Transformation of heavy metal fractions on soil urease and nitrate reductase activities in copper and selenium co-contaminated soil

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A B S T R A C T

This study aims to explore the effects of the distribution, transformation and bioavailability of different fractions of copper (Cu) and selenium (Se) in co-contaminated soils on soil enzymes, providing references for the phytoremediation of contaminated areas and agriculture environmental protection. Pot experiments and laboratory analysis were used to investigate the transformation and bioavailability of additional Cu and Se for pakchoi (Brassica chinensis) in co-contaminated soil. In the uncontaminated soil, Cu mainly existed in residual form, whereas Se was present in residual form and in elemental and organic-sulfide matter-bound form. In the contaminated soil, Cu mainly bound to Fe–Mn oxides, whereas Se was in exchangeable and carbonates forms. After a month of pakchoi growth, Cu tended to transfer into organic matter-bound fractions, whereas Se tended to bound to Fe–Mn oxides. The I_R (reduced partition index) value of Cu decreased as the concentrations of Cu and Se gradually increased, whereas the I_R value of Se decreased as the concentration of Se increased. The I_R value before pakchoi planting and after it was harvested was not affected by the concentration of exogenous Cu. Soil urease and nitrate reductase activities were inhibited by Cu and Se pollution either individually or combined in different degrees, following the order nitrate reductase > urease. The significant correlation between the I_R value and soil enzyme activities suggests that this value could be used to evaluate the bioavailability of heavy metals in soil. Path analysis showed that the variations in exchangeable Cu and organic-sulfide matter-bound and elemental Se had direct effects on the activities of the two enzymes, suggesting their high bioavailability. Therefore, the I_R value and the transformation of metals in soil could be used as indicators in evaluating the bioavailability of heavy metals.

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1. Introduction

Heavy metals are common environmental pollutants that not only exist individually but also co-exist with other contaminants; thus, the combined effects of heavy metals have attracted much attention in recent decades (Bayramoglu et al., 2012; Santorufo et al., 2012). The adsorption, desorption, and coordination among different heavy metals can affect their distribution, bioavailability and toxicity in soil (Wang et al., 2009a). Therefore, the effects of combined pollution depend on the constituents of the mixture and may vary significantly (Jensen and Sverdrup, 2002).

Copper (Cu), as an enzymatic cofactor in several metabolic processes, is an essential trace element for crop growth at low concentrations and a common soil contaminant (Santorufo et al., 2012). In recent years, copper mining, the widespread use of feed additives, fungicides, and organic fertilizers, irrigations, and urban sewage sludge compost utilization have worsened Cu pollution (Susanne et al., 2007; Gonzalez et al., 2011; Wang et al., 2012; Zhang et al., 2012). Selenium (Se), an essential metalloid trace element for organisms, is associated with Keshena and Kashin–Beck diseases (Semnani et al., 2010). This element may be released through wet and dry deposition (Gregori et al., 2002). According to a survey, the concentration of Se in wastewater surrounding Cu ores is beyond the acceptable levels because the content of Se in mining area soil is far beyond the background value (Gregori et al., 2000). Meanwhile, suspended solids in atmospheres near mining areas contain large amounts of Se, which enter the soil through wet and dry deposition (Gregori et al., 2002; Semnani et al., 2010). Soils around coal mines and power plants are heavily contaminated with Se, with 50 times higher than the background (Huang et al., 2009a).

The mobility and bioavailability of heavy metals depend not only on their total contents but also on their physical and chemical forms (Wang et al., 2009b). Therefore, the study of heavy metal
fractions is significant. Sequential extraction, although operationally defined, can give information about the association of heavy metals with the geochemical phases of soil. Thus, sequential extraction is widely used to reveal the distribution of heavy metals in fractions and to assess the mobility and toxicity of metals in soil (Guo et al., 2011).

Soil enzymes participate in biochemical reactions, material recycling, and energy metabolism; enzyme activity reflects the direction and strength of biochemical processes in soil (Yang et al., 2006). Given that soil enzyme activities are sensitive to heavy metal contamination (Chen et al., 2005); they are widely used as biological indicators for estimating the adverse effects of various pollutants on soil quality (Gao et al., 2010a). Single Cu and single Se pollution studies showed that nitrate reductase and urease activities could be used as sensitive indicators for Cu or Se pollution (Margésin et al., 2000; Nowak et al., 2002; Arora et al., 2010).

Clay surfaces, which specifically bind heavy metals and interact with soil enzymes, may also have mutual effects (Gao et al., 2010a,b). Simple correlation analysis and multiple stepwise regressions are widely used in studying the relationship between chemical fractions and soil enzyme activities (Chen et al., 2005; Yang et al., 2006; Gao et al., 2010b). Considering the good correlations between these two factors, soluble and exchangeable metals (e.g., Cd, Cu, and Zn) are considered as the most bioavailable and toxic fractions in soil (Yang et al., 2006; Huang et al., 2009b). However, single correlation analysis simply determines the relationship between two variables (Yap et al., 2010); and multiple stepwise regressions only analyze the main effect on dependent variables (Bernstein et al., 1988). Accordingly, these two methods ignore the inner link of variables, and could not reflect the relationship between dependent variable and independent variable. Path analysis can distinguish the correlation between predictor and response variables into both direct and indirect effects, exploring the causal relationships and the direction of causal path (Fjeld et al., 1994). Thus, path analysis could directly reflect the influences on soil enzyme activity, considering the interaction between different fractions.

Combined heavy metal pollutions between metal cations such as Cu and Cd; Cu, Pb; and Cd; and Pb and Cd were studied, which showed that Cu exist antagonism effects with Pb and Cd (Huang et al., 2009b; Gao et al., 2010b). But the joint-effects between Cu and anion were less reported. Varying degrees of antagonism or detoxification between Se and Pb, Pb, As, and Hg were also reported (Rosen and Liu, 2009). However, little is known regarding the combined pollution effects of Cu and Se. In soil, the main absorbed forms of Cu and Se are Cu$^{2+}$ and SeO$_4^{2-}$, respectively (Banuelos and Lin, 2005). The co-existence of Cu and Se in soil may produce different ecological effects on their mobility, bioavailability, and toxicity. Many studies reported on heavy metal fractions in polluted soil and even heavy metal aging progress in soil (Han et al., 2004; Jalali and Khanlari, 2008; Arora et al., 2010; Guo et al., 2011). To the best of our knowledge, only a few studies investigated the transformation of heavy metal fractions in soil during the entire growth season and the relationship between fraction transformation and soil enzyme activity (Han and Banin, 1997; Wang et al., 2009a).

Accordingly, this study has the following objectives: (1) to confirm the fractions and transformation of Cu and Se in polluted soil before and after planting; (2) to evaluate the effects of urease and nitrate reductase under combined pollution; and (3) to explore the relationship between metal fraction transformation and soil enzyme activities.

2. Materials and methods

2.1. Experimental materials

All reagents used in this study were of analytical grade. The tested forms of Cu and Se were CuSO$_4$ and Na$_2$SeO$_3$, respectively. Seeds of pakchoi (Brassica chinensis L, Qinbai no. 2) were provided by Northwest A&F University Seeds Co. Ltd., Shaanxi, China. Non-contaminated soil classified as cinnamon soil was collected at a depth of 0–20 cm from the Northwest A&F University (34°16′ N, 108°04′ E) farm in Shaanxi Province, China. The basic physicochemical properties of the soil are as follows: pH 7.95, 16.47 g kg$^{-1}$ organic matter content, 39.5 percent clay, 23.41 cmol kg$^{-1}$ cation exchange capacity, 35.6 percent calcium carbonate content, 1.2 mg kg$^{-1}$ amorphous iron content, 26.56 mg kg$^{-1}$ total Cu content, and 0.221 mg kg$^{-1}$ total Se content. These soil properties were determined according to the methods described by Bao (2000). Soil pH was determined in water extracts using a soil-to-solution ratio of 1:2.5. Organic matter content was measured with hot K$_2$Cr$_2$O$_7$ oxidation and FeSO$_4$ titration. Cation exchange capacity was conducted using the NH$_4$OAC method, and the total content of soil heavy metal elements was determined using atomic absorption spectroscopy and atomic fluorescence spectroscopy after digestion with aqua regia.

2.2. Experimental design

Soil was air-dried at room temperature, homogenized, and allowed to pass through a 5 mm sieve. Chemical fertilizers, including 100 mg kg$^{-1}$ N (urea), 75 mg kg$^{-1}$ P$_2$O$_5$ (calcium superphosphate), and 75 mg kg$^{-1}$ K$_2$O (potassium chloride), were mixed thoroughly with 1.0 kg air-dried soil (bulk soil) in plastic pots (10 cm in height and 15 cm in diameter). The soil samples were spiked with four Cu$^{2+}$ concentrations (0, 200, 400, and 800 mg kg$^{-1}$ soil; added as CuSO$_4$) and four Se$^{4+}$ concentrations (0.2, 2.5, 10, and 20 mg kg$^{-1}$ soil; added as Na$_2$SeO$_3$). The experiment adopted two-factor completely randomized designs, including a total of 16 treatments (Table 1). All treatments were with four replicates. The resulting soil mixtures were equilibrated for 14 d at 25 °C and 50 percent humidity. Ten pakchoi seeds were sown in each pot, and the seedlings were thinned to five in each pot after 10 d. The soil moisture content was kept at 70 percent water holding capacity (18 percent) by quantitative watering once every 2 d. The plants were harvested after 30 d.

2.3. Soil sampling and analyses

Soil samples were collected from all pots before planting and after harvest. The samples were air-dried and ground. Part of them were sieved to <2 mm for analysis of soil enzyme activity, and the other parts were ground to pass through a mesh nylon sieve (0.15 mm) for chemical analyses of soil Cu and Se concentrations. Urease activity was measured by mixing 5 g air-dried soil samples, 1 mL methylbenzene, 10 mL urea solution (10 percent), and 20 mL citrate buffer (pH 6.7) in a reaction flask. The mixture was incubated for 24 h at 37 °C. The indophenols colorimetric method was used to measure the NH$_4^+$ released by the enzyme enzymatic hydrolysis of urea. The indophenols were colorimetrically determined at 578 nm. The activity was expressed as mg NH$_4^+$ N kg$^{-1}$ h$^{-1}$ (Guan 1986).

Nitrate reductase activity was measured by mixing 1 g soil samples, 1 mL 0.8 mmol L$^{-1}$ 2,4-dinitrophenol solution, 1 mL 0.05 percent KNO$_3$ solution, 1 mL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg kg$^{-1}$)</th>
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<tr>
<td>Cu</td>
<td>Se</td>
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<tr>
<td>CK</td>
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<tr>
<td>Cu$_{800}$</td>
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</tr>
<tr>
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<tr>
<td>Se$_{2.5}$</td>
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<td>Se$_{10}$</td>
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<td>Se$_{2.5}$</td>
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<td>Se$_{10}$</td>
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<td>0</td>
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<tr>
<td>Se$_{2.5}$</td>
<td>10</td>
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<tr>
<td>Se$_{10}$</td>
<td>20</td>
</tr>
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</table>

Table 1
The design and metal contents of single combined treatments.
one percent glucose solution, and 7 mL anaerobic deionized water. After incubation for 24 h at 30 °C, the mixture was filtered and determined by colorimetry at a wavelength of 520 nm. The activity was expressed as mg NO$_2$–N kg$^{-1}$ d$^{-1}$ (Nowak et al., 2002).

Control tests without soils or substrates were carried out to evaluate the spontaneous or abiotic transformation of substrates in all the enzyme activities analyzed. The results were expressed as an average of three replicates in the analysis for the soil samples.

2.4. Cu and Se fractions analyses

The soil Cu and Se fractions were analyzed using the methods proposed by Tessier et al. (1979), Chen et al. (2010) and Qu et al. (1997), respectively (Table 2). The extracts were determined by adding one gram of soil into a 100 mL centrifuge tube. The tube was shaken and then centrifuged at 4000 rpm for 10 min. Cu and Se were determined by atomic absorption spectroscopy and atomic fluorescence spectroscopy, respectively (Bao, 2000).

2.5. Statistical analyses

All results were reported as the mean of four replicates, and all data were subjected to one-way ANOVA using SPSS 13.0 statistical software. The least significant difference test was used to detect significant differences among the means of the different treatments ($p < 0.05$). Path analysis was achieved by Amos18.0 under the scale-free least square method.

The parameter $I_R$ (reduced partition index) was used to describe the relative binding intensity and fractional redistribution of heavy metal in soils according to Han [Han and Banin, 1997; Han et al., 2004]:

$$ I_R = \frac{\sum f_i \times \bar{p}_i}{25} $$

where $f_i$ is the fractional percentage content of heavy metal in soil and $i$ is the extraction step number ($i = 1, 2, 3 \ldots$). $I_R$ was introduced to describe quantitatively the relative binding intensity of heavy metal in soils. If the total heavy metal in soil resides in exchangeable fraction, the value of $I_R$ has the minimum value (0.04); if it resides in residual fraction, the value of $I_R$ has the maximum value (1).

3. Results

3.1. Transformation of Cu fractions

The changes in the Cu fractions in contaminated soils before planting and after harvest exhibited the same trend (Fig. 1a and b). In the single Cu-polluted soil, Cu was dominantly the most stable species in residual fraction (> 95 percent). However, in combined pollution soils, Cu mainly presented in exchangeable fraction (16–29 percent), Fe–Mn oxide-bound fraction (26–35 percent), and residual fraction (32–53 percent). The percentages of exchangeable fraction and Fe–Mn oxide-bound fraction that occupied total Cu were higher in the combined treatments than in the single Cu pollution. These percentages increased with increasing soil Cu and Se concentrations. By contrast, the organic matter-bound Cu and residual Cu fraction showed an opposite trend.

The five Cu fractions in soil were compared before planting and after harvest. The organic matter-bound Cu and the Fe–Mn oxide-bound Cu increased by 1.6–7.1 times and 12–63 percent, respectively, whereas the exchangeable Cu decreased by approximately 30–59 percent. This result shows that the exogenous Cu in soil transformed into Fe–Mn oxide-bound Cu and organic matter-bound Cu with time and plant absorption. The exogenous Se in soil could enhance this transformation.

3.2. Transformation of Se fractions

In both single Se and combined soil pollution, Se mainly existed as exchangeable and carbonate-bound Se with a percentage 51–71 percent. No differences were observed among the other four fractions. The percentages of soluble Se, exchangeable and carbonate-bound Se, and Fe–Mn oxide-bound Se increased with both Se and Cu concentrations. By contrast, the residual Se and the organic-sulfur matter and elemental Se decreased (Fig. 1c and d). The five fractions in soil were compared before planting and after harvest. The soluble Se and the organic sulfide matter and elemental Se decreased by 7–67 percent and 4–82 percent, respectively, whereas the Fe–Mn oxide Se and the exchangeable and carbonate-bound Se increased by 60–191 percent and 9–16 percent, respectively. These results indicate that the exogenous Se in soil transformed into exchangeable Se and bound to carbonates and Fe–Mn oxidates with time and plant absorption. The exogenous Cu in soil could enhance this transformation.

3.3. $I_R$ value of Cu and Se in soil before planting and after harvest

The $I_R$ value could reflect the bioavailability to some degree. A low $I_R$ value suggests that the heavy metals exist as unstable fractions (e.g., exchangeable), and a high $I_R$ value suggests that the heavy metals exist mainly in stable fractions. The $I_R$ value of Cu in soils before planting and after harvest exhibited the same trend (Fig. 2a and b): the native (non-amended) soil had the highest $I_R$ value of Cu (0.97 and 0.91). The $I_R$ value of Cu decreased with increasing soil Cu concentration, and the largest decline reached up to 54.6 percent for Cu$_{1000}$. When Cu concentration was the same, the $I_R$ value of Cu decreased with increasing Se concentration. The native (non-amended) soil had the highest $I_R$ value of Se (0.57–0.66) (Fig. 2c and d). The $I_R$ value of Se decreased with increasing soil Se concentration, and the largest decline was up to 71.3 percent for Se$_{100}$. The $I_R$ value varied with Cu concentrations in

Table 2

Sequential extraction methods for copper and selenium fractions.

<table>
<thead>
<tr>
<th>Name</th>
<th>Cu Fractions</th>
<th>Analysis methods</th>
<th>Se Fractions</th>
<th>Analysis methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_1$ (EX-Cu)</td>
<td>0.1 mol L$^{-1}$ NH$_4$HAC 25 °C, shaken for 2 h, liquid/soil$=10:1$</td>
<td>Soluble (SOL-Se)</td>
<td>0.25 M KCl 25 °C, shaken for 1 h, liquid/soil$=10:1$</td>
<td></td>
</tr>
<tr>
<td>$F_2$ (CAB-Cu)</td>
<td>1 mol L$^{-1}$ NaCl 25 °C, shaken for 2 h, liquid/soil$=10:1$</td>
<td>Exchangeable and bound to carbonates (EXC-Se)</td>
<td>0.7 M KH$_2$PO$_4$ (pH 5.0), 25 °C, shaken for 4 h, liquid/soil$=10:1$</td>
<td></td>
</tr>
<tr>
<td>$F_3$ (FEM-Cu)</td>
<td>0.1 mol L$^{-1}$ HNO$_3$ + 0.01 mol L$^{-1}$ HCl 25 °C, shaken for 0.5 h, liquid/soil$=10:1$</td>
<td>Bound to Fe–Mn oxides (FEM-Se)</td>
<td>2.5 M HCl, heated for 50 min in bath at 90 °C, shaken intermittently, liquid/soil$=10:1$</td>
<td></td>
</tr>
<tr>
<td>$F_4$ (OM-Cu)</td>
<td>0.01 mol L$^{-1}$ HNO$_3$, 30% H$_2$O$_2$ heated for 2 h in 85 °C, shaken intermittently, liquid/soil$=10:1$</td>
<td>Bound to organic–sulfide matter and elemental (OM-Se)</td>
<td>5% K$_3$C$_6$H$_5$O$_7$ 8 mL and (1:1) HNO$_3$ 2 mL, heated for 3 h in bath at 95 °C, shaken intermittently, liquid/soil$=10:1$</td>
<td></td>
</tr>
<tr>
<td>$F_5$ (RES-Cu)</td>
<td>15 mL HNO$_3$, 5 mL HF, 5 mL HClO$_4$ heated to 300 °C for 2 h, until the solutions become clear</td>
<td>Residual (RES-Se)</td>
<td>10 mL HNO$_3$ and HClO$_4$ (4:1), heated to 150–165 °C for 2 h, until the solutions become clear</td>
<td></td>
</tr>
</tbody>
</table>
soil: Cu800 could inhibit the $I_R$ value of Se before planting, whereas the Se $I_R$ value remained constant after harvest. When soil Se concentration was less than 10 mg kg$^{-1}$, the $I_R$ value of Se peaked at Cu400. When Se concentration was 20 mg kg$^{-1}$, the $I_R$ value of Se decreased with increasing Cu concentration.

### 3.4. Combined effects of Cu and Se on soil enzyme activity

#### 3.4.1. Urease activity

Compared with the control, single Cu treatments of 200, 400, and 800 mg kg$^{-1}$ significantly reduced urease activity by 31.5...
percent, 38.1 percent, and 59.6 percent, respectively ($p < 0.05$). No significant inhibition was found in single Se pollution. Urease activity decreased by 4.4 percent, 5.4 percent, and 3.6 percent for Se 2.5, 10, and 20 mg kg$^{-1}$ treatment, respectively (Fig. 3a).

For the combined treatments, the inhibition rate of urease activity increased with the exogenous Cu concentrations under the same Se concentrations. The maximum inhibition rate was 60.0 percent for the Cu$^{800} + $Se$^{2.5}$ treatment. When Cu concentration was less than 400 mg kg$^{-1}$, soil urease activity initially increased with exogenous Se concentration and then decreased, reaching a peak at Se 2.5 mg kg$^{-1}$. When Cu concentration was 800 mg kg$^{-1}$, the highest urease activity was reached at the Se 20 mg kg$^{-1}$ treatment. The inhibition rates of urease activity were slightly higher in soil after harvest than before planting with the same trend (Fig. 3b).

3.4.2. Nitrate reductase activity

As presented in Fig. 3c, both single and combined pollution strongly inhibited soil nitrate reductase activity. At 200, 400, and 800 mg kg$^{-1}$ Cu treatments, the soil nitrate reductase activity in single Cu pollution significantly declined by 26.5 percent, 69.3 percent, and 86.1 percent, respectively, compared with control ($p < 0.05$). At 2.5, 10, and 20 mg kg$^{-1}$ treatments, the soil nitrate reductase activity in single Se pollution decreased by 10.2 percent, 20.7 percent, and 34.9 percent, respectively ($p < 0.05$).

For the Cu and Se combined pollution treatments, the inhibition rate of soil nitrate reductase activity increased with increasing exogenous Cu concentration under the same Se concentration. The maximum inhibition rate was 89.4 percent for the Cu$^{800} + $Se$^{20}$ treatment. When the Cu concentrations were same, nitrate reductase activity decreased after the initial upward trend, with the highest at the Se$^{2.5}$ and Se$^{10}$ treatments. Nitrate reductase activity in soil after harvest showed slightly higher inhibition rates than which in the soil before planting with the same trend (Fig. 3d).

3.5. Effects of Cu and Se fractions on soil enzyme activity

3.5.1. Correlation between the different Cu and Se fractions and soil enzyme activity

The relationship between heavy metal fractions and soil enzyme activities was studied by some researchers (Margesin et al., 2000; Chen et al., 2005; Gao et al., 2010a,b). However, most studies ignored the variation of heavy metal content or soil enzyme activity during the entire plant growth period. In this study, we considered the changes in five fractions of heavy metal and soil enzyme activity in the correlation analysis. The changes in each indicator were the values before planting minus the values after harvest (Table 3).

The variation in urease activity was significantly correlated with the variation in each Cu fraction. Nitrate reductase activity was significantly correlated with exchangeable Cu, organic matter-bound Cu, and total Cu content. It also showed a significant relationship with organic sulfide matter-bound and elemental Se and total Se content. The variation in the $I_R$ value had a significant correlation with the variations in urease and nitrate reductase activities during the plant growth period, suggesting that the variation in the $I_R$ value could indicate the variation in soil enzyme activity.

3.5.2. Path analysis between Cu/Se fractions and soil enzyme activity

The relationship of the variation in soil Cu and Se fractions with soil urease and nitrate reductase activities determined by path analysis on Amos 18.0 is presented in Fig. 4. The values are standardizations with weight coefficients, representing relatively positive or negative effects. The significant correlation between two variables was connected by an arrow ($p < 0.05$). Carbonate-bound Cu and exchangeable Se were ignored because of their insignificant coefficients.
Table 3
Correlation between fractions and soil enzyme activity

<table>
<thead>
<tr>
<th></th>
<th>$\Delta F_1$</th>
<th>$\Delta F_2$</th>
<th>$\Delta F_3$</th>
<th>$\Delta F_4$</th>
<th>$\Delta F_5$</th>
<th>$\Delta T$</th>
<th>$\Delta I_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu Urease</td>
<td>0.931*</td>
<td>–0.812**</td>
<td>–0.845**</td>
<td>–0.893**</td>
<td>0.887**</td>
<td>0.941**</td>
<td>0.809**</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>–0.534</td>
<td>0.302</td>
<td>0.391</td>
<td>0.645**</td>
<td>–0.474</td>
<td>–0.533</td>
<td>–0.532</td>
</tr>
<tr>
<td>Se Urease</td>
<td>0.081</td>
<td>–0.111</td>
<td>–0.395</td>
<td>–0.347</td>
<td>0.037</td>
<td>–0.268</td>
<td>–0.513</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>0.223</td>
<td>0.372</td>
<td>–0.079</td>
<td>0.604</td>
<td>0.224</td>
<td>0.541</td>
<td>0.562</td>
</tr>
</tbody>
</table>

$\Delta F_{1-5}$ means the variation of each fraction between before plant and after harvest; $\Delta T$ and $\Delta I_r$ means the variation of total Cu, Se and I_r before plant and after harvest;

* $p < 0.05$.
** $p < 0.01$, $n = 16$.

Fig. 4. Effects of different fractions on soil urease and nitrate reductase activities.

Note: All the values are standardizations with weight coefficients, and the significant correlation was connected by an arrow ($p < 0.05$). The positive or negative values represent relatively positive or negative effects. EX: exchangeable; FEM: bound to Fe–Mn oxidates; OM: bound to organic matter; Tot: total concentration.

A negative effect was observed between exchangeable Cu content and soil urease activity, with a path coefficient of –0.90. By contrast, organic matter-bound Cu and exchangeable Cu showed a positive effect on nitrate reductase activity, with a path coefficient of 2.50 or 3.12. The total Cu content and Fe–Mn oxidate Cu independently affected the activities of these two enzymes through the contents of exchangeable Cu and organic matter-bound Cu. The contents of Fe–Mn oxidate Se and organic sulfide matter-bound and elemental Se exhibited a negative effect on urease activity (–0.41 and –0.45), whereas the content of organic sulfide matter-bound and elemental Se had a positive effect on nitrate reductase activity (0.56). The total Se and soluble Se independently affected the activities of these two enzymes through Fe–Mn oxidate Se and organic sulfide matter-bound and elemental Se.

4. Discussions

The fractions of heavy metals in soil are influenced by pH, redox conditions, organic matter content, and microorganisms (Chen et al., 2010). This study found that exogenous single Cu mainly bound to Fe–Mn oxidates, whereas exogenous single Se existed in exchangeable and carbonate forms after entering the soil (Fig. 1). Similar results were also obtained in other studies. Luo et al. (2003) found that Fe–Mn oxidate Cu is the dominant fraction in Cu-polluted soil, whereas residual Cu is dormant in unpolluted soil. Guo et al., 2011 also found that Cu exists as residual and reducible fractions in acid mine drainage soil. This result may be attributed to the fact that Cu does not exist as ion in soil; most Cu is adsorbed by iron and aluminum oxides in Cu$^{2+}$–O–Fe$^{3+}$ or Cu–O–Al forms (Banuelos and Lin, 2005). It may also due to the tested soil in this study was low in organic carbon, but high in clay-size fraction and Fe–Mn oxides (in Section 2.1). By contrast, selenite is much more strongly adsorbed on the adsorbing surfaces of soils (Hawkesford and Zhao, 2007). Thus, Se mainly exists in exchangeable and carbonate-bound form.

In this study, the soil content of Fe–Mn oxidate Cu after harvest increased by 12–33 percent compared with that before planting, whereas the soil content of organic matter Cu after harvest was 1.6– to 7.1-fold that before planting (Fig. 1a and b). During the entire growth season of pakchoi, exchangeable Se content decreased by 9–16 percent and Fe–Mn oxidate Se content increased by 60–191 percent (Fig. 1c and d). This result may be attributed to the exogenous heavy metals that were adsorbed on the surface of soil solid as the exchangeable form rapidly entered the soil environment and then slowly transformed into other stable forms with time (Jalali and Khanlari, 2008). The distribution of added metals into different solid phase fractions is a multi-step process (Tang et al., 2006). The first step is initiated by the rapid adsorption to soil surfaces by forming outer sphere complexes driven by the difference in the concentration gradient from the solution phase to the surface of soil minerals and negatively charged organic matter. The slow continuing sorption is due to the diffusive penetration from its outer sphere to its inner sphere (such as the surface of carbonates, Fe–Mn oxides, and other residuals) (Tang et al., 2006). Many studies also found that the added Cu and Se transformed from soluble and exchangeable to stable fractions with time (e.g., Fe–Mn oxidate-bound Cu and Se) (Lu et al., 2009).

Heavy metals in soils can inhibit enzyme activity through many pathways: (1) by reducing the production of enzymes through
their toxic effects on soil microflora; (2) by combining with the active protein groups of enzymes; (3) through complexation of the substrate; and (4) by reacting with the enzyme–substrate complex (Wang et al., 2009b). However, different metals affect soil enzymes in different ways. In the present study, the activity of soil nitrate reductase diminished with increasing amended Cu or Se concentrations. This finding is in agreement with an earlier report that soil enzyme activities diminish with increasing available concentrations of Cu and Se (Nowak et al., 2002; Kızılkaya et al., 2004). Moreover, soil urease activity was also inhibited with increasing Cu concentration, but no significant inhibition was observed in the Se-amended soil. The significant inhibitory effects of Cu on soil urease activity could be explained by the theories mentioned above (Fig. 3), whereas the insignificant effects of Se on it may be attributed to the fact that Se could replace sulfur in the unstable active centers of these enzymes (Fig. 3). Thus, the varied speeds or degrees of disintegration of the enzyme substrate complex may cause different effects on enzymatic reactions (Nowak et al., 2002).

Han and Banin (1997) first provided the relative binding intensity (Ig), which was used in single heavy metal pollution (Han et al., 2004). A low Ig value suggests higher bioavailability of metals in soil. In this experiment, the Ig value decreased with increasing Cu and Se concentrations (Fig. 2). The reason for this result is that heavy metals are fixed in solid phase in natural soil, making them difficult to be released. However, when high concentrations of exogenous heavy metals enter the soil, most of them initially exist in unstable forms (Han et al., 2004). The bioavailability of heavy metals is related not only to the fractions but also to the plant uptake, soil microorganism, and enzyme activity (Han et al., 2004). Although the Ig value was provided as an indicator of bioavailability, insufficient evidence was provided. Previous studies on Ig value paid much attention to the fractions and distribution of metals, but the relationship between Ig value and soil enzyme or plant uptake was ignored (Han and Banin, 1997; Han et al., 2004). In this study, significant correlations between Ig values and urease or nitrate reductase activity were observed, which indicated that the Ig value could be used as an indicator of soil enzyme activities.

In this study, the good correlation between the variation in each fraction of heavy metals and soil enzyme activity (Table 3) confirmed the high bioavailability of soluble, exchangeable, and organic matter-bound heavy metals. Path analysis (Fig. 4) also showed that the contents of exchangeable Cu and organic matter-bound Se directly affected soil urease and nitrate reductase activities, suggesting the higher bioavailability or toxicity of the two fractions. The extractable fraction of heavy metals is considered a more bioavailable fraction of metals in soil (Wang et al., 2009a); thus, the concentration of this fraction is usually used for ecologic risk assessment (Nagel et al., 2003). Soil enzyme activities have significant relation with organic matters, which could improve bioavailability of Se in soil (Susanne et al., 2007). So this could explain why these two fractions have directly effects on soil enzyme activities. However, further research is needed to improve current understanding of the mechanisms underlying the different concentrations of Cu and Se in soils.

5. Conclusions

Cu mainly bound to Fe–Mn oxides, whereas Se was present in exchangeable and carbonate forms before planting. After a month of pakchoi growth, Cu tended to transfer into organic matter-bound fractions, whereas Se tended to bind to Fe–Mn oxides. The Ig value of Cu decreased as the concentrations of Cu and Se gradually increased, whereas the Ig value of Se decreased as the concentration of Se increased. Soil urease and nitrate reductase activities were inhibited by Cu and Se pollution either individually or combined in different degrees, and they exhibited a good correlation with Ig value. Correlation and path analyses showed that exchangeable Cu and organic matter-bound Se have direct effects on the activities of the two enzymes. Therefore, the Ig value and the transformation of metals fraction in soil could be used to evaluate the bioavailability of heavy metals.

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References


