Effects of Selenium–Zinc Interactions on the Bioavailability of Selenium/Zinc in Soil and Its Mechanism

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Abstract

Purpose Biofortification is a core strategy in solving the malnutrition of selenium (Se) and zinc (Zn) for global human being. However, whether Se and Zn co-biofortification can manipulate soil Se/Zn bioavailability and its mechanism still remains unclear.

Methods The pot experiment was conducted to investigate the co-amendment of selenate and Zn sulfate on the growth of pak choi, especially on the uptake of Se and Zn, and to elucidate the effect of soil pH and soil enzyme activity on the bioavailability of Se/Zn in soil and its mechanism.

Results Results showed that plant growth inhibition caused by the application of high Se rate was significantly alleviated with Zn supplements, and the biomass in the shoots and roots of pak choi in Se2.5Zn20 and Se2.5Zn50 treatments significantly increased (67.0%–112.8%) compared with the Se2.5Zn0 treatment. Additionally, Se and Zn co-amended application significantly enhanced soil available Se/Zn content compared with correspond single Se/Zn treatments. The increase of soil available Zn content could be attributed to the significant decrease in the soil pH, while the increase of soil available Se was from the biochemical conversion caused by the activity of catalase, urease, and alkaline phosphatase.

Conclusion Se–Zn co-amendment can ameliorate bioavailability of Se/Zn in soil by regulate the pH and enzyme activity of soil.

Introduction

Selenium (Se) and zinc (Zn) are essential micronutrients for humans and animals, and inadequate intake of these micronutrients could threaten human health because it weakens the immune system. Meanwhile, they have been associated with an increase in the incidence of cardiovascular diseases, asthma, epilepsy, growth retardation, decreased fertility, cognitive decline, and various cancers (Mangueze et al. 2018). Estimates pointed that ca. 30% (2.7 billion) and 15% (0.5–1 billion) of the world's population experience Zn and Se deficiency, respectively, which constitute a serious problem of public health, especially in developing countries (Li et al. 2015). In China, approximately 51% of soil is affected by Se deficiency, and most of this area is Zn deficient as well (Dinh et al. 2018; Yang et al. 2007). Therefore, to meet the human nutrition's needs for Se and Zn, effective and efficient co-biofortification strategies of Se and Zn must be developed (Mangueze et al. 2018). Among them, agronomic biofortification by applying Se and Zn fertilizer in soil is an effective and reliable solution that can supplement Se and Zn in co-deficient areas to increase the Se and Zn contents in plants and then in the human body (Bañuelos et al. 2015; Xue et al. 2020). Notably, a narrow range exists among Se dietary deficiency (< 40 ug/d), suitability (~ 110 ug/d), and toxicity (> 400 ug/d) levels, which are quite difficult to regulate (Qin et al. 2016). When Zn was applied to soil, especially in calcareous soil with high pH and carbonate content, a series of physical and chemical reactions will occur with the soil components, thereby resulting in an extremely low available Zn content for plants (Lu et al. 2012). Furthermore, Se usually exists as selenite oxyanions in oxidizing conditions, whereas Zn exists largely as Zn^{2+} in aqueous environments (Xue et al. 2020). Therefore, the interaction of Se and Zn in soil must be clearly understood in their co-biofortification.

When different elements enter the soil–plant system, their interaction will affect the bioavailability of elements in soil and the growth of crops. Previous studies found that application of L-SeMet (L-selenomethionine) and EDTA-Zn (sodium ethylenediamine tetraacetate-Zn) in soil significantly ($p < 0.05$) increased the biomass of pak choi (Dai et al. 2019). Selenate and Zn sulfate co-amended treatments could reduce the binding intensity of Zn, thereby making Zn the available forms for plants and promoting the uptake and translocation of Zn by pak choi (Xue et al. 2020). Previously, Poblaciones and Rengel (2017) and Dai et al. (2019) verified that the co-amendment of Se and Zn could promote the uptake of Se/Zn in plants. By contrast, in a field experiment, Ramezani et al. (2016) reported that soil co-application of selenite and Zn sulfate meliorated the uptake of Se but restrained Zn uptake of alfalfa. Fang et al. (2008) found that foliar application of sodium selenite and Zn sulfate had no significant influence ($p > 0.05$) on the absorption of Se/Zn in rice, which may be due to the discrepancy in the metabolic pathways between Se and Zn in plants. Generally, Se enters the roots via the S transport system, P transporter (*OsPT2*), or a silicon transporter [*Lsi1* (*OsNIP2;1*)] (Dinh et al. 2018), and Zn uptake by plant roots involves an active process through Zn transporter (*ZIP*) (Nguyen et al. 2017). Based on the findings, different responses on soil Se/Zn bioavailability induced by Se–Zn co-amended condition was closely related to plant species and soil properties and relied on Se/Zn species and application methods as well.

Previous studies involved in the Se–Zn interaction were mostly focused on the effect of selenite and Zn sulfate co-amendment on plant growth. Given that selenite was feasibly adsorbed onto carbonate, Fe/Mn oxides, and organic matter in soil, soil Se bioavailability was inhibited. Nevertheless, Se initially exists in the soluble fractions and binds weakly to soil particles with the

addition of selenate; Se easily migrates with pore water in soil and becomes more available than other soil fractions (Wang et al. 2012). Consequently, selenate was the major Se fertilizer that is commonly used in Se biofortification, whereas the effect of selenate and Zn sulfate co-amendment on plant growth have not yet to be systematically conducted.

The accumulation of Se and Zn in crops not only depends on the availability of Se and Zn contents in soil (Xu et al. 2020) but also affected by the physicochemical properties soil, such as pH, cation exchange capacity, redox potential, enzyme activity, and microbe activity (Dhal et al. 2013). Soil pH plays an incomparable role in governing Se/Zn bioavailability in the soil (Antoniadis et al. 2017). Zn availability decreases as soil pH increases. At pH values below 7.7, Zn^{2+} predominates, but $ZnOH^+$ is predominant between pH 7.7 and 9.1 and $Zn(OH)_2$ is predominant above pH 9.1 (Zhao et al. 2018). On the contrary, the bioavailability of Se gradually decreases with pH, which determines the valence state of Se in soil, thereby, influencing soil Se bioavailability. The defined available Se in soil mainly appears as inorganic Se (IV) and Se(VI) (Dinh et al. 2018).

Soil enzymes are involved in all nutrient cycling, and its availability to plants can be used as an index of soil function (Tan et al. 2018), which could also be widely used to assess the impact of various heavy metals on soil microbial function, both in a long and short period of time (Yang et al. 2007). Studies showed that the application of Se/Zn markedly influenced soil enzyme activity (Kunito et al. 2001; Nowak et al. 2004). Soil is a multiphase complex medium. The surfaces of soil particles, such as clay, can not only adsorb heavy metal ions but also interact with soil enzymes (Hu et al. 2014). Thus, the relationship between soil enzyme activity and the bioavailability of metals in soil needs to be further explored.

Given above, the main objectives of this study were to (1) clarify the effect of co-amendment of selenate and Zn sulfate on plant growth, especially the uptake of Se and Zn, and (2) elucidate the effect of soil pH and soil enzyme activity on the bioavailability of Se/Zn in soil and its mechanism.

Materials And Methods

Experimental materials

Soil (Eum-Orthic Anthrosol) was collected from the surface layer (0–20 cm) of the test field located at the Northwest A&F University (34°15'N, 108°3'E) in Shaanxi Province, China. The soil used for the experiments presented the following properties: pH (7.9), organic matter (8.53 g/kg), cation exchange capacity (CEC: 23.34 cmol/kg), clay (39.50%), calcium carbonate (118.0 g/kg), total nitrogen (1.11 g/kg), total phosphorus (0.98 g/kg), available phosphorus (11.62 mg/kg), total Se (0.14 mg/kg), total Zn (89.23 mg/kg), soil available Se (0.022 mg/kg), and soil available Zn (0.25 mg/kg).

Seeds of pak choi (*Brassica chinensis* L.) were provided by the Northwest A&F University Seeds Co. Ltd., Shaanxi, China. Pak choi seeds were immersed in 0.5% NaClO solution for 30 min and washed with deionized water before planting.

Selenate and Zn sulfate were used as fertilizer sources. Selenate (Na_2SeO_4 , $\geq 97\%$) and Zn sulfate ($ZnSO_4 \cdot 7H_2O$, $\geq 99.5\%$) were purchased from a reagent factory in Tianjin, China.

Experimental design

According to previous studies, Se (Na_2SeO_4) exposure levels were set as 0, 0.5, 1.0, and 2.5 mg Se/kg dry soil, and Zn ($ZnSO_4 \cdot 7H_2O$) application rates were set as 0, 20, and 50 mg Zn/kg dry soil (Dai et al. 2019; Xue et al. 2020). Each treatment has three replicates for a total of 12 treatments (Table 1).

Table 1
Treatment levels of amendments with Se and Zn (mg/kg).

Treatments		Content		Treatments		Content		Treatments		Content		Treatments		Content	
		Se	Zn			Se	Zn			Se	Zn			Se	Zn
Se0	Zn0	0	0	Se0.5	Zn0	0.5	0	Se1	Zn0	1	0	Se2.5	Zn0	2.5	0
	Zn20	0	20		Zn20	0.5	20		Zn20	1	20		Zn20	2.5	20
	Zn50	0	50		Zn50	0.5	50		Zn50	1	50		Zn50	2.5	50

After the soil was homogenized and equilibrated for 21 days, basal fertilizer (N, P, and K) that comprised 0.15 g/kg N (urea, AR) and 0.033 g/kg P (monopotassium phosphate, AR) were thoroughly mixed into the equilibrated soil (Qin et al. 2016). Different concentrations of Se and Zn solutions were applied to the soil using a plastic nebulizer. A total of 15 seeds were sown in each pot, thinned to 5 seedlings per pot after 10 days, and grown under natural lighting conditions. Deionized water was supplied once every 2 to 3 days to maintain 70% of the soil water-holding capacity during the entire experiment.

In 30 d after sowing, the pak choi was harvested and then were divided into shoots and roots. The shoots and roots were washed thoroughly with deionized water after being washed with tap water and then dried with absorbent tissue. Both parts of the pak choi were oven-dried at 90°C for 30 min and 60°C to a constant weight, and then dry weight (DW) was recorded. Dry samples were used to analyze Zn and Se concentration. Soil samples were collected from each pot and then completely air-dried at room temperature, homogenized, and finally ground to pass through 2, 1, and 0.149 mm nylon sieves for chemical analyses, respectively.

Plant and soil analysis

Plant samples (1.000× g) were digested in HNO₃: HClO₄ (4: 1; V/V) mixture. The digested solution was cooled and diluted using deionized water. The diluted solution was filtered, and a part of those samples was directly taken to determine the Zn concentrations by AAS (Flame atomic-absorption spectrometry, PinAAcle 900F, America). Another part was heated for 30 min at 100°C boiling water bath in 6 M HCl solution and stored in PE bottles for the determination of total Se concentration by AFS (Atomic fluorescence spectrometry-930, Jitian, Beijing, China) (Bao 2000).

Soluble and exchangeable Se fractions were easily available to plants and were thereby defined as bioavailable Se (Wang et al. 2012). The details are described as follows: (1) Soluble Se: 10 mL 0.25M KCl was added to 1.0 g dry soil in a 50 mL polypropylene centrifuge tube. Subsequently, the tube was shaken at 200 rpm/m at 25°C for 1 h. The mixture was centrifuged for 10 min at 4000 rpm and filtered; (2) Exchangeable Se: 20 mL 0.7 M KH₂PO₄ (pH = 5.0) was added to the above tube and was shaken at 200 rpm/m and 25°C for 4 h, and then centrifuged for 10 min at 4000 rpm. The two step's supernatant was collected for Se content analysis by AAS (Wang et al. 2012).

Soil available Zn (DTPA-Zn): 25 mL 0.005 mol/L DTPA in 0.01 mol/L CaCl₂ buffered at pH 7.3 with 0.1 mol/L triethanolamine (TEA) was added to 12.5 g dry soil in a 100 mL polypropylene centrifuge tube. Then, the tube was shaken at 160 rpm/m at 25°C for 2 h and filtered. The filtrate can then be directly used for AAS analysis (Bao 2000).

Soil pH was extracted with CO₂-free deionized water (water : soil = 2.5:1) and measured by a pH electrometer (Bao 2000). The activity of invertase (INV) in soil was determined by 3,5-dinitrosalicylic acid colorimetric method, and the amount of reducing sugar produced by enzymatic reaction with sucrose as substrate, that is, the mg number of glucoses produced 1 g soil after 24 h was expressed. The urease activity (URE) in soil was determined by phenol sodium–sodium hypochlorite colorimetric method and by enzymatic reaction of urea as substrate NH₄⁺-N, expressed by the number of NH₄⁺-N milligrams per 24 h per 1 g soil sample. The alkaline phosphatase (APS) activity in soil was determined by the colorimetric method of benzene disodium phosphate, and the conversion of benzene disodium phosphate to phenol was expressed by the number of micrograms P₂O₅ in soil 1 h later. The catalase (CAT) activity in soil was titrated with standard potassium permanganate solution by adding a certain amount of hydrogen peroxide solution to the soil, and the enzyme activity was expressed as the KMNO₄ milliliter number of 0.1 mol/L per 1 g soil sample (Guan et al. 1986).

Quality control and data analysis

Different quality assurance and control measures were adopted in sample preparation and chemical analyses, including the use of certified reference materials for instrumental calibration, determination of the method detection limit, and analyses of reagent blanks, sample duplicates, and spiked samples. In plant standard substances, the measured Se concentrations were 0.19 ± 0.05 mg/kg (GBW-10014 (GSB-5), cabbage leaves, China, 0.20 ± 0.03 mg/kg) and Zn concentrations were 25 ± 0.57 mg/kg (GBW-10014 (GSB-5), cabbage leaves, China, Zn = 26 ± 2 mg/kg). In soil standard substances, the measured Se concentrations were 0.67 ± 0.12 mg/kg (GBW-07404 (GSS-4), calcareous soil, China, Zn = 0.63 ± 0.18 mg/kg) and Zn concentrations were 226.91 ± 1.92 mg/kg (GBW-07404 (GSS-4), calcareous soil, China, Zn = 210 ± 13 mg/kg).

The differences among different Se/Zn treatments with the same Zn/Se application concentration were evaluated using a one-way analysis of variance (one-way ANOVA, $p < 0.05$). Two-way ANOVA was used to further investigate the effects of Se and Zn treatments and their interaction on plant growth. Pearson correlation analysis, linear stepwise regression (SR), and path analysis were conducted

to clarify the effect of soil pH, enzyme activity, and available Se/Zn on Se/Zn bioavailability. All tests were performed using Microsoft Excel 2013 and SPSS 13.0.

Results

Plant growth

The biomass (dry weight; DW) in pak choi shoot and root was significantly affected by single Se/Zn and Se–Zn co-amended treatments ($p < 0.01$) (Table 2). When compared with control treatment, the biomass (DW) in pak choi shoot and root showed no significant ($p > 0.05$) difference at the single Se concentration of 0.5 and 1 mg/kg but reduced by 40.4% and 27.7% at single Se concentration of 2.5 mg/kg, respectively. Meanwhile, for single Zn treatments, the biomass of pak choi shoot had no significant ($p > 0.05$) effect, and the root biomass increased by 33.4–51.0% compared with the control treatment ($p < 0.05$).

Table 2
Soil pH, biomass of shoot and root (DW: dry weight) of pak choi with Se and Zn applications.

Treatments		Soil pH value	Shoot biomass (g/plant)	Root biomass (g/plant)
Se0	Zn0	7.92 ± 0.00 ^{Aab}	6.79 ± 0.6 ^{Ab}	0.24 ± 0.03 ^{Aab}
	Zn20	8.23 ± 0.01 ^{Ab}	6.85 ± 0.59 ^{Ab}	0.37 ± 0.01 ^{Bc}
	Zn50	8.08 ± 0.11 ^{Ab}	6.37 ± 0.31 ^{Ab}	0.33 ± 0.05 ^{Bab}
Se0.5	Zn0	7.82 ± 0.06 ^{Aa}	5.96 ± 0.74 ^{Ab}	0.20 ± 0.04 ^{Aa}
	Zn20	8.27 ± 0.08 ^{Bb}	7.71 ± 0.21 ^{Bb}	0.20 ± 0.03 ^{Aa}
	Zn50	8.34 ± 0.01 ^{Bc}	7.80 ± 0.56 ^{Bc}	0.26 ± 0.05 ^{Aa}
Se1	Zn0	8.12 ± 0.13 ^{Bab}	7.20 ± 0.69 ^{Bb}	0.28 ± 0.05 ^{Ab}
	Zn20	7.68 ± 0.07 ^{Aa}	5.05 ± 0.82 ^{Aa}	0.20 ± 0.02 ^{Aa}
	Zn50	7.80 ± 0.04 ^{Aa}	5.64 ± 0.13 ^{ABb}	0.26 ± 0.02 ^{Aa}
Se2.5	Zn0	8.15 ± 0.16 ^{Ab}	4.04 ± 0.43 ^{Aa}	0.17 ± 0.01 ^{Aa}
	Zn20	8.05 ± 0.18 ^{Ab}	6.87 ± 0.38 ^{Bb}	0.29 ± 0.03 ^{Bb}
	Zn50	7.84 ± 0.08 ^{Aa}	3.72 ± 0.75 ^{Aa}	0.37 ± 0.02 ^{Cb}
ANOVA (F values)				
Se		26.9**	38.7**	14.9**
Zn		2.2	8.3**	18.6**
Se * Zn		38.4**	20.8**	11.1**
Note: Different upper-case letters indicate the significant differences ($p < 0.05$) among different Zn treatments with the same Se application concentration, and different lower-case letters indicate the significant differences ($p < 0.05$) among different Se treatments with the same Zn application concentration. Two-way ANOVA results (F value) for two groups (Se; Zn) are presented in Table 2, * and ** represent $p < 0.05$ and $p < 0.01$, respectively.				

Plant growth inhibition caused by high Se concentration was alleviated significantly ($p < 0.05$) with Zn supplements. For example, Se2.5Zn20 treatment resulted in a significant increase in biomass in shoots (69.9%) and roots (67.0%) of pak choi, and Se2.5Zn50 treatment resulted in a significant increase in biomass in roots (112.8%) ($p < 0.05$) when compared with Se2.5Zn0 treatment. However, the significant ($p < 0.05$) decrease (21.7–46.7%) in the biomass in shoots and roots was observed in the same Zn treatments, as a result of application of Se ($p < 0.05$).

Se and Zn uptake

The Se content in shoot and root of pak choi was significantly affected by single Se/Zn and Se–Zn co-amended treatments ($p < 0.01$) (Figs. 1c and d). For single Se treatments, Se content in shoot and root of pak choi significantly increased with the application rates of Se application ($p < 0.05$) (Fig. 1a). Compared with single Se treatments, the Se content of shoots in the combined application of Se and Zn treatments declined by 2.8–63.1% (only showed significant difference when Se was applied for Zn 20 mg/kg treatment). The Se content of the pak choi root increased by 26.8–149.9% in comparison with the corresponding single Se treatment (only showed significant difference in Se0.5Zn50 and Se2.5Zn50 treatments) (Fig. 1a).

The Zn content in pak choi shoot and root was significantly affected by single Zn treatment ($p < 0.01$), whereas Zn content was not significantly influenced by the single Se treatments or Se*Zn interaction (Figs. 1e and f) ($p > 0.05$). For single Zn treatments, Zn content in shoot and root of pak choi significantly increased (0.6–3.1 folds) with the increase in Zn application rates ($p < 0.05$) (Fig. 1b). No significant change in Zn contents was observed in plants exposed to Se–Zn co-amended treatments in comparison with the corresponding single Zn treatment ($p > 0.05$) (Fig. 1b).

Available Se/Zn in soil

The available Se/Zn contents in soil with different treatments are shown in Fig. 2. The available Se/Zn content in soil was significantly influenced by Se and Zn treatments or their interaction ($p < 0.01$) (Figs. 2c and d).

For single Se treatments, available Se contents in soil significantly increased (11.7–62.6 folds) with the increasing Se rates ($p < 0.05$) (Fig. 2a). Compared with the corresponding single Se treatments, the available Se contents in soil in the combined application of Se and Zn treatments increased by 9.6–41.6% (only showed significant difference in Se1Zn20 treatment) ($p < 0.05$) (Fig. 2a).

For single Zn treatments, the available Zn contents of soil significantly increased (4.5–11.0 folds) with the Zn application rates ($p < 0.05$) (Fig. 2a). Compared with corresponding single Zn treatments, the available Zn contents of soil in the combined application of Se and Zn treatments increased by 0.12–70.7% (only showed significant difference in Se1Zn20 and Se2.5Zn50 treatments) ($p < 0.05$) (Fig. 2a).

Soil pH and enzyme activities

In the present study, single Se/Zn application showed no significant effect on soil pH. Conversely, the pH of Se–Zn co-amended soil changed with the Se/Zn content in soil ($p < 0.01$) (Table 1). Compared with Se0.5Zn0 treatment, the soil pH in Se0.5Zn20 and Se0.5Zn50 treatments increased by 0.46 and 0.53 units, respectively ($p < 0.05$); When Se was applied at 1.0 mg/kg and 2.5 mg/kg with the addition of Zn into the soil, a slight decrease (0.10–0.44 units) in soil pH was observed as compared with the corresponding single Se treatments. When soils were treated with 1.0 and 2.5 mg/kg Se, the pH slightly decreased (0.19–0.55 units) at 20 and 50 mg/kg Zn treatments in comparison with the corresponding single Zn treatment (Table 1).

The activities of the selected soil enzymes, including invertase (carbon cycle), urease (nitrogen cycle), alkaline phosphatase (phosphorus cycle), and catalase (hydrogen dioxide degradation), are presented in Fig. 3. Compared with control treatments, no change in invertase activity was observed in single Se/Zn treatments (Fig. 3a). Meanwhile, urease and alkaline phosphatase activities were stimulated by low level of Se/Zn (0.5–1 mg/kg Se or 20 mg/kg Zn) in soil, but were inhibited by high level (i.e., 2.5 mg/kg Se or 50 mg/kg Zn) (Figs. 3b and c). For the catalase activity, although a significant increase (28.3–48.8%) in single Se treatments ($p < 0.05$) was noted, no significant difference in single Zn treatments was observed compared with those in the control treatments (Fig. 3d).

Compared with corresponding single Se treatment, a significant decrease in urease activity (12.1–31.8%) were observed in the Se–Zn co-amended treatments (Fig. 3b). Moreover, invertase activity (7.0–50.3%) in Se application with Zn supplements treatments, as well as alkaline phosphatase activity (11.4–27.4%) (Figs. 3a and c) increased. Additionally, no significant difference in the catalase activity with Zn supplements was observed (Fig. 3d) ($p > 0.05$). Compared with corresponding single Zn treatment, the application of Se resulted in an increase in invertase (15.2–51.8%) and alkaline phosphatase (17.2–20.2%) activities and a decrease in urease (17.1–24.3%) and catalase (19.2–48.2%) activities (Fig. 3).

Effect of soil pH, enzyme activity, and availability of Se/Zn on Se/Zn content in pak choi

The content of Se/Zn in plants is the most direct indicator of the bioavailability of soil Se/Zn.

Correlation analysis

Correlation analysis estimated with the linear regression programs showed that pH, enzyme activities, and the availability of Se/Zn on soil were related to the Se/Zn content in shoots and roots of pak choi (Table 3).

Table 3
The correlation coefficient between soil characteristics and the uptake of pak choi.

Index	pH	INV	URE	APS	CAT	Available Se	Available Zn	Shoot Se	Root Se	Shoot Zn	Root Zn
pH	1										
INV	-0.400*	1									
URE	0.566**	-0.429**	1								
APS	0.093	0.175	-0.125	1							
CAT	0.218	-0.433**	0.307	-0.331*	1						
Available Se	-0.173	0.241	-0.147	0.135	-0.215	1					
Available Zn	-0.134	0.430**	-0.549**	0.272	-0.372*	0.119	1				
Shoot Se	-0.062	0.209	0.076	-0.124	-0.249	0.823**	0.130	1			
Root Se	-0.045	0.328	0.069	-0.109	-0.387*	0.901**	0.290	0.927**	1		
Shoot Zn	0.013	0.335*	-0.498**	0.253	-0.270	0.021	0.966**	0.019	0.185	1	
Root Zn	0.014	0.289	-0.496**	0.326	-0.294	-0.070	0.922**	0.001	0.180	0.924**	1
Note: n = 36, * indicates a significant correlation of 95%; ** indicates a significant correlation of 99%.											

Se content in shoots and roots of pak choi had the best positively correlation with the available Se in soil ($R^2 > 0.82$, $p < 0.01$). Additionally, Se content in pak choi roots was significantly and negatively correlated with the catalase activities ($R^2 = -0.38$, $p < 0.05$).

The Zn content in pak choi shoots and roots was significantly positively correlated with the available Zn content in soil ($R^2 > 0.92$, $p < 0.01$). However, this correlation with soil urease was negative ($R^2 > -0.49$, $p < 0.01$). The available Zn in soil also had negative correlation with soil urease activities ($R^2 = -0.54$, $p < 0.01$). Notably, a significant positive correlation was found among the Zn content and invertase activities in pak choi shoots ($R^2 = 0.33$, $p < 0.05$).

Stepwise regression (SR) modeling

Table 4 summarizes the results in applying the SR modeling with Se/Zn (in mg/kg) in the shoots and roots of pak choi as dependent variables, and soil pH, soil enzyme activities (e.g., invertase (in mg glucose/g/d), urease (in mg $\text{NH}_4^{+}\text{-N/g/d}$), alkaline phosphatase (in ug/g/h), catalase (in ml KmnO_4/g)), and availability of Se/Zn in soil (in mg/kg) as independent variables. The “B” values correspond to the beta coefficients for each independent variable in the regression model. The adjusted R^2 value reflects the explanatory power of the regression model. We can determine whether the model offers a good fit for the data based on the F-test and its associated significance level. The significance (Sig.) figure is 0.05 or below shows a statistically significant) relationship between independent and dependent variables (Xu et al. 2020).

Table 4
Summary of stepwise regression results.

	Independent variables	B	Intercept	Adj.R ²	F	Sig
Shoot Se	Available Se, APS	167.434, -6.7222	292.909	0.717	45.239	0.000
Root Se	Available Se, CAT, available Zn, URE, APS	79.169, -8.644, 16.509, 57.817, -1.617	-16.669	0.910	72.029	0.000
Shoot Zn	Available Zn	18.945	25.426	0.846	193.584	0.000
Root Zn	Available Zn, pH	36.712, 19.485	-123.125	0.944	298.146	0.000

The results from the SR models showed that the main influencing factors of shoot Se content were the content of available Se and alkaline phosphatase activities in soil; the main influencing factors of Se content in root were the content of available Se/Zn, urease, catalase, and alkaline phosphatase activities in soil. The adjusted R² values for Se in shoot and root associated with the main influencing factors are 0.717 and 0.910, respectively, indicating that the models separately explain 71.7% and 91.0% of the variations in the Se content of shoot and root, respectively (Table 4). Furthermore, the indirect path coefficients indicated that catalase activities affected the available Se in soil ($p < 0.01$) (Table S1). This finding reveals that the effect of catalase activities in soil on the Se content of root is through the available Se in soil.

Discussions

Effects of Se–Zn interaction on plant growth and Se/Zn uptake

Plant growth is a symbolic index for evaluating the ecological effects induced by the addition of Se/Zn in soil. In the present study, plant growth was significantly inhibited by the high level of Se (Se2.5Zn0) in soil, and the biomass in pak choi shoot decreased by 40.4% compared with that in the control treatment. This finding was also verified in rape (Prins et al. 2011), rice (Boldrin et al. 2013), and maize (Longchamp et al. 2016), which caused by oxidative stress and/or the nonspecific replacement of S in proteins and other S compounds (Wang et al. 2019). Plant growth inhibition caused by high Se concentration was significantly alleviated with Zn supplements. Specifically, the biomass in shoot and root of pak choi in the addition of Zn significantly increased compared with that of the corresponding single high Se rate treatment (Table 2). Similar results also have been reported by Dai et al. (2019). Zn is an essential element in plants that is involved in the physiological processes of growth and metabolism and is regarded as a necessary co-factor of six classes of enzymes in plants, which include oxidoreductases (Mao et al. 2015). Therefore, these observations suggest that Zn may exert a Se detoxification effect at high Se doses.

This study found that Zn supply significantly increased the Se content in the root of pak choi exposed to exogenous Se treatments. Interestingly, the shoot Se content decreased with the increase in Zn compared with the single Se treatments (Fig. 1a). Da Silva et al. (2011) also reported similar results, which may be attributed to the following reasons. First, it may due to the biodilution (Keskinen et al. 2010), where in the biomass of the pak choi shoot was slightly increased (69.9%) with the application of 2.5 mg/kg of Se and 20 mg/kg of Zn compared with the single Se treatment (Se2.5Zn0) (Table 2), which induced a significant decrease (55.7%) of the Se content in shoot of pak choi in the corresponding concentration treatment (Fig. 1a). Second, Zn supply may inhibit the translocation of Se in pak choi. The O-acetylserine (OAS) levels are positively correlated with Zn hyperaccumulation in plants, and the expression of both ATP sulfurylase (ATPS) and APS reductase (APSR) was positively regulated by OAS in plants. Furthermore, the expressions of ATPS and APSR increase the reduction of Se from selenite to selenide (Na and Salt 2011). Selenite can easily be converted into organic Se species, such as selenocystine, Se-methyl-selenocysteine, selenomethionine, and selenomethionine Se-oxide. They are mostly accumulated in roots and are rarely transported to the shoots (Li et al. 2008). Third, Cd, Cu, Se, and Zn exposure increased the expression of sulfate transporters (Na and Salt 2011). In general, selenate enters the root of plants via S transport system (Wang et al. 2019). Accordingly, the expression of sulfate transporters enhances the ability of plant roots to absorb Se (Fig. 1a), thereby suggesting a positive correlation between the available Zn and Se contents of the soil in shoot of pak choi (Table S1). However, this observation was in contrast to a previous study that concluded that the soil application of Se (L-Selenomethionine) and Zn (EDTA-Zn) significantly ($p < 0.05$) increased Se/Zn accumulation in shoot and root of pak choi without affecting its formal growth (Dai et al. 2019). This discrepancy may be due to the different exogenous Se species: (1) different soil amendments, such as the addition of

EDTA chelates, which have been used to increase the availability of trace elements (Cu and Zn) to plant (Jalali and Khanlari 2008); and (2) the uptake of L-SeMet by the roots was higher than that of inorganic Se (Wang et al. 2019).

The co-amendment of selenate and Zn sulfate had no significant ($p > 0.05$) effect on the Zn content in the shoot and root of pak choi (Figs. 1b, e, and f). This finding is in agreement with many previous studies on rice (Fang et al. 2008; Manguoze et al. 2018). However, Xue et al. (2020) found that the combination of exogenous selenite and Zn sulfate markedly elevated the uptake and translocation of Zn in pak choi. The cause of this phenomenon is that selenite was feasibly adsorbed onto carbonate, Fe/Mn oxides, and organic matter in soil, which left selenite remains in the soil in turn (Qin et al. 2016). Therefore, selenite can be wrapped on the surface of soil particles through electrostatic adsorption, which inhibits the contact between soil particles and Zn, thereby reducing the fixation effect of soil on Zn and improving the bioavailability of Zn in the soil (Xue et al. 2020). This aspect needs to be confirmed by further investigation.

Mechanism of Se–Zn interaction that affects Se/Zn content in plants

Se–Zn co-amend treatments significantly ($p < 0.05$) increased the available Se/Zn content in the soil (Figs. 2a and b). Moreover, the available Se and Zn contents in soil were significantly and positively ($R^2 > 0.82$, $p < 0.01$) correlated with the Se and Zn content in the shoots and roots of pak choi, respectively (Table 2); such result is consistent with the previous findings (Qin et al. 2016; Wang et al. 2018; Xue et al. 2020). The bioavailability of Se and Zn in soil may be affected by the following factors: (1) Soil pH (Rengel 2015): In this study, compared with the single Zn treatment, Se–Zn co-amended treatments significantly ($p < 0.05$) reduced the soil pH by 0.19–0.55 (Table 2) and then increased the available Zn content of the soil by 0.12–70.7% (Fig. 2b), which coincided with the existing studies (Alloway 2009; Xu et al. 2020). Meanwhile, path analysis also indicated that pH negatively affected the available Zn content in soil and then affected the Zn content in the roots of pak choi (Table S1). Additionally, the pH of the soil treated with low Se rate (0.5 mg/kg) and Zn significantly ($p < 0.05$) increased by 0.46–0.53 compared with Se0.5Zn0 treatment (Table 2), and the de-protonated Se^{6+} was the main speciation of Se (Dinh et al. 2018), which increased the available Se content in soil (Fig. 2a) and uptake of Se in pak choi roots (Fig. 1a). Notably, Se (1 and 2.5 mg/kg)–Zn co-amended treatments decreased soil pH compared with that of the corresponding single Se treatment because of the rhizosphere acidification caused by root exudation (Dinh et al. 2018). Low molecular weight organic acids released by plant roots could dissolve and release stable Se in soil (Wang et al. 2018), and then the available Se content of soil with Se–Zn co-amended treatments increased by 9.6–41.6% compared with the corresponding single Se treatment (Fig. 2a). (2) Se–Zn interaction: Selenate could be reduced to selenite in the soil, which forms an immobile compound (ZnSeO_3) with Zn^{2+} (Feroci et al. 2005). To some extent, it reduced the bioavailability of Se and increased the bioavailability of Zn by reducing the precipitation effect of CaCO_3 on Zn in calcareous soil. (3) Root–Se–Zn interaction: The roots of plant can secrete low molecular weight organic acids with high affinity for metal species, which could form Se–organic acid complex (Dinh et al. 2018), Zn–organic acid complex (Zhao et al. 2018), or a ternary complexes (Se–metal–organic) combined by the metal bridge bonding of Se and humic acid (Dinh et al. 2018; Xue et al. 2020) and then ameliorated the bioavailability of Se and Zn in the soil (Figs. 1 and 2). (4) Microbe–Se–Zn: microorganisms oxidized organic matter, thereby releasing Zn bound to organic matter in the rhizosphere (Zhao et al. 2018). Moreover, microorganisms take up Se(VI) as terminal electron acceptors during respiration, which promoted the reduction of high-valent Se to low-valent Se and its combination with low molecules of humus (Wang et al. 2018), thereby increasing the potential available Se content.

In general, enzymes associated with nutrient cycling and energy flow are considered as the indicators of soil health (Tan et al. 2018). Invertase, urease, and alkaline phosphatase are involved in the C, N, and P cycles in soil, and catalase is an oxidoreductase that protects organisms from the toxicity of hydrogen peroxide (Hu et al. 2014; Li et al. 2012). In this study, the activities of these four enzymes were significantly ($p < 0.01$) affected by Se and Zn treatments in the soil (Fig. 3). The effect of Se/Zn on the activity of enzyme in soil are mainly discussed in the following ways: (1) Through complexation of the substrate (Hu et al. 2014); (2) by reacting with the enzyme–substrate complex (Chen et al. 2000); (3) by combining with the active groups of the enzyme (Das and Varma 2011); and (4) by influencing microbial activity, which synthesizes enzymes (Bin et al. 2013). In the present study, the activity of urease and alkaline phosphatase was promoted at low concentration and inhibited at high concentration with single Se/Zn treatment (Figs. 3b and c), which was regarded as hormesis. The hormetic dose response was highly generalizable, that is, it is independent of biological model, endpoint measured, and chemical agent (Calabrese and Baldwin 2003). Previous studies regarding the effect of single Se/Zn application on soil enzyme activity were also consistent with hormesis (Nowak et al. 2004; Yang et al. 2006). In addition, similar to the studies of Nowak et al. (2004) and Bin et al. (2013), single Se application had no significant ($p > 0.05$) effect on the activity of sucrase but significantly ($p < 0.05$) reduced the activity of catalase (Figs. 3a and d). No significant ($p > 0.05$) effect was

observed on the activity of sucrase and catalase with single Zn treatment (Figs. 3a and d). However, significant ($p < 0.05$) inhibition was observed on the activity of catalase of single Zn treatment (70–700 mg Zn /kg) by Yang et al. (2006), indicating that the effect of exogenous Se and Zn on the activity of soil enzyme was related to the content of Se and Zn.

Compared with single Se/Zn treatment, Se–Zn co-amended treatments significantly ($p < 0.05$) increased the activity of invertase and alkaline phosphatase in soil but inhibited the activity of urease (Fig. 3). Thus, it improved the soluble nutrient content in soil, the ability of soil to release simple sugars, the decomposition and conversion rate of organophosphorus, and reduced the transformation capability of the organic nitrogen into inorganic nitrogen (Ciarkowska et al. 2014). Monosaccharides were the main energy source of soil microorganisms (Ciarkowska et al. 2014). The increase in the activity of sucrase indicated that the microbial activity was improved, which promoted biological oxidation and released Zn related to the organic matter in rhizosphere soil (Zhao et al. 2018). Therefore, the activity of sucrase was significantly positively correlated with the available Zn content in soil (Table 3). The increase in the activity of alkaline phosphatase enhanced the mineralization of soil organophosphates (Zhang et al. 2013), and the generated inorganic phosphorus compounds may form competitive adsorption with Se (Dinh et al. 2018). In turn, it will affect the Se content in the shoots and roots of pak choi (Table 4). Organic nitrogen in soil can be hydrolyzed to NH_3 and CO_2 by urease with the increase in soil pH (Das and Varma 2011). This finding was consistent with the conclusion of this study that the activity of urease was significantly positively correlated with soil pH. Thus, the significant ($p < 0.05$) negative correlation between the content of Zn in shoots and roots of pak choi, available Zn content in soil, and the activity of urease can be attributed to the relationship between urease and soil pH (Table 3). As for catalase, only at the same Zn application rate, application of Se significantly ($p < 0.05$) inhibited the activity of catalase compared with single Zn treatment (Fig. d). This phenomenon may be due to the exogenous water-soluble Se can complicate the enzymatic substrate or mask the catalytic active group, thereby leading to the disintegration of the enzyme–substrate complex and reducing the reaction rate of the enzyme (Gianfreda et al. 2005; Nowak et al. 2002). Catalase is an intracellular enzyme involved in the metabolism of microbial oxidoreductase (Bin et al. 2013). Therefore, the reduction of the catalase activity could affect the oxidation-reduction of microorganisms, which in turn influences the form and valence of Se. Meanwhile, the form and valence of Se in soil are closely related to the bioavailability of Se in soil (Dinh et al. 2018). This finding also explains that catalase can indirectly affect the Se content of roots in pak choi by negatively affecting the available Se content in the soil in this study (Table S1).

Conclusion

In the present study, although Se stress significantly inhibited plant growth compared with the control treatment, the inhibition of plant growth at high Se rate was completely alleviated by Zn. More importantly, Se combined with Zn amendment significantly increased the available soil Se/Zn content compared with the single Se/Zn treatment, and in turn increased the uptake of Se of roots in pak choi. No significant difference was observed in the Zn content of shoots and roots in pak choi among single Se/Zn with their added combination. Notably, Se–Zn co-amendment can regulate the pH and enzyme activity of soil, and then affected the bioavailability of Se/Zn in soil. For example, the increase in the available Zn content could be attributed to the significant decrease in the pH of the soil treated with Se–Zn co-amendment compared with single Zn treatments. The activity of catalase, urease, and alkaline phosphatase could induce changes in the soil available Se/Zn affecting Se/Zn uptake by pak choi. Therefore, soil amendment of Se and Zn sulfate can be used as a potential strategy for the co-biofortification of Se and Zn.

Declarations

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

Figure 1

Se (a) and Zn (b) content in shoot and root of pak choi with Se and Zn applications. Error bars represent the standard error of the means (n = 3). Different upper-case letters indicate the significant differences (p < 0.05) among different Zn treatments with the same Se application concentration, and different lower-case letters indicate the significant differences (p < 0.05) among different Se treatments with the same Zn application concentration. Two-way ANOVA results (F value) for two groups (a: Se; b: Zn) are presented in c (shoot Se), d (root Se), e (shoot Zn), and f (root Zn), * and ** represent p < 0.05 and p < 0.01, respectively.

Figure 2

Available Se (a) and Zn (b) content of soil with or without Se application. Two-way ANOVA results (F value) for two groups (a: Se; b: Zn) are presented in c (available Se) and (available Zn)), * and ** represent p < 0.05 and p < 0.01, respectively.

Figure 3

Soil enzyme activities (invertase (a), urease (b), alkaline phosphatase (c), catalase (d)) of soil with or without Se and Zn applications. Two-way ANOVA results (F value) for two groups (a: Se; b: Zn) are presented in e (invertase), f (urease), g (alkaline phosphatase) and h (catalase), * and ** represent $p < 0.05$ and $p < 0.01$, respectively.

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