study. The other four treatment levels of As and Se were calculated using the following equation:

$$x = \frac{X - X_0}{\Delta j} \quad (1)$$

where X represents the actual treatment level of As or Se, X_0 represents the central value of As or Se (2.5 mg L^{-1}), x is the coded value (Table 1, column codes) and Δj is the scaling factor for As or Se (1.768 mg L^{-1}).

Experiment I was a single-factor experiment designed to investigate the simple effects of individual of As and Se concentrations on uptake of As and Se by Chinese brake fern. To investigate the effects of As on plant uptake of Se, Se was imposed at its central value of 2.5 mg L^{-1} while increasing the As concentration from 0 to 5 mg L^{-1}. Similarly, to study the effects of Se on As uptake, As was imposed at its central value of 2.5 mg L^{-1} while increasing the Se concentration from 0 to 5 mg L^{-1} (Table 1). Each treatment was replicated three times.

In order to investigate the interactive effects of As and Se on the uptake of As and Se by Chinese brake fern, a two-factor (As and Se) and five-level (0, 0.73, 2.5, 4.27 and 5 mg L^{-1}) orthogonal rotation regression design consisting of 16 experimental runs was employed. In this experiment, three replications were employed for each treatment except for run 9, which had eight replications in order to minimize the experimental error (Experiment II, runs 1–16 of Table 2) (Box et al., 1978).

Since some of the treatment levels of Experiment I were the same as that of Experiment II, Experiment I was nested in Experiment II by adding additional single-factor treatments of As and Se to Experiment II (runs 17–20, Table 2), and the two experiments were carried out simultaneously.

2.2. Cultivation of Chinese brake fern

Spores were obtained from fertile fronds of Chinese brake fern grown in southern China. The spores were sown in pottery pots that were filled with As- and Se-free fine sandy soil (<2 mm). The pots were covered with a transparent thin plastic film. Humidity was maintained at a constant level by placing the pot in a larger plastic container containing a depth of 5 cm of water in its base. Two months later, when the sporelings reached a height of 3–4 cm with 2–3 fronds, the plants were transplanted to a plastic seedbed (5 cm in size) filled with a mixture of 1/2 peat moss, 1/4 fine sand and 1/4 vermiculate. After transplantation, the fern sporelings were left to grow for another 3 months. During the latter stage, the small sporelings were fertilized once per week with Hoagland–Arnon nutrient solution (Hoagland and Arnon, 1938) at 1/5 strength to accelerate the growth of the plants.

2.3. Details of the hydroponic experiments

Healthy and uniform ferns with 8–12 fronds were selected for the hydroponic experiments. Ferns were transplanted to hydroponic systems containing Hoagland–Arnon nutrient solution at 1/5 strength with vigorous aeration. The nutrient solution was replaced once per week. Controlled temperatures in the greenhouse ranged from 25 to 28 °C and relative humidity was roughly 75%. A 14-h photoperiod with an average photon flux density of 820 μmol m^{-2} s^{-1} was supplied by cool-white fluorescent lamps.

Before initiating the experiments, healthy Chinese brake ferns of a similar size (i.e., with 8–12 fronds) were selected. These had acclimated in the hydroponic systems for 2 weeks. The roots were rinsed carefully and thoroughly with tap water and then with de-ionized water. Plants were then transplanted to an opaque plastic pot containing 1 l of treatment solution. For each replication, one plant was placed in a hole on a styrofoam sheet that covered each pot. As and Se were added in the form of $Na_2HAsO_4{\cdot}7H_2O$ and Na_2SeO_3, respectively. The pH of the solution was adjusted with diluted HCl or NaOH. After 14 days of growth under single Se or As treatments or co-exposure to Se and As, the plants were harvested. The plants were rinsed carefully and thoroughly with tap water followed by de-ionized water, after which the fern samples were separated into fronds and roots, dried, and ground for As and Se analysis.

2.4. Determination of As and Se concentrations

The ground-up tissues were digested with concentrated HNO_3–$HClO_4$, and the concentrations of Se and As were determined using a hydride generation atomic fluorescence spectrometer

Table 2
Treatment levels of Se and As for Experiment II to study the interactive effects on Chinese brake fern.

Run	Codes		Treatments	
	As	Se	As (mg L^{-1})	Se (mg L^{-1})
1	1	1	4.27	4.27
2	1	−1	4.27	0.73
3	−1	1	0.73	4.27
4	−1	−1	0.73	0.73
5	1.414	0	5.00	2.50
6	−1.414	0	0	2.50
7	0	1.414	2.50	5.00
8	0	−1.414	2.50	0
9	0	0	2.50	2.50
10	0	0	2.50	2.50
11	0	0	2.50	2.50
12	0	0	2.50	2.50
13	0	0	2.50	2.50
14	0	0	2.50	2.50
15	0	0	2.50	2.50
16	0	0	2.50	2.50
17	−1	0	0.73	2.50
18	1	0	4.27	2.50
19	0	−1	2.50	0.73
20	0	1	2.50	4.27

The treatments were based on a two-factor and five-level orthogonal rotation regression design. All runs were performed in triplicate except run 9, which was replicated eight times to minimize the experimental errors according to orthogonal rotation regression design.

(AFS820, Beijing Titan Instruments Co., China) (Wei et al., 2006). Accuracy of the elemental analysis was verified using standard reference material from the Center for Standard Reference of China.

2.5. Statistical analysis

Data from the uptake of As and Se in Experiment II were modeled by fitting a polynomial quadratic equation with the effects of the linear, quadratic and interactive terms of As and Se concentrations (Al-Attar and Nickless, 1988; Tu and Ma, 2003) as shown in the following equation:

$$y = b_0 + \sum_{j=1}^{m} b_j x_j + \sum_{i \neq j}^{m} b_{ij} x_i x_j + \sum_{j=1}^{m} b_{jj} x_j^2 \quad (2)$$

where y represents As or Se uptake, x represents the concentration of As or Se in the solution, b_j, b_{ij} and b_{jj} represent the regression coefficients, m is the number of factors, and i and j are the order numbers of the variables.

Three-dimensional surface plots were drawn according to the polynomial quadratic equation to illustrate the interactive effects of As and Se on the uptake of As and Se by Chinese brake fern.

Data from Experiment I were $\log_{10}$-transformed to reduce the variation. One-way ANOVA with multi-comparisons using Tukey's test at $P < 0.05$ was performed to compare the means among different treatments. For Experiment II, modeling and graphing of the experimental results were completed through REG and GRAPH procedures. All statistical analyses were completed using SAS software.

3. Results

3.1. Simple effects of As on Se uptake

When As was imposed at its central value of 2.5 mg L^{-1}, the uptake of Se by both fronds and roots was found to increase significantly with the addition of Se (for fronds: $F = 435.907$, $P < 0.001$; for roots: $F = 1009.492$, $P < 0.001$) (Fig. 1a and b). In contrast, when Se was imposed at its central value of 2.5 mg L^{-1}, the uptake of Se by the fronds of Chinese brake fern remained nearly constant with the addition of As (Fig. 1a) compared to the control; only at the treatment level of 4.27 mg As L^{-1} was a small, but significant, decrease observed ($F = 6.966$, $P = 0.006$). At the same time, Se uptake of roots was also found to be unchanged, except at a concentration of 0.73 mg L^{-1} As for which there was a small, but significant, decrease compared to the control ($F = 11.283$, $P = 0.001$) (Fig. 1b).

It is interesting to note that the ratios of frond-to-root Se concentrations, known as translocation factors (TFs), expressed different trends for Se versus As addition (Fig. 2a and b). When As was imposed at its central value of 2.5 mg L^{-1}, the average TFs of Se increased from 0 to 0.34 (Fig. 2a) with the addition of Se; whereas, when Se was maintained at its central value of 2.5 mg L^{-1}, the average TFs of Se decreased from 0.36 to 0.24 with the addition of As, except at the level of 0.73 mg L^{-1} As (Fig. 2b).

3.2. Simple effects of Se on As uptake

When Se was imposed at its central value of 2.5 mg L^{-1}, the uptake of As by fronds was observed to increase substantially with the addition of As (for fronds: $F = 141.701$, $P < 0.001$; for roots: $F = 403.494$, $P < 0.001$) (Fig. 1c). The average As uptake by fronds increased only 3.3-fold at the imposed level of 4.27 mg As L^{-1}; whereas, the uptake increased 16.4-fold at an As concentration of 5 mg L^{-1} (relative to the control). Similar phenomena were found for the uptake of As by roots, in which increasing rates of As uptake at the imposed level of 4.27 mg As L^{-1} were much lower than at that of 5 mg As L^{-1}. When As was imposed at its central value of 2.5 mg L^{-1}, As uptake by fronds and roots was found to decrease substantially with the addition of Se (for fronds: $F = 17.577$, $P < 0.001$; for roots: $F = 140.781$, $P < 0.001$). The only exception was found for the fronds at the imposed level of 5 mg Se L^{-1} (Fig. 1c).

The average TFs of As were found to increase with the addition of Se except for an unexplainable undulation at the level of 4.27 mg Se L^{-1} (Fig. 2c). The TFs decreased at the moment when As levels increased (Fig. 2d).

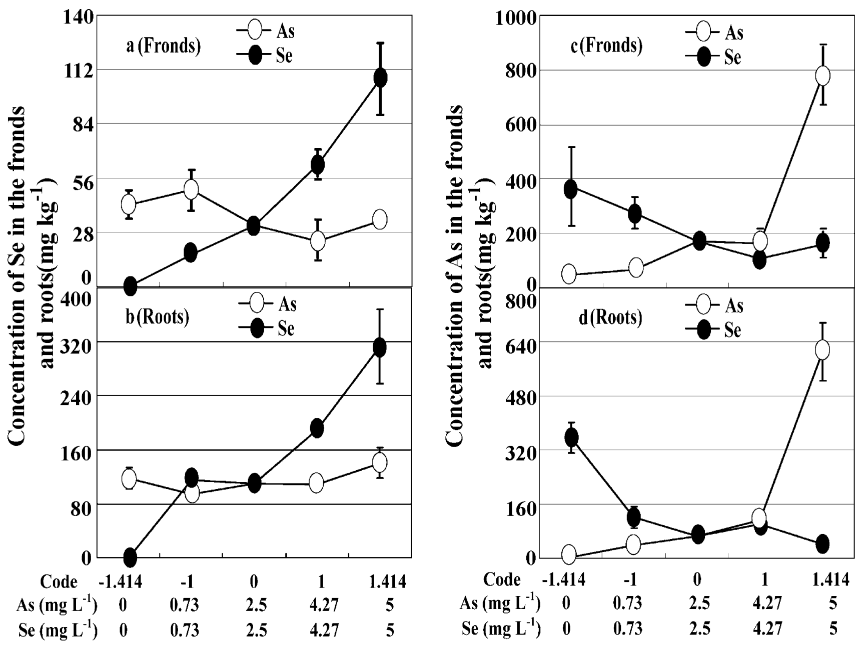

Fig. 1. Simple effect of addition of As and Se in the solution on the uptake of As and Se by Chinese brake fern after being grown for 14 days under hydroponic conditions. Each treatment was replicated three times. Symbols and vertical lines in the curves are means and standard error of means. Each of the curves was drawn based on the related single-factor experiment. (a and b) The effects of As and Se on the uptake of Se in the fronds and roots and (c and d) the effects of As and Se on the uptake of As in the fronds and roots, respectively. Line –○– As represents the experiments in which Se was maintained at its central value of 2.5 mg L^{-1} while increasing the As concentration from 0 to 5 mg L^{-1}; line –●– Se represents the experiments in which As was maintained at its central value of 2.5 mg L^{-1} while increasing the Se concentration from 0 to 5 mg L^{-1}.

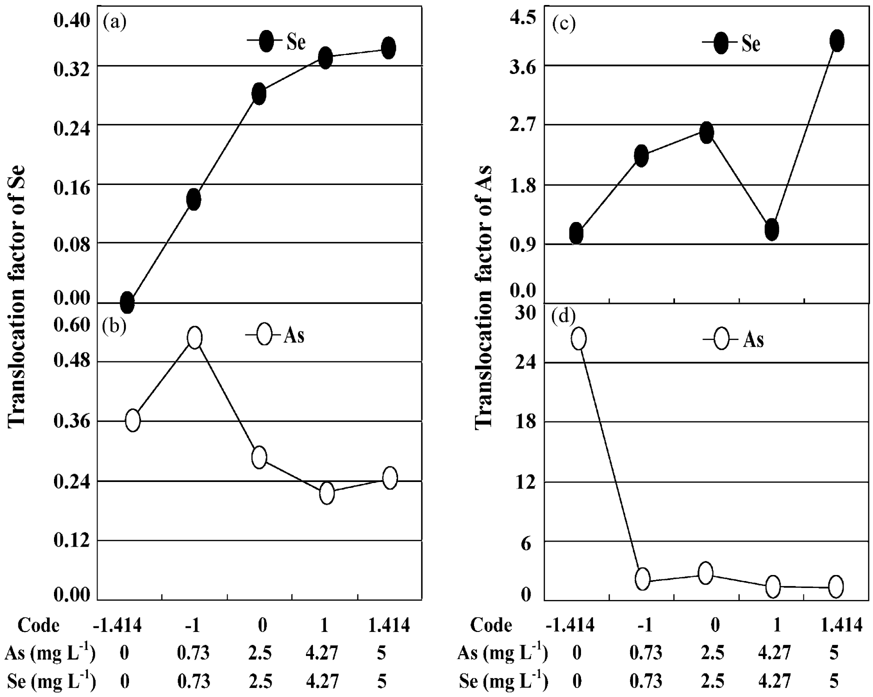

Fig. 2. Simple effect of the addition of As and Se in solution on the translocation factor (the ratio of As or Se concentration in fronds to that in roots) in Chinese brake fern after being grown for 14 days under hydroponic conditions. Each treatment was replicated three times. (a and b) The effects of increasing Se or As levels on the translocation factors of Se, and (c and d) the effects of increasing Se or As levels on the translocation factors of As. For an explanation of the lines –●– Se and –○– As refer to the caption for Fig. 1.

3.3. Interactive effects of Se and As on uptake

At exposure of lower than 2.5 mg L^{-1} Se levels, the uptake of Se by roots of Chinese brake fern increased with increasing amounts of As except at Se levels less than 1.221 mg L^{-1} and As levels less than 2.568 mg L^{-1}, where Chinese brake fern took up very little Se in the roots (Fig. 3a, low area). The calculated transitional value for Se uptake by roots was 2.5 mg L^{-1}. This was the concentration at which the uptake of Se by roots changed from increasing to decreasing with increasing As levels. Near this transition value, the uptake of Se by roots was almost unchanged with increasing levels of As (Fig. 3a). When exposed to low levels of As, as Se levels increased from low to high, Chinese brake fern could accumulate more Se in the roots (Fig. 3a, high area); however, when exposed to high As levels, the uptake of Se by roots of Chinese brake fern decreased sharply.

Interactive effects of the two elements on As uptake by roots were demonstrated in the contour plot shown in Fig. 3b. At relatively high As (5 mg L^{-1}) but low Se (0 mg L^{-1}) levels, the fern took up most of the As in roots, and the uptake of As increased steadily with increasing As levels (Fig. 3b, high area); however, with the same initial concentrations, increasing Se levels led to decreased uptake of As by Chinese brake fern in the roots. It is interesting to note that, when Se was at a relatively high level of 5 mg L^{-1}, as As levels increased, the uptake of As first decreased (Fig. 3b, low area) and then subsequently increased (Fig. 3b, increased area).

4. Discussion

4.1. Stimulation effect of As on Se uptake by Chinese brake fern

The effects of As on uptake of Se by Chinese brake fern are illustrated in Figs. 1a and b and 3a, which indicate that the effects of As on Se uptake might switch from stimulating to antagonistic, depending on the levels of Se. Since As is a non-essential element and can cause oxidative stress in plants (Hartley-Whitaker et al., 2001; Cao et al., 2004), the stimulation by As of Se uptake in the

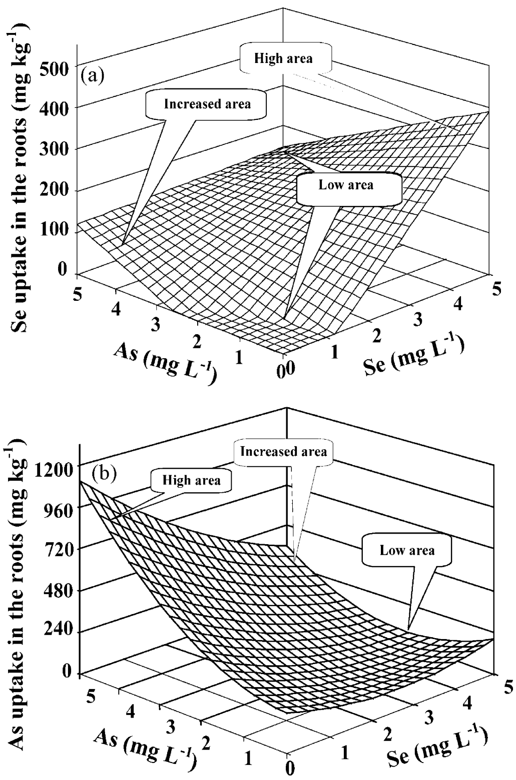

Fig. 3. Interactive effects between As and Se in the roots of Chinese brake fern after being grown for 14 days under hydroponic conditions. Each treatment was replicated three times except run 9, which was replicated eight times to minimize the experimental error. (a) The interactive effects between As and Se in the roots of Chinese brake fern on the uptake of Se by the roots and (b) the interactive effects between As and Se in the roots of Chinese brake fern on the uptake of As by the roots.

roots might be related to the antioxidative functions of low levels of Se. In other words, the addition of As might induce oxidative stress, which, in turn, could be alleviated by lowering Se concentrations to less than 2.5 mg L^{-1}. Similar stimulation of Se uptake by As has been found by Ebbs and Weinstein (2001), who reported that 1 mg L^{-1} As remarkably enhanced the uptake of Se in barley (*Hordeum vulgare* L., cv. Spring Ontario) exposed to 0.01 mg Se L^{-1} in a hydroponic experiment. When Se was maintained at its central value of 2.5 mg L^{-1} (i.e., the transition value for Experiment II), the uptake of Se in both fronds and roots remained almost constant despite increases in As concentration (Fig. 1a and b), suggesting the stimulating effect of As on Se uptake was weak at this point. This result was subsequently confirmed in Experiment II (Fig. 3a).

4.2. Antagonistic effect of As and Se in Chinese brake fern

When Se levels increased above 2.5 mg L^{-1}, the antagonistic effect of As on Se uptake replaced the stimulating effect. This result was demonstrated by the roots of Chinese brake fern taking up more Se at low As levels (Fig. 3a, high area) and less Se at high As levels (Fig. 3a, low area). The antagonistic effect was more obvious for Se affecting As uptake than for As affecting Se uptake in the roots of Chinese brake fern (Figs. 1a–d and 3a and b). The relatively slowly increasing rates of As uptake both in fronds and roots under the imposed As values of 0–4.27 mg L^{-1} indicate that As uptake was clearly retarded by the presence of 2.5 mg L^{-1} Se (Fig. 1c and d); however, as As levels increased from 4.27 to 5 mg L^{-1}, sharp increases in As uptake rates were observed (Fig. 1c and d). This result demonstrates the As-hyperaccumulating nature of Chinese brake fern, a characteristic that is observed with or without co-exposure to Se. Further evidence shows the antagonistic effect of Se on As uptake. The uptake of As was clearly inhibited by high Se levels relative to low Se levels (Fig. 3b, high, low and increased areas). These findings are consistent with those of Khattak et al. (1991), who found that in alfalfa (*Medicago sativa*), Se concentrations of less than 0.1 mg L^{-1} levels supplied as Na_2SeO_4 suppressed the uptake of As. Other reports have also confirmed the antagonistic effects of Se on other heavy metals, such as Pb and Cd in the plants of Chinese cabbage (*B. rapa*) and lettuce (*L. sativa*) (He et al., 2004). In their study, He et al. (2004) found that enrichment of Se slowed the accumulation of Pb and Cd.

4.3. Effect of As and Se on the translocation of As or Se

The variation of TFs of Se showed that, when Chinese brake fern was maintained at the central value of 2.5 mg As L^{-1}, as the concentration of Se increased from 0 to 2.5 mg L^{-1}, increased translocation of Se from roots to fronds was observed (Fig. 2a). A sharp reduction of As uptake by roots occurred simultaneously (from 357 to 40 mg kg^{-1}) (Fig. 1d), resulting in increases in the TFs of As (Fig. 2c). In general, this fern demonstrates stable rates of As accumulation and translocation from roots to fronds (Ma et al., 2001; Chen et al., 2002), with most of the As being taken up and stored in fronds. Consequently, Chinese brake fern might take up and transport more Se to fronds in the presence of As, possibly to counteract the oxidative stress induced by As. Low levels of Se may act as an antioxidant, which in turn could reduce the accumulation of As in the fronds of Chinese brake fern (Fig. 1c), thereby decreasing the TFs of As (Fig. 2d) and demonstrating the ability of As to stimulate Se uptake by roots (Fig. 3a, increased area). When Se levels increased to values greater than 2.5 mg L^{-1}, the TFs of Se remained almost unchanged. This result suggests that the toxic effects of Se had begun to operate in Chinese brake fern, leading to less translocation of Se from roots to fronds. This property is in agreement with the definition of "accumulator" suggested by Baker (1981), indicating that Chinese brake fern is a Se-accumulator.

Interestingly, when Se was imposed at its central value, Chinese brake fern preferentially increased As uptake in roots, which led to antagonistic effects on Se and resulted in a sharp decrease of the TFs (Fig. 2d). Unexpectedly, when As was maintained at the value of 0.73 mg L^{-1}, the TF of Se increased (Fig. 2b), possibly due to the weak beneficial effect of Se.

5. Conclusions

The interaction of As and Se on their uptake by Chinese brake fern, an As-hyperaccumulator and Se-accumulator, was fully explored in this study. The results show that at low levels of Se, As enhanced both Se uptake and the translocation of Se from roots to fronds. In contrast, at higher levels of Se, As suppressed the uptake of Se by Chinese brake fern. These results suggest that As serves to both stimulate and suppress Se uptake. The result is also in agreement with the well-known fact that Se is an element with both beneficial and toxic properties. The effect can change from beneficial to toxic based on the concentration of Se in plants. In this study, Se addition invariably reduced As uptake as well as the translocation of As from roots to fronds in Chinese brake fern, suggesting Se only plays an antagonistic role in As uptake.

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