Title: Root application of selenite can simultaneously reduce arsenic and cadmium accumulation and maintain grain yields, but show negative effects on the grain quality of paddy rice

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Abstract

Most current technologies can hardly simultaneously reduce the accumulation of arsenic (As) and cadmium (Cd) in crops. In this study, root application of selenite [Se (IV)] and selenate [Se (VI)] was used to assess their abilities to reduce the accumulation of As and Cd, and maintain the yields and quality of rice grains. The results show that Se (IV) showed a weaker ability than Se (VI) to maintain the grain contents of many essential elements, but a stronger ability to decrease As and Cd contents in rice grains, and maintain the yields, photosynthesis rate and stomatal conductance, and increase the grain contents of several amino acids (AAs), total Se, selenomethionine (SeMet) and selenocysteine (SeCys). The best outcomes resulted at a relatively high application of 5 mg kg⁻¹ Se (IV), reflecting in the highest total Se, SeCys and SeMet content (14.95, 118.70 and 864.73 µg kg⁻¹, respectively) in the grains, highest grain yield, and lowest grain As and Cd content (0.36 and 0.07 mg kg⁻¹, respectively). In addition, the application of 1-5 mg kg⁻¹ Se (IV) seemed to facilitate the formation of SeMet in the grains, but most inorganic Se in the grains were transformed into SeCys and SeMet under Se (VI) treatments. This study provides a new idea to resolve the problems of high accumulation of As and Cd in rice grains and insufficiency of Se intake in China.

Keywords: rice growth; photosynthesis; dry biomass; grain production and quality; remediation

1. Introduction

Cadmium (Cd) and arsenic (As) are highly toxic to organisms and are becoming more widely distributed with increasing pollution (Chaney et al., **2004**), especially in China (Zheng et al., **2013**). For examples, the As and Cd concentration in the grains of 107 rice plants sampled from the downstream area of the Lianhuashan tungsten mine in China reaches up to 1 and 0.79 mg kg⁻¹, respectively, being far higher than their individual China food safety national standard (GB 2762—2012, for both As and Cd: 0.2 mg kg⁻¹) (Yu et al., **2016**); Zhou et al. (**2008**) reported that the grain As and Cd concentration in 23.1% of 25 rice samples collected around a smelting plant in the Guixi city, Jiangxi province, China, exceeds their individual China national standard.

Neither Cd nor As are essential nutrients for plants. However, both elements can accumulate extensively in plants, reduce crop yield and even cause plant death (Feng et al., **2013b**; Garg and Singla, **2011**; Kozhevnikova et al., **2007**; Zeng et al., **2008**). Long-term Cd intake can cause functional changes in the urinary system and affect bone development, and long-term As intake may cause various cancers such as those of the bladder, lungs, and liver (Farnese et al., **2014**; Kobayashi et al., **2002**).

Selenium (Se) is an essential nutrient for humans and low levels of Se are reported to be beneficial to plants (Ding et al., **2014**). Se can inhibit the uptake of heavy metals (metalloids) in plants (Ding et al., **2015**; Filek et al., **2008**; Feng et al., **2013**, **2013b**; Lin et al., **2012**; Malik et al., **2012**; Saidi et al., **2014**) including Cd or As, and promote plant growth and improve crop production (Shah et al., **2001**; Zahedi et al., **2009**). Studies on the roles of low levels of Se in reducing the phytotoxicity of heavy metals (metalloids) have mainly focused on its antioxidant function, regulation of the photosynthetic

system, and the repair of damaged cells (Cartes et al., **2010**; Djanaguiraman et al., **2005**; Hartikainen et al., **2000**; Mora et al., **2008**). Thus far, few studies (Liao et al., **2016**) have reported the ability of Se to reduce the accumulation and the phytotoxicity of As and Cd in agricultural products. Moreover, few studies (Liao et al., **2016**) have evaluated the effects of Se on crop yields and quality under heavy metal (metalloid) stresses. This has greatly limited the practical use of Se to reduce heavy metal (metalloid) accumulation in agricultural products.

After being taken up by the root system, Se (IV), Se (VI) and organic Se compounds show different routes in their transport and distribution in plants (Zhu et al., **2009**). Generally, the majority of Se (VI) in the plant roots will be retained in its original form and be rapidly transported through the xylem to the shoots, while Se (IV) is rapidly assimilated into organic forms that are less mobile (Zayed et al., **1998**; Carey et al., **2012**). The variations in the uptake and the metabolism of different Se species may influence the ability of Se to reduce the uptake and the phytotoxicity of heavy metals (metalloids) in plants. Research on this topic has been scarce thus far.

It is reported that the average Se content in rice grains in different regions of China is $0.025 \mu\text{g g}^{-1}$ (seriously low-Se rice), which results in the Se deficiency of inhabitants in China because the cereal Se is the main Se source in the Chinese diet (Chen et al., **2002**). In China, the general population in 72% of the total land area is facing a Se deficiency problem (Feng et al., **2013a**). Se deficiency can directly endanger human health and cause diseases such as Keshan, cardiovascular, liver diseases, cataracts, and even cancers (Cox and Bastiaans, **2007**; Feng et al., **2013a**). Furthermore, Mahmoud and

Edens (2003) reported that inorganic Se has a higher toxicity than organic Se (SeCys and SeMet); however, organic Se is safer, and more easily assimilated by humans (Cantor et al., 1975; Fox et al., 1981; Rayman, 2008), and has a higher antioxidant capacity (Mahmoud and Edens, 2003) than inorganic Se. Therefore, it is of practical importance to protect the population's health by increasing the Se content, especially organic Se content in foods, and thereby increase the Se intake of the population in Se-deficient regions.

Here, we hypothesize that the root application of different Se species can reduce the grain contents of As and Cd, and at the same time maintain the yields and grain quality in paddy rice. To this end, the present study applied base fertilizers to address the following objectives: 1) to investigate the ability of selenite or selenate to reduce the phytotoxicity and the uptake of As and Cd, and compare the differences between various Se species; 2) to examine the effect of different Se species on the plant growth, grain yield, and amino acid (AA) contents of rice grains (*Oryza sativa* L.); and 3) to determine the changes in Se species in rice grains.

2. Materials and methods

2.1. Soil pretreatment

The test soil was sampled from an agricultural field. According to the China Soil Environmental Quality Standards (GB 15618-2008, pH within a range of 5.5 to 6.5 for a paddy field, 30 mg kg⁻¹ for As and 0.30 mg kg⁻¹ for Cd), the soil Cd and As concentration was 4.83 and 1.78 times higher than the individual Quality Standard, respectively. The sampling site is located in the Diaojiang catchment in Duan County,

Guangxi Zhuang Autonomous Region, China. The basic physical and chemical properties of the test soil are shown in Table 1. The methods for the determination of soil properties were described in the study of Tang et al. (1999). The soil sample was placed in a greenhouse and air dried naturally for at least one month. The air-dried soil was ground and passed through a 0.84 mm sieve, thoroughly mixed and placed in the dark until use. In the experiments, the rice plants were cultured in plastic barrels. There were three barrels (i.e., replicates) in each treatment, and each barrel contained 5 kg soil pre-mixed with base fertilizers and Se treatments. The base fertilizers were applied to the soil in the following doses: N [$\text{CO}(\text{NH})_2$], 0.20 g kg^{-1} ; P (KH_2PO_4), 0.12 g kg^{-1} ; and K (K_2SO_4), 0.26 g kg^{-1} (all base fertilizers are supplied with analytical reagents). Se (Na_2SeO_3 and Na_2SeO_4 , analytical grade) was applied at the following rates: CK (0 mg kg^{-1} , control, without Se addition), 0.1 mg kg^{-1} [Se(IV)0.1, Se(VI)0.1], 1 mg kg^{-1} [Se(IV)1, Se(VI)1] and 5 mg kg^{-1} [Se(IV)5, Se(VI)5]. The soil mixed with base fertilizers and Se treatments was flooded for one week before the rice seedlings were transplanted.

2.2. Plant culture

Hybrid white rice (H-Liangyou 6839) was used in the greenhouse experiment. The cultivation of the rice seedlings followed the methods of Feng et al. (2013). Three-week-old seedlings with three true leaves were transplanted into the plastic barrels, with two plants per barrel. During the subsequent growth process, the rice plants were maintained in a glass greenhouse with natural light at temperatures of $15\text{--}38^\circ\text{C}$ night/day. The soil was flooded at a depth of 3–4 cm above the ground of soil

throughout the rice-growing period.

A photosynthesis system (LI-6400 XT) was used to measure flag leaf photosynthesis in the heading stage. After the growth for 135 days, plant height was measured, and then the rice plants were separated into roots, shoots and panicles. The roots and shoots were carefully washed with de-ionized water and dried in an oven at 70°C to a constant weight. The dry weight of the shoots was then measured to estimate the biomass. The panicles were dehulled and the grain yield per barrel was measured. The roots, shoots, husks and grains were ground with a small ceramic grinder before digestion.

2.3. Sample preparation and analysis

The soil and plant samples were digested using a DigiBlock ED54 digestion system (LabTech, Beijing, China). The digestion methods of the soil and plant samples refer to that of Chand and Prasad (2013) and Wei et al. (2006), respectively.

The Cd, As, Se and essential nutrients in the digested plant and soil samples were determined using an iCAP Qc inductively coupled plasma mass spectrometer (Thermo Fisher, USA). Standard reference materials (bush leaves, GBW07603, GSV-2; CRM soil, GBW07408, GSS-8) were used to ensure the accuracy of the elemental analysis, which were purchased from the Center for Standard Reference of China.

2.4. Determination of amino acids in rice grains

Briefly, approximate 0.2 g grain powder samples were weighed into 20 mL ampoules, to which a 6 mol L⁻¹ HCl solution was added. The mixture was frozen in liquid nitrogen and vacuumed to 7 Pa before sealing. The vacuum hydrolysis tubes were placed in a

110°C thermostat oven for 24 h of hydrolysis. After being cooled and mixed, the tubes were opened and the solutions were filtered. A portion of the solutions was collected using a pipette and evaporated to dryness at 60°C under reduced pressure using a rotary evaporator. Sodium citrate buffer [$c(\text{Na}^+) = 0.2 \text{ mol L}^{-1}$, $\text{pH} = 2.2$] was added to the products, which were then shaken and filtered. The AAs in the supernatant were analyzed according to the Chinese national standard method GB/T 18246–2000. Standard reference materials for AAs analysis (AAS18-10ML) were purchased from the Sigma Aldrich Company.

Instrument conditions: The 835–50 AA analyzer (Hitachi Limited, Japan) was operated at a buffer flow rate of $0.225 \text{ mL min}^{-1}$, a ninhydrin flow rate of 0.30 mL min^{-1} , a column temperature of 53°C , and a reaction cell temperature of 97°C .

2.5. Determination of different Se species in rice grains

The combined instrument system used to determine the different Se species was comprised of three parts: high performance liquid chromatography (HPLC), hydride generation (HG), and atomic fluorescence detection (AFS). The HPLC system was comprised of a high-pressure pump (Shimadzu, Tokyo, Japan) equipped with a $200 \mu\text{L}$ sample loop of a six-port injection valve (7725i, Rheodyne, Cotati, USA) and a PRP-X100 anion exchange column (Hamilton, Reno, NV, USA); the mobile phase consisted of $60 \text{ mmol L}^{-1} (\text{NH}_4)_2\text{HPO}_4$ ($\text{pH} 6.0$, 1.0 mL min^{-1}). For the HG system, the reducing agent and carrier solution were $2.0\% \text{ KBH}_4$ (m/v) + $0.35\% \text{ KOH}$ (m/v) and $10\% \text{ HCl}$ (v/v), respectively. The detection system was an AFS-820 atomic fluorescence spectrometer, in which the excitation light source was a high-performance Se hollow

cathode lamp (General Research Institute for Nonferrous Metals, Beijing, China) operated at 80 mA; the photomultiplier tube was operated at a high negative voltage of 300 V; the carrier and shielding gas flow rates were 400 and 600 mL min⁻¹, respectively.

Rice grain powder samples (0.2 g each) were weighed into 15 mL centrifuge tubes before adding 10 mL of methanol and water (1 : 2). After being vortexed, the mixture was sonicated for 15 min (room temperature) and then centrifuged for 30 min (at least 5000 r min⁻¹). The supernatant was transferred into dark-purple Erlenmeyer flasks and concentrated under reduced pressure at 40°C using a rotary evaporator. The methanol was removed and the remaining sample was filtered through a 0.45 µm membrane filter before analysis (Han et al., 2013).

The standards for the different species of Se included: SeO₄²⁻ standard solution (GBW10033) and Se (IV) standard solution (GSB04-1751-2004) purchased from China Standards Network, and SeMet (S3875) and selenocysteine (SeCys, S1650) purchased from Sigma–Aldrich. The spike-recovery chromatogram of four Se species in standard solution and the chromatogram of Se species in rice grains treated with 5 mg kg⁻¹ Se (VI) are listed in Figure 1.

2.6. Bioconcentration factor (BCF) and translocation index (TI)

The BCFs of Cd and As were calculated using Eq. (1):

$$BCF = c_{Cd/As, plant} \times 100\% / c_{Cd/As, soil} \quad (1)$$

Where $c_{Cd/As, plant}$ represents the grain (or shoot) Cd or As content; and $c_{Cd/As, soil}$ represents the soil Cd or As content.

The TIs of Cd and As were calculated using Eq. (2):

$$TI = C_{Cd/As, \text{ grain}} \times 100\% / C_{Cd/As, \text{ shoot}} \quad (2)$$

Where $C_{Cd/As, \text{ grain}}$ represents the grain Cd or As content; and $C_{Cd/As, \text{ shoot}}$ represents the shoot Cd or As content (Ghosh and Singh, 2005; Jia et al., 2011).

2.7. Data analysis

Each treatment had three replications. The data were analyzed using one-way ANOVA combined with multiple comparisons (Tukey's test) to determine significance differences between different treatments ($P \leq 0.05$). In the text, if there was no special elucidation, all mentioned increases or decreases refer to "comparing with the control". Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Plots were generated using SigmaPlot (Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1. Effect of different Se species on the growth and photosynthesis of rice

The addition of Se (IV) or Se (VI) showed a limited effect on the plant height, dry matter weight and total grain yield except for the Se(IV)1 treatment which significantly reduced the plant height compared to the control (Table 2).

Overall, only a certain concentration range of Se could significantly affect the photosynthesis parameters in this study. The Se(IV)0.1 treatment significantly reduced the photosynthetic rate, however, the values of photosynthetic rate in the Se(IV)1 and Se(IV)5 treatments were comparable to that of the control. The addition of Se (IV) did not affect the stomatal conductance and the intercellular CO₂ concentration. The transpiration rate was significantly enhanced by the Se(IV)1 treatment, however, the Se(IV)5 treatment resulted in the transpiration rate being comparable to that of the

control (Table 2).

The Se(VI)5 treatment significantly reduced the photosynthetic rate and stomatal conductance but increased the transpiration rate. The CO₂ concentration was not affected by the Se(VI) treatments (Table 2).

3.2. Changes in Se and heavy metal (metalloid) contents in different organs of rice plants

The Se contents in the different organs of the rice plants increased as the concentrations of Se (IV) and Se (VI) increased (Figure 2a). With the Se (IV) treatment concentration being $\leq 0.1 \text{ mg kg}^{-1}$, the root Se content was highest among all organs, followed by the shoots, husks and grains. However, when Se (IV) treatment concentration was higher than 0.1 mg kg^{-1} , the order changed to roots > shoots $\approx$ grains > husks. In the Se(VI)0.1 and Se(VI)1 treatments, the Se content in the roots was highest among all organs, followed by the shoots, grains and husks; however, in the Se(VI)5 treatment the order changed to: roots > grains > shoots > husks. The Se content in the grains, shoots or husks under the Se(IV)0.1 treatment was similar to that under the Se(VI)0.1 treatment. However, the shoot or husk Se content under the Se(IV)5 treatment was higher than that under the Se(VI)5 treatment (Figure 2a).

The application of Se (IV) and Se (VI) both significantly reduced the Cd content in the roots, shoots and grains of the rice plants compared to the control (Figure 2b). However, the Cd content in the husk was significantly suppressed by the Se(IV)1 and Se(IV)5 treatments, but was not affected by the treatments containing Se (VI) (Figure 2b). The Se(IV)5 treatment was the most effective in reducing the grain Cd content

compared with the other Se treatments, where the resultant grain Cd content was only 0.07 mg kg⁻¹ (data not shown), far below the national standard (0.2 mg kg⁻¹).

Both the Se (IV) and Se (VI) treatments remarkably reduced the As content in the grains and husks, however, did not influence the shoot and root As contents (Figure 2c). The Se(IV)5 treatment was the most effective in reducing the grain As content compared with the other Se treatments. Although, the resultant grain As content (0.36 mg kg⁻¹) remained higher than the national standard (0.2 mg kg⁻¹), and this value was up to 66.04% lower than in the control.

The Se (IV) treatments significantly reduced the BCF of Cd/As in the grains and Cd in the shoots, and the TIs of Cd and As. Compared with other Se (IV) treatments, the Se(IV)5 treatment was the most effective in reducing the values of above parameters although this treatment did not reduce the BCF of As in the shoots. Meanwhile, the Se (VI) treatments reduced the BCF of Cd/As in the grains and Cd in the shoots; however, there was no decrease in the BCF of As in the shoots or the TIs of Cd and As after the addition of Se (VI) (Table 3).

3.3. Effect of different Se species on the macronutrient contents in various organs of rice plants

The Se (IV) and Se (VI) treatments did not result in major changes in the grain Mg, K and Ca contents, except for the Se(VI)5 treatment where the grain Ca content was significantly reduced compared to the control (Table 4). The Se (IV) and Se (VI) treatments did not affect the Mg, K and Ca in the rice husk except for the Se(VI)1 treatment, where rice husk K content was significantly reduced. The shoot Mg content

significantly decreased with increasing Se (IV) treatment concentrations. However, the shoot Ca content was not affected by all Se (IV) treatments, and the shoot K content was obviously lowered by $\leq 1 \text{ mg kg}^{-1}$ Se (IV). Unlike Se (IV) treatments, the Se (VI) treatments significantly reduced the shoot Mg, K and Ca contents. The addition of Se (IV) resulted in the significant increases in the root Mg and K contents, and did not affect the root Ca content. The treatments containing Se (VI) did not affect the root Mg and Ca contents, but significantly increased the K content (Table 4).

3.4. Effect of different Se species on the micronutrient contents in various organs of rice plants

The application of Se (IV) indicated an inhibitory effect on most of the micronutrients in the various organs of the rice plants. The Se (IV) treatments significantly decreased the contents of grain Fe and Zn, husk Mn, shoot Mn and Zn, and root Cu. However, they did not affect the contents of grain Mn and Cu, shoot Cu and root Fe, Zn and Mn, but marked increased the shoot Fe content. Little changes occurred in the husk Fe, Zn, and Cu contents (Table 5).

The Se (VI) treatments had a lower effect on the contents of most tested microelements in the various organs of the rice plants compared with the Se (IV) treatments. The Se (VI) treatments largely decreased the contents of grain Zn, shoot Mn and Zn, and root Cu. The Se(VI)1 treatment remarkably reduced the husk Mn content, while the Se(VI)5 treatment resulted in the husk Mn being comparable to that of the control. However, the Se (VI) treatments showed limited effects on the contents of grain Mn and Cu, husk Fe, Cu, and Zn, shoot Cu, and root Mn, Zn, and Fe. However, an

increase was found in the contents of grain and shoot Fe after the addition of Se (VI) to the soil (Table 5).

3.5. Effect of different Se species on AAs contents in rice grains

The Se(IV)0.1 treatment increased the contents of the AAs except for serine, valine, methionine and tryptophan (Table 6). In particular, the contents of aspartic acid, glutamic acid, isoleucine, leucine, phenylalanine and histidine were markedly increased. The Se(IV)0.1 treatment resulted in a 6.2% increase in the total AAs and 18.6% increase in the essential AAs in the rice grains. However, the Se(IV)1 and Se(IV)5 treatments did not show a beneficial effect on the contents of most AAs in the grains. For the Se(IV)1 treatment, the total and essential AAs increased by 3.6% and 16.5%, respectively; for the Se(IV)5 treatment, the total AAs decreased by 0.4%, while the essential AAs increased by 11.9% (Table 6).

Unlike the Se (IV) treatments, the Se (VI) treatments showed a negative effect on the contents of most AAs. The Se(VI)5 treatment significantly decreased the contents of 14 AAs (except for tryptophan and proline), and resulted in a 62.5% reduction in the total AAs and a 45.7% reduction in the essential AAs. The Se(VI)0.1 and Se(VI)1 treatment reduced the total AAs by 9.0% and 4.9%, and the essential AAs by 4.2% and 6.8%, respectively (Table 6).

3.6. Changes in the contents and proportions of inorganic and organic Se in grains

After the addition of Se (IV) and Se (VI), only three species of Se were detected in rice grains, i.e., Se (IV), SeCys and SeMet (Figure 1 and 3). The Se (IV), SeCys and SeMet contents in the grains all increased with increasing Se (IV) concentration and reached

their highest levels of 64.02, 118.70, and 864.73 $\mu\text{g kg}^{-1}$, respectively, at 5 mg kg^{-1} Se (IV).

When Se (VI) was supplied, the grain Se (IV) reached the highest level of 15.58 $\mu\text{g kg}^{-1}$ at the Se(VI)1 treatment, but it was comparable to that of the control at Se(VI)5 treatment. Both the SeCys and SeMet contents in the grains increased with increasing Se (VI) concentration.

The Se (VI) treatments resulted in a decrease in the proportion of inorganic Se (IV), but an increase in the proportions of organic SeCys and SeMet (Figure 4). After the addition of Se (IV) to the soil, the proportion of SeMet increased with increasing Se (IV) concentration; however, the proportion of SeCys peaked at 26.6% at the Se(IV)1 treatment and then decreased at the Se(IV)5 treatment. As the Se (VI) concentration increased, the proportions of grain SeMet and SeCys increased, while that of Se (IV) constantly decreased.

4. Discussion

In the present study, except for the Se(IV)1 treatment, the rest of treatments containing Se (IV) and Se (VI) had no negative effects on plant growth, indicating that Se (IV) and Se (VI) treatments at $\leq 5 \text{ mg kg}^{-1}$ have limited impacts on the growth of rice. However, the Se(IV)0.1 and Se(IV)1 treatments caused a 12.81% and a 9.16% reduction in the total grain yield, respectively, suggesting a certain negative effect of these Se (IV) treatments. A low dose of Se (IV) did reduce the grain yield, which was possibly related to a decrease in the photosynthetic efficiency. The correlation between photosynthetic efficiency and crop yield is constrained by multiple factors and contrasting results have

been obtained in some cases (Islam, **2015**). However, numerous studies have shown that reducing the photosynthetic efficiency will lead to lower crop yields (Furbank et al., **2015**; Sekhar et al., **2015**). Meanwhile, it has been demonstrated that increasing the photosynthesis of C3 plants can improve crop yields (Lawson et al., **2012**). Moreover, the addition of exogenous Se can repair damaged chloroplasts, increase the chlorophyll content, inhibit the excessive production of reactive oxygen species, and improve the photosynthetic efficiency of plants (Zhong et al., **2015**). In the present study, the Se(IV)0.1 treatment inhibited the photosynthetic rate, however, a further increase in the Se (IV) concentrations resulted in the photosynthetic rate being a bit higher than that in the control. The above results indicate that the effect of Se on the photosynthetic efficiency of plants may be associated with the level of Se exposure. We did not observe a positive correlation between the photosynthetic efficiency and grain yield of rice for the Se (VI) treatments, which inhibited the photosynthetic rate of rice but did not affect the grain yield. The reduced photosynthetic rate measured in response to the Se (VI) treatments may be associated with the reduced stomatal conductance. This result indicates that the Se (VI) treatment may affect the degree of stomatal opening of rice leaves. It has been reported that Se (VI) can reduce the value of stomatal conductance to induce a partial stomatal closure in two *Prunus* rootstock genotypes (Pezzarossa et al., **2009**), which is consistent with the results of this study.

A reduction in stomatal conductance generally inhibits transpiration in plants (Boyer, **2015**). However, both the Se(VI) and Se(IV) treatments increased the transpiration rate of the rice leaves in the present study, which is inconsistent with the

reduction in the stomatal conductance. The Se (VI) treatment may have affected other pathways, such as the stratum corneum of leaves, and thereby increased the transpiration of the rice plants. Improving transpiration intensity can facilitate the uptake and transport of mineral elements. It was surprising that in the present study, the root application of Se (IV) and Se (VI) reduced the content of most of the essential elements as well as Cd in the various organs of the rice plants. This finding indicates that the uptake of these elements and their translocation to shoots are limited by certain unknown factors, possibly by root morphology. Because, our previous study showed that Se (IV) can inhibit root development and reduce the proportion of fine roots in rice, thereby reducing the uptake of heavy metals and certain nutrient elements (e.g., shoot Mg and K, root Fe, shoot and root Mn) (Feng et al., **2013**). Taking into consideration the above findings, we infer that the Se (IV) treatment can inhibit root development and reduce the root pressure of rice plants, thereby negatively affecting the nutrient uptake and translocation capacities of roots.

The results of the present study showed that the root application of Se (IV) and Se (VI) could both reduce the Cd and As content in the various organs of the rice plants, especially the grains. However, Se (IV) shows a higher ability than Se (VI) to reduce the accumulation of Cd and As. Because the Se(IV)5 treatment resulted in the lowest Cd and As and the highest Se (14.95 mg kg^{-1}) contents in the rice grains; the grain yield remained stable and there was no loss of essential AAs. Furthermore, we found that the Se (IV) treatments reduced the TIs of Cd and As from the shoots to the grains. A report has shown that Se (IV) may chelate with Cd and As compounds and thereby reduce

their transport in plants (Barrow and Whelan, **1989**). Therefore, the inhibited TIs of Cd and As in this study might be related with their chelating reactions with Se in rice plants. Reports have shown that adding Se only to the culture medium of crops can alter the AA composition and content of the edible part to a certain degree (Munshi and Mondy, **1992**). For example, Wang et al. (**1989**) found that Se can increase multiple AAs in wheat grains and therefore improve the quality and nutritional value of wheat, as found in this study for rice.

Se (VI) is more soluble in soil water than Se (IV) which tends to react with iron and form oxides and hydrated oxides with low water solubility, and it is less easily absorbed by plants (Hamdy and Gissel-Nielsen, **1977**). However, in this study the soil application of Se (IV) at high concentrations resulted in higher Se content in the various organs of the rice plants compared with the equivalent Se (VI) treatments (Figure **2a**). A report has shown that most dryland plants have a higher absorption capacity for Se (VI), while aquatic plants have a higher absorption rate for Se (IV) (Huang et al., **2013**). Therefore, above results indicated that other unknown facts may have enhanced the ability of rice plants to take up more Se under the Se (IV) treatments than under the Se (VI) treatments.

In the present study, regardless of the Se species added, i.e., Se (IV) or Se (VI), the major species detected in the rice grains were Se (IV), SeMet, and SeCys, while Se (VI) was not detected. This is in agreement with the results reported by Han et al. (**2015**). However, reports also showed that when Se (VI) is added, Se (VI) is the major Se species in plants (Kahakachchi et al., **2004**), which is inconsistent with the current

results. The underlying mechanism, which remains unclear, may be associated with the redox processes of Se (VI) in paddy soil.

It is well established that the daily intake of Se should be within the range of 50–300 µg for adults (Whanger, **1989**). On the basis of 400 g of daily consumption of rice for Chinese adults, the daily average intake of Se from the rice produced in the present study would far surpass the standard of 300 µg (up to 5.98 mg).

5. Conclusion

This trial provides a new idea about how to remediate As- and Cd- polluted paddy rice and at the same time to enhance the daily intake of Se in order to improve public health in China. Our results suggest that it is better to use Se (IV) rather than Se (VI) to reduce the As and Cd contents in the grains of rice plants. The Se(IV)0.1 treatment could significantly enhanced the grain contents of aspartic acid, glutamic acid, isoleucine, leucine, phenylalanine, histidine and total AA contents; however the Se(IV)1 and Se(IV)5 treatment did not further show a beneficial effect on the contents of most AAs in the grains. The Se(VI)5 treatment reduced almost all of the AA contents in the rice grains. Three shortcomings of this technology should be mentioned: (1) the addition of Se (IV) suppressed the contents of essential nutrients in the grains; (2) the content of total Se in the grains is higher than the daily needs of people; and (3) the As content in the grains is still higher than the national standard of China. Therefore, when this technology is used in practice, further studies on the regulation of soil moisture content are warranted so as to reduce the grain As content below the national standard.

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References

- Barrow, N.J., Whelan, B.R., 1989. Testing a mechanistic model. VIII. The effects of time and temperature of incubation on the sorption and subsequent desorption of selenite and selenate by a soil. *Eur. J. Soil Sci.* 40, 29–37.
- Boyer, J.S., 2015. Impact of cuticle on calculations of the CO₂ concentration inside leaves. *Planta* 242 (6), 1405–1412.
- Cantor, A.H., Scott, M.L., Noguchi, T., 1975. Biological availability of selenium in feed stuffs and selenium compounds for prevention of oxidative diathens in chicks. *J. Nutr.* 105, 96–105.
- Carey, A.M., Scheckel, K.G., Lombi, E., Newville, M., Choi, Y., Norton, G., Price, A.H., Meharg, A.A., 2012. Grain accumulation of selenium species in rice (*Oryza sativa* L.). *Environ. Sci. Technol.* 46, 5557–5564.
- Cartes, P., Jara, A.A., Pinilla, L., Rosas, A., Mora, M.L., 2010. Selenium improves the antioxidant ability against aluminium–induced oxidative stress in ryegrass roots. *Ann. Appl. Biol.* 156, 297–307.
- Chand, V., Prasad, S., 2013. ICP–PES assessment of heavy metal contamination in tropical marine sediments: A comparative study of two digestion techniques. *Microchem. J.* 111, 55–63.
- Chaney, R.L., Reeves, P.G., Ryan, J.A., Simmons, R.W., Welch, R.M., Angle, J.S., 2004. An improved understanding of soil Cd risk to humans and low cost methods to phytoextract Cd from contaminated soils to prevent soil Cd risks. *Biometals* 17 (5), 54–553.

Chen, L.C., Yang, F.M., Xu, J., Hu, Y., Hu, Q.H., Zhang, Y.L., Pan, G.X., 2002.

Determination of Selenium Concentration of Rice in China and Effect of Fertilization of Selenite and Selenate on Selenium Content of Rice. *J. Agric. Food Chem.* 50, 5128–5130.

Cox, D.N., Bastiaans, K., 2007. Understanding Australian consumers' perceptions of selenium and motivations to consume selenium enriched foods. *Food Qual. Prefer.* 18 (1), 66–76.

Ding, Y.Z., Feng, R.W., Wang, R.G., Guo, J.K., Zheng, X.Q., Xu, Y.M., 2014. A dual role of Se on Cd toxicity: evidence from plant growth, root morphology and responses of the antioxidative systems of paddy rice. *Plant Soil* 375, 289–301.

Ding, Y.Z., Wang, R.G., Guo, J.K., Wu, F.C., Xu, Y.M., Feng, R.W., 2015. The effect of selenium on the subcellular distribution of antimony to regulate the toxicity of antimony in paddy rice. *Environ. Sci. Pollut. R.* 22 (7), 5111–5123.

Djanaguiraman, M., Devi, D.D., Shanker, A.K., Sheeba, J.A., Bangarusamy, U., 2005. Selenium—an antioxidative protectant in soybean during senescence. *Plant Soil* 272, 77–86.

Farnese, F.S., Oliveira, J.A., Farnese, M.S., Gusman, G.S., Silveira, N.M., Siman, L.I., 2014. Uptake arsenic by plants: Effects on mineral nutrition, growth and antioxidant capacity. *IDESIA (Chile)* 32, 99–106.

Feng, R.W., Wei, C.Y., Tu, S.X., 2013. Interactive effects of selenium and antimony on the uptake of selenium, antimony and essential elements in paddy-rice. *Plant Soil* 365, 375–386.

- Feng, R.W., Wei, C.Y., Tu, S.X., 2013a. A review: The roles of selenium in protecting plants against abiotic stresses. *Environ. Exp. Bot.* 87, 58–68.
- Feng, R.W., Wei, C.Y., Tu, S.X., 2013b. A dual role of Se on Cd toxicity: evidences from the uptake of Cd and some essential elements and the growth responses in paddy–rice. *Biol. Trace Elem. Res.* 151, 113–121.
- Filek, M., Keskinen, R., Hartikainen, H., 2008. The protective role of selenium in rape seedlings subjected to cadmium stress. *J. Plant Physiol.* 165 (8), 833–844.
- Fox, M.R.S., Jacobs, R.M., Jones, A.O.L., Fry, B.E.Jr., Rakowska, M., Hamilton, R.P., Harland, B.F., Stone, C.L., Tao, S.H., 1981. Animal Models for Assessing Bioavailability of Essential and Toxic Elements. *Cereal Chem.* 58, 6–11.
- Furbank, R.T., Quick, W.P., Sirault, X.R.R., 2015. Improving photosynthesis and yield potential in cereal crops by targeted genetic manipulation: Prospects, progress and challenges. *Field Crop Res.* 182, 19–39.
- Garg, N., Singla, P., 2011. Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. *Environ. Chem. Lett.* 9, 303–321.
- Ghosh, M., Singh, S.P., 2005. A comparative study of cadmium phytoextraction by accumulator and weed species. *Environ. Pollut.* 133 (2), 365–371.
- Hamdy, A.A., Gissel-Nielsen, G., 1977. Fixation of selenium by clay minerals and iron oxides. *Z. Pflanzenernähr. Bodenk.* 140, 63–70.
- Han, D., Li, X.H., Xiong, S.L., Tu, S.X., Chen, Z.G., Li, J.P., Xie, Z.J., 2013. Selenium uptake, speciation and stressed response of *Nicotiana tabacum* L. *Environ. Exp. Bot.* 95, 6–14.

- Han, D., Xiong, S.L., Tu, S.X., Liu, J.C., Chen, C., 2015. Interactive effects of selenium and arsenic on growth, antioxidant system, arsenic and selenium species of *Nicotiana tabacum* L. Environ. Exp. Bot. 117, 12–19.
- Hartikainen, H., Xue, T.L., Piironen, V., 2000. Selenium as an anti-oxidant and pro-oxidant in ryegrass. Plant Soil 225, 193–200.
- Huang, Q.Q., Du, W., Wang, Q., Zhang, J.S., Jiang, R.F., Li, H.F., 2013. Uptake and accumulation of Se by rice in different soils supplied with selenite or selenate (in Chinese). Acta Scien. Circum. 33 (5), 1423–1429.
- Islam, M.T., 2015. Effects of high temperature on photosynthesis and yield in mung bean. Bangladesh J. botany 44 (3), 451–454.
- Jia, Y., Tang, S.R., Ju, X.H., Shu, L.N., Tu, S.X., Feng, R.W., Giusti, L., 2011. Effects of elevated CO₂ levels on root morphological traits and Cd uptakes of two *Lolium* species under Cd stress. J. Biomed. Biotechnol. 12 (4), 313–325.
- Kahakachchi, C., Boakye, H.T., Uden, P.C., Tyson, J.F., 2004. Chromatographic speciation of anionic and neutral selenium compounds in Se-accumulating *Brassica juncea* (Indian mustard) and in selenized yeast. J. Chromatogr. A. 1054, 303–312.
- Kobayashi, E., Okubo, Y., Suwazono, Y., Kido, T., Nishijo, M., Nakagawa, H., Nogawa, K., 2002. Association between total cadmium intake calculated from the cadmium concentration in household rice and mortality among inhabitants of the cadmium-polluted Jinzu River basin of Japan. Toxicol. Lett. 129, 85–91.
- Kozhevnikova, A.D., Seregin, I.V., Bystrova, E.I., Ivanov, V.B., 2007. Effects of heavy metals and strontium on division of root cap cells and meristem structural

organization. Russ. J. Plant Physiol. 54, 257–266.

Lawson, T., Kramer, D.M., Raines, C.A., 2012. Improving yield by exploiting mechanisms underlying natural variation of photosynthesis. Curr. Opin. Biotech. 23 (2), 215–220.

Liao, G.J., Wu, Q.H., Feng, R.W., Guo, J.K., Wang, R.G., Xu, Y.M., Ding, Y.Z., Fan, Z.L., Mo, L.Y., 2016. Efficiency evaluation for remediating paddy soil contaminated with cadmium and arsenic using water management, variety screening and foliage dressing technologies. J. Environ. Manage. 170, 116–122.

Lin, L., Zhou, W.H., Dai, H.X., Cao, F.B., Zhang, G.P., Wu, F.B., 2012. Selenium reduces cadmium uptake and mitigates cadmium toxicity in rice. J. Hazard. Mater. 235, 343–351.

Mahmoud, K.Z., Edens, F.W., 2003. Influence of selenium sources on age-related and mild heat stress-related changes of blood and liver glutathione redox cycle in broiler chickens (*Gallus domesticus*). Comp. Biochem. Phys. B. 136 (4), 921–934.

Malik, J.A., Goel, S., Kaur, N., Sharma, S., Singh, I., Nayyar, H., 2012. Selenium antagonises the toxic effects of arsenic on mung bean (*Phaseolus aureus* Roxb.) plants by restricting its uptake and enhancing the antioxidative and detoxification mechanisms. Environ. Exp. Bot. 77, 242–248.

Mora, M.L., Pinilla, L., Rosas, A., Cartes, P., 2008. Selenium uptake and its influence on the antioxidative system of white clover as affected by lime and phosphorus fertilization. Plant Soil 303 (1), 139–149.

Munshi, C.B., Mondy, N.I., 1992. Glycoalkaloid and nitrate content of potatoes as

- affected by method of selenium application. Biol. Trace Elem. Res. 33, 121–127.
- Pezzarossa, B., Remorini, D., Piccotino, D., Malagoli, M., Massai, M., 2009. Effects of selenate addition on selenium accumulation and plant growth of two *Prunus* rootstock genotypes. J. Plant Nutr. Soil Sci. 172, 261–269.
- Rayman, M.P., 2008. Food–chain selenium and human health: emphasis on intake. Brit. J. Nutr. 100, 254–268.
- Saidi, I., Chtourou, Y., Djebali, W., 2014. Selenium alleviates cadmium toxicity by preventing oxidative stress in sunflower (*Helianthus annuus*) seedlings. J. Plant Physiol. 171, 85–91.
- Sekhar, K.M., Sreeharsha, R.V., Reddy, A.R., 2015. Differential responses in photosynthesis, growth and biomass yields in two mulberry genotypes grown under elevated CO₂ atmosphere. J. Photoch. Photobio. B. 151, 172–197.
- Shah, K., Kumar, R.G., Verma, A., Dubey, R.S., 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Sci. 161, 1135–1144.
- Tang, S.R., Wilke, B.M., Huang, C.Y., 1999. The uptake of copper by plants dominantly growing on copper mining spoils along the Yangtze River, the People's Republic of China. Plant Soil 209, 225–232.
- Wang, X., Ren, S., Liu, W., 1989. A study on selenium application for improving the soil in the environment at lower selenium level. J. Environ. Sci–China 1, 115–119.
- Wei, C.Y., Sun, X., Wang, C., Wang, W.Y., 2006. Factors influencing arsenic accumulation by 531 *Pteris vittata*: A comparative field study at two sites. Environ.

Pollut. 141, 488–493.

Whanger, P.D., 1989. A country with both selenium deficiency and toxicity: some thoughts and impressions. J. Nutr. 119, 1236–1239.

Yu, H.Y., Ding, X.D., Li, F.B., Wang, X.Q., Zhang, S.R., Yi, J.C., Liu, C.P., Xu, X.H., Wang, Q., 2016. The availabilities of arsenic and cadmium in rice paddy fields from a mining area: The role of soil extractable and plant silicon. Environ. Pollut. 215, 258–265.

Zahedi, H., Noormohammadi, G., Rad, A.H.S., Habibi, D., Boojar, M.M.A., 2009. Effect of zeolite and foliar application of selenium on growth, yield and yield component of three canola cultivar under conditions of late season drought stress. Nat. Sci. Biol. 1, 73–80.

Zayed, A., Lytle, C.M., Terry, N., 1998. Accumulation and volatilization of different chemical species of selenium by plants. Planta 206 (2), 284–292.

Zeng, X., Li, L., Mei, X., 2008. Heavy metal content in Chinese vegetable plantation land soils and related source analysis. Agr. Sci. China 7, 1115–1126.

Zheng, M.Z., Li, G., Sun, G.X., Shim, H., Cai, C., 2013. Differential toxicity and accumulation of inorganic and methylated arsenic in rice. Plant Soil 365, 227–238.

Zhong, Y., Chen, T.F., Zheng, W.J., Yang, Y.F., 2015. Selenium enhances antioxidant activity and photosynthesis in *Ulva fasciata*. J. appl. phycol. 27 (1), 555–562.

Zhou, J., Cui, J., Liang, J.N., 2008. Assessment of heavy metals pollution on water quality and rice around the spoil area of the smeltery (in Chinese). Acta Agr. Boreali-Sinica 23: 349-352.

Zhu, Y.G., Pilon-Smits, E.A.H., Zhao, F.J., Williams, P.N., Meharg A.A., 2009.

Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation. *Trends Plant Sci.* 14, 436–442.

Figure legends

Figure 1 The chromatograms of Se-species in standard substances and in rice grains treated with 5mg kg⁻¹ Se (VI).

Figure 2 Effects of different species Se on the contents of Se, Cd and As in different organs of paddy rice. Error bars represent standard error of mean ($n = 3$). Lowercases letters (Grains), capital letters (Rice husks), lowercases letters with back quote (Shoots), capital letters with back quote (Roots) indicate significant differences (one way ANOVA, $P \leq 0.05$) between treatments including CK treatment, Se (IV) treatments and Se (VI) treatments, respectively.

Figure 3 Effects of different species Se on the contents of Se (IV), SeCys and SeMet in grains. Error bars represent standard error of mean ($n = 3$). Lowercases letters (Se (IV)), capital letters (SeMet) and lowercases letters with back quote (SeCys) indicate significant differences (one way ANOVA, $P \leq 0.05$) between treatments involving CK treatment, Se (IV) treatments and Se (VI) treatments, respectively.

Figure 4 Effects of different species Se on the percentages of Se (IV), SeCys and SeMet in grains.

Figure 1

Figure 2

Figure 3

Figure 4

Table 1 Soil background values for selected physicochemical properties

Soil property	Value
Total Cd (mg kg ⁻¹)	1.45
Total As (mg kg ⁻¹)	53.46
Total Cr (mg kg ⁻¹)	22.64
Total Ni (mg kg ⁻¹)	9.72
Total Cu (mg kg ⁻¹)	12.34
Total Zn (mg kg ⁻¹)	95.57
Total Pb (mg kg ⁻¹)	80.36
Total Se (mg kg ⁻¹)	0.38
Total N (g kg ⁻¹)	2.22
Available N (mg kg ⁻¹)	118.25
Total P (g kg ⁻¹)	1.02
Available P (mg kg ⁻¹)	63.80
Total K (g kg ⁻¹)	8.33
Available K (mg kg ⁻¹)	151.88
Organic matter (g kg ⁻¹)	33.55
pH (H ₂ O)	6.16

Table 2 The growth conditions and photosynthesis parameters of the rice plants subjected to different levels of Se (IV) and Se (VI).

Treatments	Levels (mg kg ⁻¹)	Height (cm)	Dry weight of shoots(g)	Total weight of grains (g)	
CK (control)	0	86.83±2.47ab ⁽¹⁾	16.00±3.06a	13.97±1.66a	
Se (IV)	0.1	88.33±1.15a	17.79±2.58a	12.18±2.60a	
	1	82.33±2.31c	16.63±2.91a	12.69±0.92a	
	5	86.00±2.00ab	14.13±1.19a	15.20±1.31a	
Se (VI)	0.1	85.33±3.51ab	16.42±2.66a	14.37±2.94a	
	1	86.00±1.73ab	16.18±1.08a	13.01±2.23a	
	5	86.33±2.52ab	16.77±0.66a	13.32±2.26a	

Treatments	Levels (mg kg ⁻¹)	Photosynthetic rate (μmol CO ₂ m ⁻² s ⁻¹)	Stomatal conductance (mmol H ₂ O m ⁻² s ⁻¹)	Intercellular CO ₂ concentration (μmol CO ₂ mol air ⁻¹)	Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹)
CK (control)	0	14.48±0.50ab	0.32±0.07ab	293.00±25.00a	6.37±1.33c
Se (IV)	0.1	11.90±1.42cd	0.24±0.03bc	279.67±11.85a	9.12±0.75ab
	1	13.38±0.63abc	0.35±0.04a	286.56±12.66a	9.84±0.06a
	5	15.32±1.55a	0.32±0.03ab	285.78±21.35a	7.08±0.90bc
Se (VI)	0.1	13.64±1.28abc	0.33±0.10ab	299.33±6.43a	8.81±0.61ab
	1	12.37±0.70bc	0.26±0.06abc	292.11±11.20a	9.07±2.20ab

5

10.05±1.67d

0.18±0.03c

267.00±27.38a

9.52±1.76ab

⁽¹⁾The presented data are mean values $\pm$ SE. Different lowercase letters in the same column indicate significant differences among different treatments including CK treatment, Se (IV) treatments and Se (VI) treatments ($p \leq 0.05$)

Table 3 Effects of different Se species on Cd, As bioconcentration factor and transport index in paddy rice

Treatments (mg kg ⁻¹)		Bioconcentration factor				Transport index	
		Grains		Shoot			
		Cd	As	Cd	As		
CK (control)	0	19.82±3.29a ⁽¹⁾	1.98±0.25a	49.85±5.87a	38.50±3.58ab	5.14±0.18a	40.20±8.28abc
Se (IV)	0.1	17.71±1.17ab	1.41±0.13ab	35.87±0.93b	39.17±2.87ab	3.61±0.41abc	49.44±4.32a
	1	11.56±1.81bc	1.41±0.08ab	37.41±8.35ab	40.55±4.65ab	3.53±0.64bc	32.24±9.60abc
	5	4.70±0.83d	0.67±0.21c	18.13±2.56c	41.85±1.70a	1.62±0.55d	25.90±2.78c
Se (VI)	0.1	17.26±0.57ab	1.39±0.41ab	38.31±1.63ab	35.82±2.53ab	3.89±1.25ab	45.08±1.40ab
	1	12.38±3.72bc	0.74±0.09c	40.90±3.81ab	36.49±1.99ab	2.02±0.16cd	29.89±6.72ab
	5	10.71±0.41cd	0.83±0.00bc	28.36±5.37bc	34.52±0.93b	2.39±0.06bcd	38.75±7.93bc

⁽¹⁾The presented data are mean values ± SE. Different lowercase letters in the same column indicate significant differences among different treatments including CK treatment, Se (IV) treatments and Se (VI) treatments ($p \leq 0.05$).

Table 4 The contents of Mg, K, Ca in different organs of paddy rice subjected to different levels of Se (IV) and Se (VI).

Treatments (mg kg ⁻¹)			Mg (mg kg ⁻¹)	K (mg kg ⁻¹)	Ca (mg kg ⁻¹)
Grains	CK (control)	0	1131.05±92.48a ⁽¹⁾	3656.70±17.75a	332.04±67.92a
		Se (IV)	0.1	1127.86±161.94a	307.74±70.49ab
		1	1063.35±110.32a	3288.90±410.71a	287.71±17.47ab
		5	1040.55±108.03a	3033.53±364.99a	262.85±32.34ab
	Se (VI)	0.1	1066.36±76.07a	3316.14±233.06a	263.54±33.26ab
		1	1017.71±63.50a	3335.92±320.38a	246.93±44.59ab
		5	1035.83±127.88a	3249.52±497.48a	236.65±32.92b
Rice husks	CK (control)	0	549.41±26.74a	15166.03±1459.41ab	2843.53±726.24ab
	Se (IV)	0.1	547.25±75.23a	16141.72±959.81a	2590.26±205.23ab

		1	596.96±79.44a	15601.59±872.73ab	2505.69±203.15b
		5	552.33±42.74a	14548.96±1041.92abc	2468.36±216.74b
	Se (VI)	0.1	513.03±24.96a	13540.47±371.47bc	2356.09±197.78b
		1	613.78±43.94a	12828.42±683.57c	2294.98±196.49b
		5	619.47±68.06a	16591.65±1698.23a	3316.53±676.85a
Shoots	CK (control)	0	1666.81±99.19a	14600.77±542.42a	8653.69±687.05a
	Se (IV)	0.1	1560.48±15.58ab	13154.89±866.05bcd	8373.81±283.10a
		1	1534.88±99.77b	13351.37±414.96bcd	8645.43±428.33a
		5	1476.34±47.20b	14226.85±356.48ab	8374.98±43.65a
	Se (VI)	0.1	1465.22±32.96b	13543.87±722.93abc	7947.82±678.31ab
		1	1481.62±66.48b	12243.25±855.85d	7389.54±512.29b
		5	1498.06±79.61b	12446.26±398.77cd	7316.12±116.84b
Roots	CK (control)	0	435.08±46.63bc	551.55±103.61b	5853.59±771.94ab
	Se (IV)	0.1	416.71±39.79c	566.53±31.94b	4791.33±555.71b
		1	463.08±13.16abc	523.77±69.19b	4449.26±430.80b
		5	601.15±115.85a	781.76±105.72a	5288.93±682.98ab
	Se (VI)	0.1	566.01±87.23ab	544.89±28.15b	5085.41±762.24ab
		1	571.38±29.47ab	496.82±34.52b	5006.92±1060.74ab
		5	555.28±107.43ab	687.50±42.33a	6448.99±1145.26a

⁽¹⁾The presented data are mean values $\pm$ SE. Different lowercase letters in the same column indicate significant differences among different treatments including CK treatment, Se (IV) treatments and Se (VI) treatments ($p \leq 0.05$).

Table 5 The contents of Mn, Fe, Cu, Zn in different organs of paddy rice subjected to different levels of Se (IV) and Se (VI).

Treatments (mg kg ⁻¹)			Mn (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)
Grains	CK (control)	0	23.09 $\pm$ 3.51ab ⁽¹⁾	13.62 $\pm$ 1.23b	4.90 $\pm$ 1.84a	32.87 $\pm$ 5.70a
	Se (IV)	0.1	22.28 $\pm$ 3.53ab	11.78 $\pm$ 0.66b	4.57 $\pm$ 0.98a	35.19 $\pm$ 8.30a
		1	24.78 $\pm$ 2.09a	10.66 $\pm$ 2.65bc	4.15 $\pm$ 0.17a	23.83 $\pm$ 2.22b

		5	17.88±1.09b	7.53±1.41c	3.58±0.24a	18.44±0.85b
	Se (VI)	0.1	22.80±2.35ab	14.00±4.04b	4.19±0.03a	24.30±1.50b
		1	22.47±4.04ab	10.25±0.06bc	4.49±0.47a	22.75±3.70b
		5	22.28±1.07ab	17.55±0.71a	4.24±0.91a	23.32±3.86b
Rice husks	CK (control)	0	443.55±30.33a	61.09±12.09a	6.64±1.63a	37.20±3.03ab
	Se (IV)	0.1	376.79±16.45ab	75.00±19.23a	6.67±0.91a	44.37±3.84a
		1	307.11±53.31b	57.94±3.47a	5.95±0.13a	33.47±2.57b
		5	315.00±16.50b	63.48±2.55a	6.44±0.58a	38.01±12.34ab
	Se (VI)	0.1	351.84±32.35ab	58.09±10.32a	6.39±0.74a	44.86±4.17a
		1	322.03±18.89b	63.71±8.35a	6.64±0.79a	42.83±2.23ab
		5	446.23±118.87a	69.86±20.93a	5.14±0.41a	46.71±2.62a
Shoots	CK (control)	0	880.94±36.63a	753.74±145.89cd	4.22±0.29ab	76.22±11.02a
	Se (IV)	0.1	819.57±70.97ab	836.89±29.51abc	4.00±0.65ab	68.98±15.10ab
		1	827.56±129.65ab	928.58±56.52ab	4.48±0.20a	63.04±13.90abc
		5	742.78±32.63bc	932.53±62.47a	3.29±0.47b	45.74±9.21c
	Se (VI)	0.1	729.32±24.25bc	667.93±62.18d	3.81±0.10ab	53.03±6.73bc
		1	746.92±34.71bc	776.33±91.69bcd	3.96±0.50ab	50.85±3.83bc
		5	694.68±48.08c	874.45±68.08ab	3.85±0.97ab	44.42±3.89c
Roots	CK (control)	0	688.79±64.77a	62864.05±5803.01ab	7.56±0.70a	87.82±14.27ab

Se (IV)	0.1	607.20±160.91a	62462.98±5393.18ab	7.82±0.16a	108.59±3.32a
	1	514.33±115.32a	48917.76±7596.04b	6.81±0.62ab	77.56±11.79ab
	5	505.88±19.08a	43004.92±2174.76b	6.33±0.81b	65.49±7.26b
Se (VI)	0.1	682.00±93.46a	66626.08±14391.48ab	7.01±0.86ab	102.36±33.82a
	1	507.57±128.06a	69229.97±13428.29ab	7.35±0.21ab	78.17±15.05ab
	5	494.72±30.53a	78959.77±27886.93a	6.31±0.52b	79.49±16.29ab

⁽¹⁾The presented data are mean values ± SE. Different lowercase letters in the same column indicate significant differences among different treatments including CK treatment, Se (IV) treatments and Se (VI) treatments ($p \leq 0.05$).

Table 6 Percentages of different amino acids in the grains of paddy rice subjected to different levels of Se (IV) and Se (VI).

Amino acids (%)	CK (control)	Se (IV)			Se (VI)		
		(mg kg ⁻¹)			(mg kg ⁻¹)		
		0.1	1	5	0.1	1	5
Aspartic acid (ASP)	0.74±0.03bc ⁽¹⁾	0.82±0.01a	0.76±0.02b	0.77±0.02b	0.71±0.03c	0.74±0.02bc	0.46±0.02d
Threonine (THR [*])	0.32±0.01abc	0.33±0.00a	0.32±0.01ab	0.31±0.01bcd	0.30±0.02cd	0.30±0.00d	0.17±0.01e
Serine (SER)	0.37±0.01abc	0.37±0.01ab	0.38±0.02a	0.35±0.00bc	0.33±0.01d	0.35±0.00cd	0.20±0.01e
Glutamic acid (GLU)	1.41±0.06bc	1.52±0.03a	1.48±0.06ab	1.40±0.02bc	1.30±0.04d	1.36±0.04cd	0.87±0.04e
Glycine (GLY)	0.37±0.01a	0.39±0.00a	0.38±0.01a	0.37±0.01a	0.34±0.02c	0.35±0.00bc	0.19±0.01d
Alanine (ALA)	0.44±0.02ab	0.47±0.00a	0.46±0.02a	0.45±0.02a	0.41±0.03c	0.42±0.01bc	0.23±0.01d
Valine (VAL [*])	0.42±0.03a	0.42±0.01a	0.42±0.03a	0.40±0.02ab	0.37±0.02b	0.38±0.00b	0.22±0.01c
Methionine (MET [*])	0.17±0.02a	0.15±0.01a	0.17±0.02a	0.17±0.02a	0.17±0.03a	0.15±0.01a	0.08±0.00b
Isoleucine (ILE [*])	0.25±0.01bc	0.29±0.00a	0.28±0.01a	0.26±0.02b	0.23±0.02d	0.24±0.01cd	0.15±0.01e
Leucine (LEU [*])	0.60±0.02b	0.65±0.01a	0.63±0.02a	0.59±0.00b	0.55±0.02c	0.58±0.01b	0.37±0.02d
Tryptophan (TRP [*])	0.18±0.06a	0.18±0.02a	0.19±0.04a	0.19±0.06a	0.17±0.05a	0.15±0.01a	0.12±0.01a
Phenylalanine (PHE [*])	0.39±0.01b	0.42±0.02a	0.39±0.01b	0.38±0.01b	0.35±0.01c	0.38±0.00b	0.29±0.01d
Lysine (LYS [*])	0.35±0.02ab	0.36±0.02a	0.35±0.01ab	0.34±0.01bc	0.32±0.01c	0.34±0.00abc	0.22±0.01d
Histidine (HIS)	0.21±0.01bc	0.23±0.01a	0.22±0.01ab	0.20±0.01bc	0.19±0.02c	0.20±0.00bc	0.14±0.01d

Arginine (ARG)	0.60±0.06ab	0.64±0.02a	0.66±0.01a	0.64±0.05a	0.55±0.06b	0.54±0.01b	0.37±0.02c
Proline (PRO)	0.33±0.02a	0.35±0.03a	0.32±0.01a	0.30±0.08a	0.27±0.04a	0.34±0.05a	0.32±0.03a
T (%) ⁽²⁾	7.15	7.59	7.41	7.12	6.56	6.82	4.4
E (%)	2.36	2.8	2.75	2.64	2.46	2.52	1.62
E/T	33.01	36.89	37.11	37.08	37.50	36.95	36.82

⁽¹⁾The presented data are mean values $\pm$ SE. Different lowercases letters in the same line indicate significant differences among different treatments including CK treatment, Se (IV) treatments and Se (VI) treatments ($p \leq 0.05$).

⁽²⁾T (%) refers to total percentages of all amino acids and E (%) refers to the percentages of essential amino acids.

* indicates the essential amino acids for humans.