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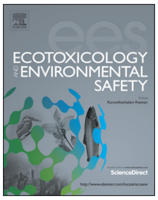

Transformation of heavy metal fractions on soil urease and nitrate reductase activities in copper and selenium co-contaminated soil

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ABSTRACT

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 oxidates, 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 oxidates. 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.

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 in the environment by fossil-fuel combustion and industrial, agricultural, and metallurgical processes, especially from mining activities of sulfide ores (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

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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 (Margesin 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, Cd, 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_3^{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_2SeO_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 $K_2Cr_2O_4$ oxidation and $FeSO_4$ titration. Cation exchange capacity was conducted using the NH_4OAC 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_2O_5 (calcium superphosphate), and 75 mg kg^{-1} K_2O (potassium chlorine), 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, 2.5, 10, and 20 mg kg^{-1} soil; added as Na_2SeO_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 sowed 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 urease enzymatic hydrolysis of urea. The indophenols were calorimetrically determined at 578 nm. The activity was expressed as mg NH_3–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 1
The design and metal contents of single combined treatments.

Treatment	Concentration (mg kg^{-1})		Treatment	Concentration (mg kg^{-1})		Treatment	Concentration (mg kg^{-1})		Treatment	Concentration (mg kg^{-1})	
	Cu	Se		Cu	Se		Cu	Se		Cu	Se
CK	0	0	$Se_{2.5}$	0	2.5	Se_{10}	0	10	Se_{20}	0	20
Cu_{200}	200	0	$Cu_{200}Se_{2.5}$	200	2.5	$Cu_{200}Se_{10}$	200	10	$Cu_{200}Se_{20}$	200	20
Cu_{400}	400	0	$Cu_{400}Se_{2.5}$	400	2.5	$Cu_{400}Se_{10}$	400	10	$Cu_{400}Se_{20}$	400	20
Cu_{800}	800	0	$Cu_{800}Se_{2.5}$	800	2.5	$Cu_{800}Se_{10}$	800	10	$Cu_{800}Se_{20}$	800	20