



# Efficiency and risks of selenite combined with different water conditions in reducing uptake of arsenic and cadmium in paddy rice<sup>☆</sup>

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## ABSTRACT

The co-contamination of arsenic (As) and cadmium (Cd) in soils is a common problem. Selenium (Se) can reduce the uptake of As and Cd in plants, and in practice, the alternate wetting and drying is a common culture mode in rice production. However, it is unknown whether Se can efficiently reduce As and Cd concentrations in crops suffering from a high-level contamination of As and Cd under different soil water conditions. In this study, we assessed the efficiency and risks of selenite [Se(IV)], in a pot experiment, to reduce the uptake of As and Cd in a rice plant (YangDao No 6) growing in a heavily contaminated soil by As and Cd (pH 7.28) under different soil water conditions. The results showed that Se(IV) failed to control the grain total As and Cd concentrations within their individual limited standard ( $0.2 \text{ mg kg}^{-1}$ ) despite that Se(IV) significantly reduced the grain total As and Cd concentrations. The soil drying treatment alone could reduce the accumulation of arsenite [As(III)] in the grains, but additional Se(IV) stimulated the accumulation of As(III) in the grains under soil drying conditions. In addition, the addition of Se(IV) enhanced the As and Cd concentrations in the shoots and/or roots of rice plants under certain conditions. The above results all suggested that the utilization of Se(IV) in a high contaminated soil by As and Cd cannot well control the total concentrations of As and Cd in plants. In this study, the available concentrations of As and Cd in the rhizosphere soil, the rhizosphere soil pH, the formation of root iron/manganese plaques and the concentrations of essential elements in the grains were monitored, and the related mechanisms on the changes of these parameters were also discussed. This study will give a guideline for the safe production of rice plants in a heavily co-contaminated soil by As and Cd.

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## 1. Introduction

The co-contamination of cadmium (Cd) and arsenic (As) in the environment is a common phenomenon around the world resulting from the anthropogenic activities or natural sources (Toppi and Gabbriellini, 1999; Ng et al., 2003; Farias et al., 2003). Cd can be retained in human body (particularly kidney) for many years and its half-time is 10–30 years (Järup et al., 1983). As accumulation in human body will increase the risk of certain diseases, such as skin

cancer, peripheral vascular disease and peripheral neuropathy (Zaloga et al., 1985). Both As and Cd will show negative effects on the growth of plants. Cd can inhibit the photosynthesis and plant growth, induce oxidative stress and decrease uptake of essential elements (Jia et al., 2011; Feng et al., 2013a; Nedjimi and Daoud, 2009). As will also show toxicity to plants, induce oxidative stress, and damage cell membrane (Rai et al., 2011; Meharg and Hartley-Whitaker, 2002).

Selenium (Se) is an essential nutrient for human beings but a beneficial element for plants (Feng et al., 2013c), and diet is the main source of Se ingestion by humans (Williams et al., 2009). The worldwide distribution of Se in the environment is uneven, which may cause the co-occurrences of deficiency and contamination of Se (Jacobs, 1990). In addition, exogenous Se can reduce the uptake

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of many heavy metals (or metalloids) in plants including Cd (Filek et al., 2008; Kumar et al., 2012; Lin et al., 2012; Feng et al., 2013a; Saidi et al., 2014; Haghghi and Teixeira da Silva, 2016) and As (Feng et al., 2009; Malik et al., 2012; Kumar et al., 2014; Chauhan et al., 2017; Moullick et al., 2017). The uptake inhibition of As and Cd by Se in plants might be due to 1) plant root system responses (Wu et al., 2017; Liu et al., 2019; Zhao et al., 2019a, b); 2) regulation of several genes for elemental uptake and transport in plants (Cui et al., 2018); 3) soil physical and chemical processes, such as pH variation and co-precipitation (Huang et al., 2018). In soils, Se and As might compete with each other for the same soil adsorption sites and the sorption of inorganic anions by hydrous ferric oxide gel (at pH 6.5) followed the order:  $\text{H}_2\text{PO}_4 > \text{H}_2\text{AsO}_4 = \text{HSeO}_3 > \text{H}_4\text{SiO}_4 > \text{MoO}_4^{2-} > \text{SO}_4^{2-} > \text{SeO}_4^{2-} > \text{Cl} = \text{NO}_3$  (Ryden et al., 1987). Therefore, the addition of Se might increase soil As bioavailability and thus make Se fail to well control the concentrations of As in plants.

The soil moisture will affect the soil Eh, pH and the dissolution of organic carbon and Fe/Mn oxides, and thus affect the bioavailability of As and Cd and their uptake in plants (Frohne et al., 2011; Shaheen et al., 2016; LeMonte et al., 2017). A long-time flooding can reduce soil Cd availability and its concentration in plants, but showed a reverse effect on As (Hu et al., 2013; Arao et al., 2009; Liao et al., 2016), which may attribute to the decreased soil redox potential (Eh), the formation of insoluble CdS (Bingham et al., 1976; Zheng and Hu, 1995; Fan et al., 2010; McLaughlin et al., 1998), the reduction of arsenate [As(V)] to more soluble arsenite [As(III)] at low Eh (0–200mv) (Masscheleyn et al., 1991), and/or the less adsorption of As by root iron plaque (Chen et al., 2005; Liu et al., 2004).

In practice, the utilization of exogenous Se to control the uptake of As and Cd in rice plants will meet with two ignored problems. 1) Whether Se can efficiently reduce the As and Cd concentrations to a safe level under a highly contaminated level of them in soils? 2) Farmers often conducts the following procedure as: flooding, soil drying and then re-flooding during the cultivation process of rice plants. These different soil water regimes may affect the efficiency of using Se to control As and Cd uptake by rice plants. Previous investigations often use anaerobic or aerobic cultivation combined with Se addition (Wan et al., 2018a, b). There were no the alternate wetting and drying process, which is a common cultivation mode in practice and may show unknown effects on the outcomes of Se in controlling the As and Cd uptake by rice plants.

To fill with the above gaps, this study was conducted under different soil water regime conditions to investigate the effects of different doses of selenite [Se(IV)] on 1) the uptake of As, Cd, Se and essential elements by a rice variety (Yangdao, No 6); 2) the bioavailability of As, Cd and Se in the soil; 3) the formation of root iron (Fe)/manganese (Mn) plaques and its relationship with the uptake of As, Cd and Se; 4) the changes of As speciation in the rice grains.

## 2. Materials and methods

### 2.1. Soil pretreatment

The soil was gathered from a Cd and As co-contaminated paddy soil in ChenZhou city, HuNan province, China. The soil contamination is mainly due to the irrigation using contaminated river water resulting from discharge of mining waste water in the region. The soil was placed in a greenhouse and air dried naturally. The air-dried soil was ground, passed through a 0.841 mm sieve (plastic), thoroughly mixed and placed in the dark until use. A portion of the above air-dried soils was passed through a 0.147 mm sieve (plastic) to determine the basic physical and chemical properties according

to the method of Tang et al. (1999). The basic physical and chemical properties of soil were as follows: pH 7.28, organic matter 49.71 g kg<sup>-1</sup>, available nitrogen (N) 238.0 mg kg<sup>-1</sup>, available phosphorus (P) 27.37 mg kg<sup>-1</sup>, available potassium (K) 96.00 mg kg<sup>-1</sup>, total As 282.27 mg kg<sup>-1</sup>, total Cd 8.10 mg kg<sup>-1</sup>, total Se 2.24 mg kg<sup>-1</sup>, available As 0.40 mg kg<sup>-1</sup>, available Cd 3.93 mg kg<sup>-1</sup>, available Se 0.14 mg kg<sup>-1</sup>; The total concentration of Mg, K, Ca and Fe is 22.86 g kg<sup>-1</sup>, 206.51 g kg<sup>-1</sup>, 22,57 g kg<sup>-1</sup> and 285.07 g kg<sup>-1</sup>, respectively, and their individually available concentration is 5.19 mg kg<sup>-1</sup>, 96.00 mg kg<sup>-1</sup>, 41.03 mg kg<sup>-1</sup> and 0.99 mg kg<sup>-1</sup>. According to Soil Environmental Quality Risk Control Standard For Soil Contamination Of Agricultural Land in China (GB 15618-2018), the As and Cd concentration in the soil is 11.29 and 13.5 times higher than their individual standard (pH of paddy soil within the range of 6.5–7.5, the standard of As and Cd is 25 mg kg<sup>-1</sup> and 0.6 mg kg<sup>-1</sup>, respectively), suggesting a heavily contamination by As and Cd for this paddy soil.

### 2.2. Experimental design and plant culture

In this study, Se was added in the form of selenite [Na<sub>2</sub>SeO<sub>3</sub>, Se(IV)] at 1 (A1) and 5 (A2) mg kg<sup>-1</sup>, and three kinds of water conditions were supplied as follows: flooding throughout the whole growth period of rice plants (C1), soil drying for 5 days at the stage of panicle differentiation (C2, and then re-flooding), and soil drying for 10 days at the stage of panicle differentiation (C3, and then re-flooding). There were in total 9 treatments in this study as following: C1, C2, C3, A1 plus C1, A1 plus C2, A1 plus C3, A2 plus C1, A2 plus C2, A2 plus C3. Each treatment replicated triple. Plastic pots were used in this study, and each pot contained 5 kg soil. The soils passed through 0.841 mm sieve were weighed and filled into the pots with basic fertilizers. The basic fertilizers included N: 0.20 g kg<sup>-1</sup>, P: 0.12 g kg<sup>-1</sup> and K: 0.26 g kg<sup>-1</sup> in the forms of CO(NH<sub>2</sub>)<sub>2</sub>, KH<sub>2</sub>PO<sub>4</sub> and KCl, respectively. Then the soil was flooded with de-ionized water and kept 3–4 cm depth of water above the soil. Before the transferring of seedlings, the water naturally dried with no visible water in soils.

A rice plant variety named YangDao No 6 was used in this study. The plump seeds were sterilized with 2% NaCl for 20 min and then were sown in a mixture of perlite and vermiculite (V: V = 1: 1). During the culture period of seedlings, the de-ionized water was supplied at regular intervals and 50% Hoagland-Arnon nutrient solution was added dropwise. When the seedlings had four leaves, the seedlings were transplanted into plastic barrels (2 seedlings in each pot). The seedlings were cultured under natural light in a greenhouse with 27 °C/32 °C (morning/noon) temperature and 48%–73% humidity. During the growth period, and 10% imidacloprid (HuLian Biology Pharmacy Limited Company in ShangHai) was supplied to kill the red spiders on the leaf surface of rice plants. For the flooding treatment, the water was remained 3–4 cm above the surface of soil, and the water management of other two soil drying treatments was performed according to the experimental design.

### 2.3. Plant harvest

When rice plants were matured, the biological parameters were recorded, including plant height, spike number, hundred grain weight and yield. The soils adhering to the root surface was collected with a brush, which was considered as the rhizosphere soil and used to determine the available concentrations of elements. The plants were separated into the shoots, roots and grains (husks were discarded). Then, the shoots and roots were rinsed with tap water and de-ionized water, and the water adhering to the surface of them was removed by filter papers. A portion of fresh root samples was collected to extract root Fe/Mn plaques. The

remained samples of shoots and roots were oven-dried at 70 °C for 48 h to constant weights.

#### 2.4. Extraction of root plaques and available elements in rhizosphere soil

The extraction procedure of root Fe/Mn plaques was performed according to the method of Liu et al. (2019). Briefly, the fresh root samples were immersed in de-ionized water for 24 h, and then rinsed twice with de-ionized water. The root samples were incubated in 50 mL of DCB solution [containing 0.03 mol L<sup>-1</sup> sodium citrate (Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·2H<sub>2</sub>O), 0.125 g sodium bicarbonate (NaHCO<sub>3</sub>), and 0.5 g sodium dithionite (Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>)] and extracted at room temperature for 1 h. The extracted solution was filtered through a 0.45 μm filter and to determine the concentrations of Fe, Mn and other elements.

Approximate 5 g rhizosphere soil samples were weighted into a plastic bottle, and at the same time 25 mL 0.1 mol L<sup>-1</sup> HCl was added. The bottles containing soil and extractor were shaken for 2 h at 25 °C and at 180 r min<sup>-1</sup>. Then the extracted solution was filtered with a 0.45 μm filter, topped up to 100 mL and stored in a freezer at 4 °C until it was used to determine the concentrations of elements.

#### 2.5. As speciation determination

The As speciation separation followed the method of Zhang et al. (2002) with some modifications. Approximate 0.4 g powders of brown rice was weighted into a 10 mL centrifuge tube and then 8 mL 50% (V/V) methanol solution was added to immerse for 1 h. Thereafter, ultrasonic apparatus (KQ-800KDE, Kunshan ultrasonic instruments co., LTD.) was used to extract for 30 min. The extraction was centrifuged at 5000 r min<sup>-1</sup> for 15 min, and the residues after centrifugation were then repeated extracted twice and the supernatant after centrifugation were mixed. The supernatant was concentrated to be dried using rotating pan (LABOROTA4000, Germany HEIDOLPH) at 40 °C, and adding de-ionized water to dissolve the extracted substances. Then above solution was transferred into a volumetric flask, topped up to 10 mL and filtered by a 0.2 μm filter. The concentration of different As speciation was determined by an atomic fluorescence spectrophotometer (AFS-8230, Beijing JiTian instrument co. LTD) equipped with a speciation analysis pretreatment device (SAP-20, Beijing JiTian instrument co. LTD). The theoretical detection limit is 0.01 μg g<sup>-1</sup>, but the actual detection limit is 2 μg g<sup>-1</sup>. The speciation of monomethylated arsenic (MMA) and dimethylated arsenic (DMA) were not detected in the grains. The standards for the different species of As included: As(III) standard solution (GBW08666), As(V) standard solution (GBW08667), MMA standard solution (GBW08668) and DMA standard solution (GBW08669) were purchased from National Institute of Metrology of China. The parameters for the instrument of speciation analysis are as follows: anion column (Agilent G3154-65001, 4.6 mm × 150 mm, 5 μm, Agilent Technologies); mobile phase: 15 mmol L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> (pH, 6.0); carrier: 7% HCl; reducing agents: 2% KBH<sub>4</sub> plus 0.5% KOH; The total current of As hollow cathode lamp is 85 mA; the negative voltage of the photomultiplier tube is 280 V; the flow rate of carrier gas 300 mL min<sup>-1</sup>; the flow rate of shielding is 500 mL min<sup>-1</sup>.

#### 2.6. Digestion of soil and plant samples

##### 2.6.1. Digestion of soil samples

The samples of plants and soils were both digested in an ED54 DigiBlock digestion system (Lab Tech, Inc., Hopkinton, MA, USA) and the digestion of these samples was performed according to the

method of Liao et al. (2016). For the soil samples, approximate 0.25 g soil sample was placed in a Teflon tube, and 10 mL HNO<sub>3</sub> and 4 mL HF were added into the tube. The mixture was incubated overnight. After that it was heated at 120 °C for 1 h and then at 150 °C for 2 h. Then the temperature was increased up to about 180 °C to let the volume of the solution being about 1 mL. After the liquid was cooled, the liquid was top up to 25 mL with de-ionized water, and then filtered via a 0.45 μm filter and stored at 4 °C for the determination of elemental concentrations.

##### 2.6.2. Digestion of plant samples

About 0.25 g of plant samples (for grains, 0.5 g) was weighted into a 50 mL glass tube, and at the same time 10 mL HNO<sub>3</sub> was added into the tube. The mixture was incubated overnight. The temperature-rise period was as follows: 80 °C for 1.5 h, 120 °C for 1.5 h, and then 150 °C for 3 h. Similar to soil samples, the liquid in the tube was heated at 180 °C to leave its volume being approximately 1 mL. The following process was the same with the digestion process of soil samples.

The concentrations of all elements in the soil samples and plant samples were determined using inductively coupled plasma mass spectrometry (iCAP Qc ICP-MS, Thermo Fisher, USA). Standard reference materials (green tea, GBW10052; soil, GBW07452; grains, GBW10045) and blanks were used to ensure the accuracy of the elemental analysis, which were purchased from the Institute of geophysical and geochemical exploration, Chinese Academy of Geological Sciences.

#### 2.7. Data analysis

One-way ANOVA with multiple comparison (Tukey test) method was used to compare the significance between various treatments ( $P \leq 0.05$ ). Multivariate analysis of variance was performed (Tukey test) to illustrate the interaction effects of Se plus different water regimes on tested parameters. All data are mean values of three replicates unless otherwise stated. The software SPSS 18.0 was used for data analysis, and the software Origin 2017 was used to plot graphs.

### 3. Results and discussions

#### 3.1. Soil addition of Se(IV) could stimulate the yield of rice plants, but its effects on plant growth depended on Se(IV) doses and water regime conditions

The interactions between soil drying treatments and Se(IV) treatments significantly affected the shoot and root fresh weights, the height, the yield, the spike number and the hundred-grain weight (Table 1). Soil drying treatments increased the yield, shoot fresh weight, height (at 10 day soil drying), and hundred-grain weight (at 5 day soil drying), but inhibited the root fresh weight (Table 1). In most cases, the addition of Se(IV) showed positive effects on the height, yield and hundred-grain weight whenever soil drying or not.

In most cases, the addition of Se(IV) did not significantly affect the spike number except for the A2C2 treatment, where spike number was significantly lower than the C2 treatment (Table 1). The addition of Se(IV) stimulated the yield of rice plants exposed to Cd stress was also observed by Huang et al. (2017). The stimulation on grain yield after the addition of Se(IV) may be due to 1) the enhanced photosynthesis. A report by Zhang et al. (2014) suggested that Se enhanced the photosynthesis of a rice variety (Xiao-NongZhan) via increasing the photosynthesis rate (Pn), the intercellular CO<sub>2</sub> concentration (Ci) and the transpiration efficiency (E), thereby increasing the grain yield. 2) the enhanced hundred-grain

**Table 1**  
The biological indexes of rice plants subjected to different levels of selenite and soil water conditions.

Treatments	Fresh weight of shoots (g)	Fresh weight of roots (g)	Height (cm)	Yield (g)	Spike number	Hundred-grain weight (g)
C1	56.45 ± 1.45f <sup>1</sup>	111.63 ± 1.50a	86.70 ± 0.62e	3.57 ± 0.19d	6.33 ± 0.58 ab	1.74 ± 0.13e
C2	80.7 ± 0.54b	72.2 ± 0.67c	89.05 ± 0.25de	5.30 ± 0.67c	6.50 ± 0.50a	2.03 ± 0.04cd
C3	74.80 ± 1.80c	53.14 ± 3.41d	91.27 ± 0.7cd	8.73 ± 0.64b	6.00 ± 0.00 ab	2.00 ± 0.13de
A1C1	65.74 ± 1.20e	83.18 ± 3.77b	86.50 ± 0.50e	9.60 ± 0.49b	5.67 ± 0.58 ab	1.92 ± 0.06de
A1C2	89.97 ± 2.27a	69.53 ± 2.03c	95.50 ± 1.50b	12.50 ± 0.67a	6.33 ± 0.58 ab	2.37 ± 0.22b
A1C3	70.41 ± 2.62d	57.82 ± 5.73d	93.55 ± 0.45bc	9.92 ± 0.12b	5.67 ± 0.58 ab	2.16 ± 0.06bcd
A2C1	64.17 ± 1.6e	70.57 ± 2.52c	94.20 ± 0.80b	9.84 ± 0.14b	5.67 ± 0.58 ab	1.89 ± 0.07de
A2C2	75.60 ± 1.77c	78.82 ± 1.44b	99.95 ± 2.55a	13.23 ± 0.76a	5.00 ± 0.00b	2.68 ± 0.12a
A2C3	78.69 ± 0.98b	55.51 ± 5.78d	94.70 ± 2.30b	13.15 ± 0.65a	5.33 ± 0.58 ab	2.30 ± 0.09bc
C	341.06** <sup>2</sup>	206.15**	43.07**	81.20**	0.78ns	46.00**
A	22.30**	24.64**	67.42**	327.17**	8.11**	23.14**
A*C	44.54**	47.01**	10.02**	27.16**	1.56ns	4.14*

<sup>1</sup> Values are means ± SD (n = 3). The lowercases letters in the same column indicate significant differences among different treatments (p ≤ 0.05).  
<sup>2</sup> F-values for the interactive effects between selenite and field drying times. ns: not significant (p > 0.05); \* and \*\* indicate significant at P ≤ 0.05 and 0.01, respectively.

weight rather than the spike number (Table 1), possibly indicating a carbon remobilization from vegetative tissues to grains, just like the stimulation of moderate wetting and drying on rice yield in the study of Yang et al. (2017).

Moderate wetting and drying can increase rice yield and reduce water use, grain As level, and methane emission (Yang et al., 2017). In this study, similar stimulation on rice yield after soil drying was also observed. For example, soil drying for 10d significantly enhanced the yield when compared to the flooding treatment and the treatment of soil drying for 5d. The drying condition during alternate wetting and drying period is the most important factor affecting yield (Yang et al., 2009; Zhang et al., 2009; Yang et al., 2017). The stimulation on rice yield after moderate alternate wetting and drying was reported to be due to 1) reducing redundant vegetative growth; 2) improving canopy structure and root growth; 3) elevating hormonal levels (abscisic acid during soil drying and cytokinin during re-watering); 4) enhancing carbon remobilization from vegetative tissues to grains (Yang et al., 2017). The soil drying did not significantly affect the spike number, but more or less enhanced the hundred-grain weight (Table 1), suggesting that the soil drying facilitated the accumulation of dry materials in the grains.

The effects of Se(IV) plus soil drying on the fresh weights of shoots and roots were observed to be dependent on the doses of Se(IV) and soil water regimes. It seemed that under flooding condition, the addition of Se(IV) stimulated the shoot growth, but inhibited the root growth (Table 1). When compared to the

treatment of soil drying for 5d, a low dose of Se(IV) at 1 mg kg<sup>-1</sup> significantly increased but a high dose at 5 mg kg<sup>-1</sup> reduced the shoot fresh weight; however, when compared to the treatment of soil drying for 10 days, the situation reversed. In terms of the root fresh weight, at a 5 day soil drying, the addition of 5 mg kg<sup>-1</sup> Se(IV) significantly increased the root fresh weight. However, at a 10 day soil drying, the addition of Se(IV) did not significantly affect the root fresh weight. Similar dose- or condition-dependent effects of Se(IV) on the growth of plants were also reported in the study of Feng et al. (2013a) and Ding et al. (2014).

3.2. Se(IV) and soil drying impacted the available concentration of As but not for that of Cd and Se in the rhizosphere soil

The soil drying significantly affected the As, Cd and Se available concentrations in the rhizosphere soil. The addition of Se(IV) only significantly influenced the As available concentration but non-significantly affected the Cd and Se concentrations. The interaction between soil drying treatment and Se(IV) showed significant effects on the As and Cd available concentrations but not on Se available concentration (Table 2).

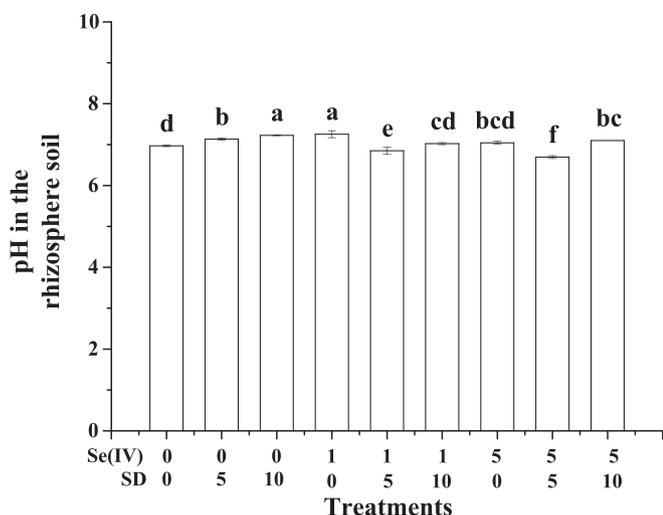
3.2.1. Available As concentration

In this study, the soil pH under the flooding treatment was significantly lower than that under the soil drying treatments (Fig. 1). Under water saturated conditions, the pH of the alkaline soil tended to decrease and converge to neutral (Fig. 1), which was in

**Table 2**  
Multivariate analysis of variance to illustrate the interactions effects of Se plus different water conditions on the concentrations of As, Cd and Se in the shoots, grains (including As(III)), roots, rhizosphere soil (including Fe and Mn) and plaques (including Fe and Mn).

Tissues	Treatments	As (mg kg <sup>-1</sup> )	Cd (mg kg <sup>-1</sup> )	Se (mg kg <sup>-1</sup> )	As(III) (μg kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )
Shoots	C	15.10** <sup>(1)</sup>	25.22ns	137.70**			
	A	2.74ns	2.17**	567.47**			
	A*C	3.82*	2.37ns	47.56**			
Grains	C	469.88**	16.86**	105.73**	7.02**		
	A	68.48**	6.00**	1073.82**	14.27**		
	A*C	3.32*	2.87ns	64.26**	2.71ns		
Roots	C	4651.50**	310.05**	72.11**			
	A	236.73**	27.47**	362.15**			
	A*C	192.41**	4.23*	18.70**			
Rhizosphere soil	C	98.86**	12.98**	4.93*		22.62**	50.09**
	A	6.34**	0.08ns	0.98ns		1.56ns	2.80ns
	A*C	8.03**	6.23**	2.31ns		3.18*	7.19**
Iron plaque	C	18.50**	128.17**	4.86*		261.82ns	1754.96**
	A	92.47**	2.94ns	33.42**		1.69**	213.12**
	A*C	48.76**	61.54**	53.10**		26.22**	902.64**

<sup>1</sup> F-values for the interactive effects between selenite and field drying times. ns: not significant (p > 0.05); \* and \*\* indicate significant at P ≤ 0.05 and 0.01, respectively.



**Fig. 1.** Effect of selenite plus different soil water conditions on the pH of rhizosphere. One-way ANOVA and Tukey' multiple range tests were used to compare the significant differences between different treatments. Bars are means and standard deviations for three replications. Different letters above bars indicate significant difference at  $P \leq 0.05$ .

line with the results of Zheng and Zhang (2011). The available As concentration in the rhizosphere soil decreased with the elongation of soil drying time (Table 3). Reports have shown that flooding will decrease the soil Eh and promote the proliferation of As-, Fe-, and sulfur-reducing bacteria (Das et al., 2016). As a result, Fe/Mn oxides will be reduced under an anaerobic condition (Zheng and Zhang, 2011), which resulted in much higher available concentrations of Fe and Mn than under an aerobic condition in soils (Figs. S1a and c), and an increase in the reduction of As(V) to As(III) (Li et al., 2009; Makino et al., 2016). As(III) has a low affinity to the soil solid phase and is easily dissolved in soil solution (Wan et al., 2019). Therefore, the above physical and chemical processes in soils under a flooding condition resulted in the higher As available concentration than soil drying treatments in this study (Table 3).

Under the flooding condition, the addition of Se(IV) significantly reduced the As available concentration in the rhizosphere soil but generally increased the soil pH (Fig. 1). Increased soil pH was also reported by Huang et al. (2018) after the addition of Se(IV). The decreases in the soil available As concentration was unlike to be due to 1) the increased soil pH. A high soil pH will stimulate the desorption of both As (Wan et al., 2019) and Se (Li et al., 2010); 2) the competition adsorption between Se and As, which was expected to result in a more release of As from the soil particles and thus increase the available As concentration. It was likely to be due to the stimulation on the uptake of As in the shoots and roots by the

addition of Se(IV) (Fig. 2), and on the fresh shoot weight (Table 1) under the flooding treatment.

Under the soil drying conditions, the addition of Se(IV) unexpectedly reduced the rhizosphere soil pH (Fig. 1), which was not in line with the situation that under the flooding condition in this study and the results of Huang et al. (2018). This decrease might be related with the stimulation on the acidic exudates from the plant roots. The soil drying tended to increase the soil pH (Fig. 1), and the high soil pH will lower down the phyto-availability of metal cations (Zeng et al., 2011), and the plants should excrete acidic materials to decrease the soil pH and then get more essential metal cations to survive. The low soil moisture and a decreased soil pH were both expected to stimulate the adsorption of As, but a generally unchanged As available concentration was observed in this study (Table 3). This may be due to the following contrary effects 1) the competition adsorption between Se and As (as previously mentioned, resulting in a more release of As); 2) the more sequestration of As onto the root plaques stimulated by the addition of Se(IV) (Table 3, see below discussion).

### 3.2.2. Available Cd and Se concentration

A low soil pH often facilitates the desorption of Cd (Wilkins et al., 1998). The study of Wan et al. (2019) observed that flooding treatment decreased the Cd in the soil solution resulting from a less formation of water-soluble Cd in soils. However, it was interesting to note that the available concentrations of Cd in most cases did not significantly change along with the soil drying time and the Se(IV) addition in this study (Table 3). The pH value of the original soil is 7.28 (an alkaline soil) and the flooding treatment decreased the soil pH to be approximate 7 (Fig. 1). The neutral to alkaline soil pH might be the predominant factor to control soil Cd available concentration under these conditions, which overcame the effects of other factors on the Cd available concentration and resulted in non-significant differences in its concentration (Table 3).

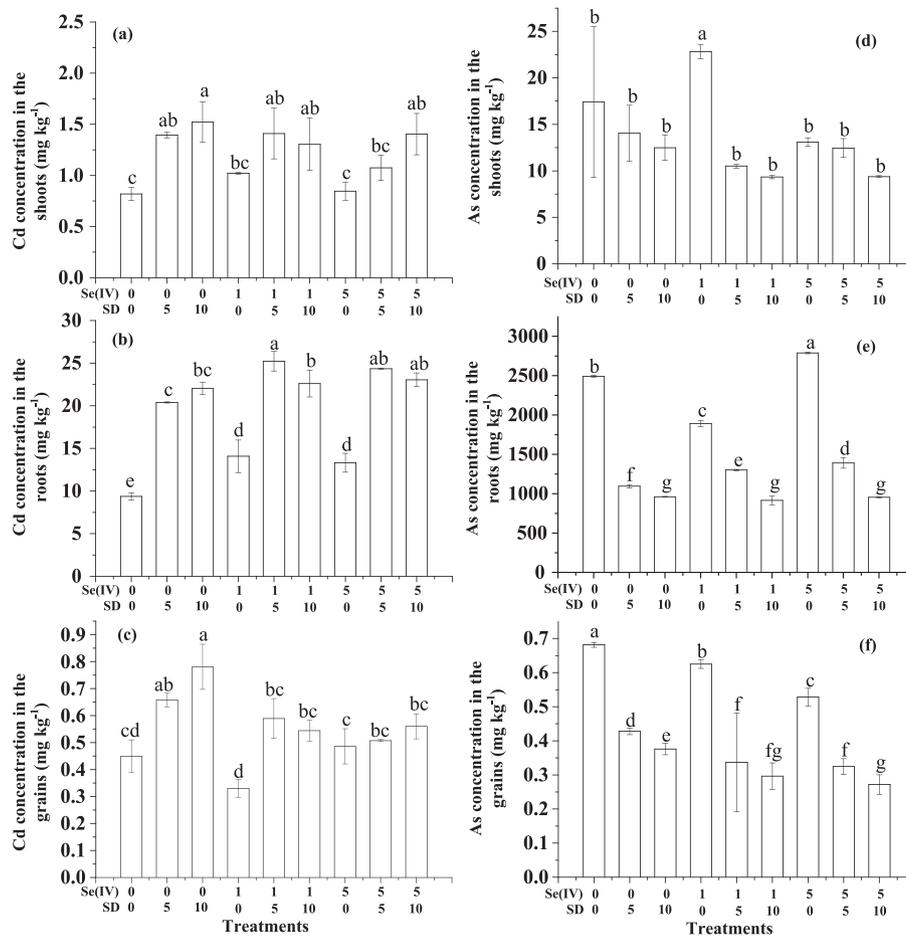
Similarly, there were no significant differences in the available Se concentration between the soil drying treatments and the flooding treatment (Table 3). The lower soil pH under the flooding treatment (pH: 6.97) tended to stimulate the adsorption of Se when compared to the soil drying treatments (pH: 7.14 to 7.23), just as the suggestion of Barrow and Whelan (1989). However, this process could be counteracted by the flooding treatment, which can increase soluble Se concentration when no Se or Se(IV) was added to the soil (Li et al., 2010), and thus resulted in the soil available Se concentration being unchanged. Interestingly, the addition of Se(IV) also did not significantly affect the soil Se available concentration whenever soil drying or not. Reports have shown that Se(IV), Se(VI), elemental Se and organic Se may all exist in anaerobic environments, and Se(VI) is the predominant soluble Se species under an oxidized condition (Yamada et al., 1999; Li et al.,

**Table 3**

The concentrations of As, Cd and Se in the rhizosphere soil (available concentrations) and plaques subjected to different levels of selenite and soil water conditions.

Treatments	As (mg kg <sup>-1</sup> )		Cd (mg kg <sup>-1</sup> )		Se (mg kg <sup>-1</sup> )	
	Rhizosphere soil	Iron plaque	Rhizosphere soil	Iron plaque	Rhizosphere soil	Iron plaque
C1	3.62 ± 0.35a <sup>(1)</sup>	475.07 ± 1.56d	3.24 ± 0.04 ab	2.49 ± 0.40b	0.36 ± 0.02a	1.64 ± 0.13e
C2	1.77 ± 0.02c	100.76 ± 5.86e	3.69 ± 0.22a	0.31 ± 0.09c	0.28 ± 0.00a	2.07 ± 0.23de
C3	1.73 ± 0.11c	193.48 ± 133.41e	3.22 ± 0.45 ab	1.02 ± 0.09c	0.34 ± 0.01a	8.56 ± 2.01b
A1C1	2.91 ± 0.10b	338.89 ± 29.23de	3.16 ± 0.33 ab	0.55 ± 0.04c	0.39 ± 0.06a	5.74 ± 1.50c
A1C2	1.57 ± 0.08c	129.27 ± 25.31e	3.69 ± 0.07a	0.83 ± 0.45c	0.36 ± 0.02a	2.44 ± 0.33de
A1C3	1.71 ± 0.06c	700.35 ± 153.84c	3.28 ± 0.09 ab	3.38 ± 0.41a	0.28 ± 0.01a	3.49 ± 0.82de
A2C1	2.96 ± 0.23b	279.89 ± 15.69de	3.79 ± 0.05a	0.46 ± 0.13c	0.37 ± 0.01a	5.72 ± 0.67c
A2C2	2.96 ± 0.23b	1265.78 ± 55.65a	3.49 ± 0.14a	0.56 ± 0.18c	0.34 ± 0.10a	12.06 ± 0.86a
A2C3	1.80 ± 0.08c	1066.73 ± 21.54b	2.76 ± 0.25b	3.47 ± 0.33a	0.35 ± 0.01a	4.10 ± 0.21cd

<sup>(1)</sup> Values are means ± SD (n = 3). The lowercases letters in the same column indicate significant differences among different treatments ( $p \leq 0.05$ ).



**Fig. 2.** Effect of selenite and different soil water conditions on the Cd concentration in the shoots (a), roots (b) and grains (c), and the As concentration in the shoots (d), roots (e) and grains (f). One-way ANOVA and Tukey' multiple range tests were used to compare the significant differences between different treatments. Bars are means and standard deviations for three replications. Different letters above bars indicate significant difference at  $P \leq 0.05$ .

2010). Se(IV) is predominant when the soil Eh ranges from 0 to 200 mV, and elemental Se and metal selenides are predominant under more reduced conditions ( $Eh < -200$  mV) (Nakamaru and Altansuvd, 2014). Therefore, the unchanged Se available concentration in the soils might be due to the following dynamic balance processes including 1) the fast adsorption of Se(IV); 2) the oxidation of Se(IV) into Se(VI) under the soil drying conditions and thus a more release of Se under an alkaline condition. Because Se(IV) was sorbed by soils more strongly than Se(VI) (Barrow and Whelan, 1989); 3) the reduction of Se(IV) under an anaerobic condition, and thus formed a lower soluble speciation (metal selenide) (Huang et al., 2018); 4) the competing adsorption between anions and Se, such as As (Ryden et al., 1987); 5) the fast uptake of Se by plants.

### 3.3. Se(IV) combined with soil drying facilitated the accumulation of As and Cd on the root Fe/Mn plaques only under a very low soil moisture condition

The soil drying treatments and adding Se(IV) treatments and their interactions all generally showed significant effects on the As, Cd and Se concentrations on the root Fe/Mn plaques except for the single treatment of Se(IV) on the Cd concentration on the Fe/Mn plaques (Table 2). The formation of root Fe/Mn plaques needs an oxidizing condition and a sufficient supply of Fe and Mn in plant growth medium (Ji et al., 2018). Under an aerated condition, rice

plants tend to have a higher formation of Fe/Mn plaques than in a water-saturated condition (Wu et al., 2012). However, in this study, the soil drying significantly reduced the Fe concentration but enhanced the Mn concentration on the Fe/Mn plaques when compared to the flooding treatment, especially at the soil drying for 5d (Figs. S1b and d). The above inconsistency was possibly due to the neutral to alkaline soil pH, which facilitated the oxidation and immobilization of Fe and Mn, thus resulting in a decreased available Fe concentration (Fig. S1a) and an insufficient Fe supply to form Fe plaque.

Wu et al. (2012) observed that root Fe/Mn plaques can sequester more As(V) than As(III) on their surface, leading to the decreased As accumulation in both roots and shoots. Root Cd concentration was found to be positively correlated with root Fe concentration (Liu et al., 2008; Zheng et al., 2012; Liu et al., 2007). As compared with flooding treatment, the increasing soil drying time only significantly enhanced the Se concentration but reduced the As and Cd concentrations on the Fe/Mn plaques (Table 3). The decreased As sequestration on the Fe/Mn plaques could be attributed to 1) The soil drying facilitated the transformation of As(III) to As(V), thus showing a higher affinity for soil adsorption (Wan et al., 2019) and resulting in the less available As concentration (Table 3) and less sequestration on the root Fe/Mn plaques. 2) The less formation of Fe plaques (Fig. S1b). The decreased Cd sequestration on the Fe plaque was probably due to the more entry of Cd from the root Fe/Mn plaques into the tissues of rice plants (Fig. 2, all tissues).

Under the flooding condition, the addition of Se(IV) significantly reduced the Cd concentration, and increased the Se concentration, but non-significantly reduced the As concentration on the Fe/Mn plaques (Table 3). When plants were subjected to a soil drying treatment at 5 days, the addition of Se(IV) did not significantly affect the As and Cd concentrations but significantly increased the Se concentration on the Fe/Mn plaques. At soil drying for 10 days, the addition of Se(IV) significantly enhanced the As and Cd concentrations but reduced the Se concentration on the Fe/Mn plaques when compared with the C3 treatment. The above results suggested that the addition of Se(IV) to an alkaline soil can stimulate the accumulation of As and Cd on the Fe/Mn plaques only under very low soil moisture, however, which needs more investigations to be identified in the future.

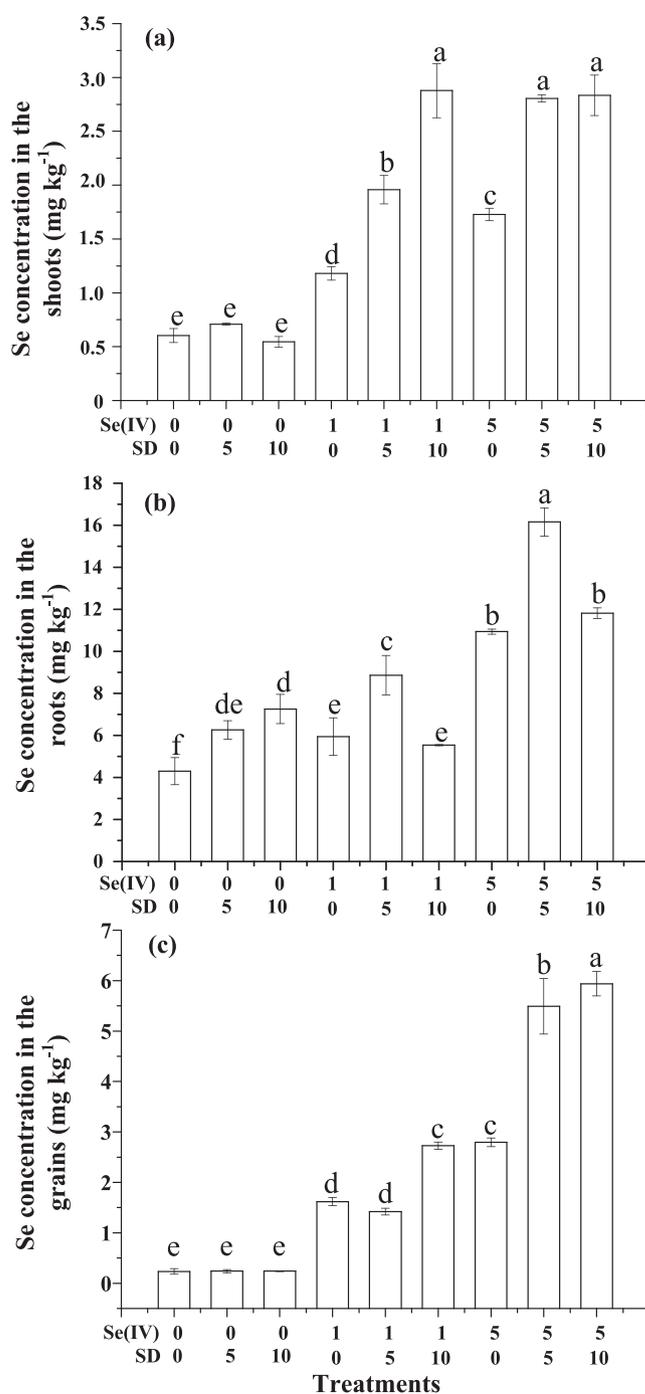
#### 3.4. Se(IV) combined with soil drying stimulated Se uptake but reduce As and Cd uptake in the grains

The single treatment of soil drying or Se(IV), and their interactions in most cases showed significant effects on the As and Se concentrations in the shoots, roots and grains (Table 2). However, the interaction effects of Se(IV) and soil drying on the shoot and grain Cd concentrations were not significant. In addition, the single effect of Se(IV) on the shoot As concentration, and the single effect of soil drying on the shoot Cd were also not significant.

The soil drying significantly enhanced the root Se concentration, but showed a limited effect on the grain and shoot Se concentrations (Fig. 3a, b, c). However, Li et al. (2010) observed that the grain Se concentration was 49% higher in the anaerobic condition than in the aerobic condition without Se addition. The above inconsistency might be due to the different soil conditions or the plant variety. Under a different soil moisture condition, the addition of Se(IV) significantly enhanced the Se concentration in all tissues of rice plants, with roots sequestering the majority of Se (Fig. 3a, b, c). When the soils were treated with a certain level of Se(IV), the soil drying stimulated Se concentration in the grains, shoots and roots (only at soil drying for 5 days). Similar results were also reported by Li et al. (2010), where the soil drying significantly enhanced the grain Se concentration by 2 fold compared to the flooding treatment when Se(IV) was added. Reports have shown that soil drying will stimulate the uptake of Cd in rice but reduced the uptake of As in rice plants (Arao et al., 2009). Similar results were also observed in this study (Fig. 2a–f).

Under the flooding condition, the addition of 1 mg kg<sup>-1</sup> and 5 mg kg<sup>-1</sup> Se(IV) significantly enhanced the shoot and root As concentration, respectively (Fig. 2d and e). This stimulation might result in the significantly decreased As available concentration in the rhizosphere soils and the slightly decreased As concentration in the Fe plaque (Table 3). The root Fe plaque often acts as a barrier or a reservoir for element uptake in plants, depending on plant species and some parameters in growth medium, such as speciation and concentrations of ions, pH, accompanying elements, Fe status, and redox state (Tripathi et al., 2014). The uptake stimulation of Cd in the roots of rice plants by Se was also observed in this study, especially upon the soil drying time being less than 5d (Fig. 2b). The above stimulation did not match well with the unchanged Cd available concentration (Table 3) but might lead to the significantly decreased Cd concentration in Fe plaque, suggesting the Fe plaque acting as a reservoir at this moment. Previous reports also found a stimulation of Se on the uptake of As in the study of Bluemlein et al. (2009) and aluminium in the study of Cartes et al. (2010).

Despite that the addition of Se increased the Cd and As concentrations in some tissues of the rice plants, the addition of Se(IV) showed significant inhibitory effects on the grain Cd (only upon soil drying 10d) and As concentrations (Fig. 2c, f). This inhibitory effect



**Fig. 3.** Effect of selenite plus different soil water conditions on the Se concentrations in the shoots (a), roots (b) and grains (c). One-way ANOVA and Tukey' multiple range tests were used to compare the significant differences between different treatments. Bars are means and standard deviations for three replications. Different letters above bars indicate significant difference at  $P \leq 0.05$ .

on As and Cd concentration might be partially due to the dilution effect because of the significantly enhanced yield after the addition of Se(IV). However, the Cd and As concentrations in all treatments were still higher than their individual national food hygiene standard (GB2715-2015, both at 0.2 mg kg<sup>-1</sup>), suggesting that the addition of Se(IV) plus different soil water regime failed to control grain As/Cd concentration within their safety levels in this study.

3.5. Se(IV) addition generally showed a negative effect on uptake of many essential elements under different water condition

Albeit the addition of Se can reduce the uptake of many heavy metals (metalloids) in plants, a negative effect on the uptake of many essential elements was also observed in the study of Feng et al. (2013b) and Hawrylak-Nowak et al. (2015). Similar results were also observed in this study. For example, under flooding condition, the addition of Se(IV) significantly enhanced the concentrations of magnesium (Mg) and potassium (K) (only at 1 mg kg<sup>-1</sup> Se(IV)), but reduced the calcium (Ca), Mn, Fe and zinc (Zn) concentrations in the grains. At a soil drying treatment for 5 days, the addition of Se(IV) significantly enhanced the concentrations of Mg (only at 1 mg kg<sup>-1</sup> Se(IV)) and Fe (only at 5 mg kg<sup>-1</sup> Se(IV)), but reduced the K and Zn concentrations, and showed non-significant effects on the Ca concentration in the grains. Reports indicated that alternate wetting and drying will affect the uptake of essential elements (Orasen et al., 2019). In this study, the concentrations of K, Ca, Fe and Zn in the grains all significantly reduced, but the grain Mg concentration enhanced with soil drying time (Table 4).

3.6. Se(IV) addition enhanced grain As(III) accumulation of thus possessed a health risk

In this study, only two types of As speciation were determined in the grains including As(III) (Fig. 4) and dimethyl arsenic (DMA). However, the DMA concentration in the grains of rice plants was detected only in the treatments with a low level of Se(IV) and with a short time (or no) soil drying, and these treatments included C1, C2 and A1C1 treatments. The DMA concentration in the C1, C2 and A1C1 treatments was 19.54, 24.47 and 30.99 µg kg<sup>-1</sup>, respectively (data not shown). The grain As(III) concentration in the soil drying treatments were significantly lower than that in the flooding treatment (Fig. 4), suggesting that soil drying could lower down the accumulation of a more toxic valence of As. However, when the rice plants were exposed to the soil drying for 5 or 10 days, the addition of Se(IV) significantly enhanced the grain As(III) concentration. The above results indicated that using Se(IV) to control the uptake of As in rice plants growing in an alkaline and high contaminated level of As soil possessed a risk of more accumulation of grain As(III). A similar stimulation on As(III) accumulation after the addition of Se(IV) was also observed by Han et al. (2015) in *N. tabacum* 'K326'.

4. Conclusions

In this study, some interesting results were observed. 1) The

Table 4 The concentrations of some essential elements in the grains of rice plants.

Treatments	Mg (g kg <sup>-1</sup> )	K (g kg <sup>-1</sup> )	Ca (g kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
C1	0.87 ± 0.01f <sup>(1)</sup>	3.51 ± 0.01b	0.64 ± 0.13a	37.83 ± 2.12a	32.59 ± 4.57a	78.75 ± 7.75a
C2	1.17 ± 0.02b	3.40 ± 0.01c	0.25 ± 0.02c	32.64 ± 0.72 ab	7.38 ± 0.35cd	48.42 ± 1.13b
C3	0.96 ± 0.06de	2.84 ± 0.01g	0.29 ± 0.03c	30.84 ± 0.76 abc	5.56 ± 0.39cd	43.28 ± 1.64c
A1C1	1.01 ± 0.05cd	6.33 ± 0.08a	0.51 ± 0.04b	27.47 ± 0.36bcd	2.69 ± 0.32d	34.92 ± 1.76de
A1C2	1.33 ± 0.00a	2.97 ± 0.01f	0.23 ± 0.02c	25.38 ± 0.84bcd	6.77 ± 0.65cd	39.30 ± 0.20cd
A1C3	1.05 ± 0.03c	2.77 ± 0.01g	0.27 ± 0.08c	23.65 ± 0.42cd	9.70 ± 1.19c	38.82 ± 0.75cd
A2C1	0.94 ± 0.03e	3.45 ± 0.02bc	0.32 ± 0.03c	27.52 ± 1.36bcd	5.49 ± 0.85cd	31.41 ± 1.88e
A2C2	1.13 ± 0.02b	3.31 ± 0.10d	0.36 ± 0.05c	33.12 ± 9.83 ab	14.23 ± 3.78b	30.03 ± 0.22e
A2C3	1.07 ± 0.01c	3.09 ± 0.05e	0.26 ± 0.05c	21.85 ± 0.70d	6.92 ± 0.57cd	33.98 ± 1.38de
C	202.55** <sup>(2)</sup>	795.55**	39.27**	7.02**	20.96**	189.34**
A	45.78**	2710.87**	4.22*	14.27**	43.26**	32.64**
A*C	11.57**	1539.20**	11.36**	2.71ns	81.77**	53.30**

<sup>(1)</sup> Values are means ± SD (n = 3). The lowercases letters in the same column indicate significant differences among different treatments (p ≤ 0.05). <sup>(2)</sup> F-values for the interactive effects between selenite and field drying times. ns: not significant (p > 0.05); \* and \*\* indicate significant at P ≤ 0.05 and 0.01, respectively.

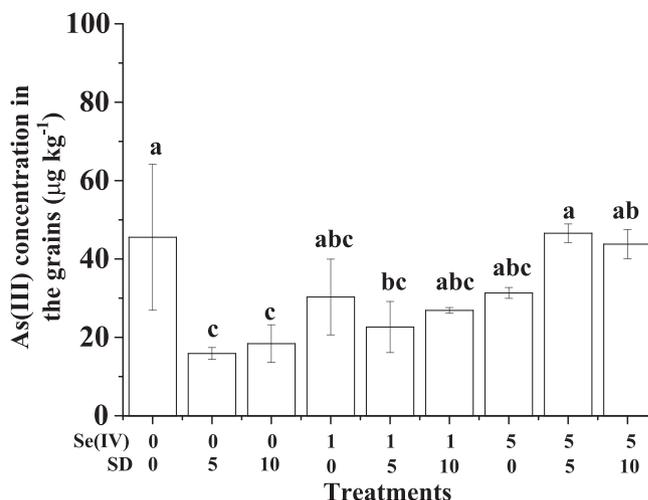


Fig. 4. Effect of selenite and different soil water conditions on the grain As(III) concentration. One-way ANOVA and Tukey' multiple range tests were used to compare the significant differences between different treatments. Bars are means and standard deviations for three replications. Different letters above bars indicate significant difference at P ≤ 0.05.

combined utilization of Se(IV) and different water regimes cannot lower down the As and Cd concentrations below their individual safety standard despite that the grain concentrations of As and Cd were significantly reduced by some combined treatments. 2) The flooding condition facilitated the uptake of As but low soil water moisture facilitated the uptake of Cd and Se in rice plants. 3) The addition of Se(IV) stimulated the sequestration of As and Cd on the root Fe/Mn plaques at a soil drying treatment for 10 days, but reduced that under a flooding condition. 4) The addition of Se(IV) stimulated the yields of rice plants subjected to As and Cd exposure, but which reduced the concentrations of many essential elements in the grains, and stimulated the accumulation of a more toxic valence of As(III), thus posing a health risk.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## CRedit authorship contribution statement

**HaiQin Lv:** Investigation. **WenXiang Chen:** Formal analysis, Writing - original draft. **YanMing Zhu:** Data curation, Visualization. **JiGang Yang:** Software. **Sohaib H. Mazhar:** Writing - review & editing. **PingPing Zhao:** Validation. **LiZhen Wang:** Methodology. **YuanPing Li:** Software. **Syed Muhammad Azam:** Validation. **Ibtissem Ben Fekih:** Validation. **Hong Liu:** Writing - review & editing. **Christopher Rensing:** Conceptualization, Writing - review & editing. **RenWei Feng:** Conceptualization, Project administration, Funding acquisition, Supervision.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2020.114283>.

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