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Aging effect on the mobility and bioavailability of copper in soil

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Abstract

Aging effect on the mobility and bioavailability of copper (Cu) was investigated using a spiked soil with different incubation periods from 3 to 56 d. Wheat was planted and earthworms were cultured separately in the incubated soils. The mobility of Cu in soil was evaluated by a chemical fractionation scheme and the toxicity and bioavailability were assessed by measuring the biomass and Cu concentration in tissues. Results showed that aging had a significant effect on Cu fraction distribution, of which Cu tended to incorporate from the exchangeable into more stable fractions such as the reducible and oxidisable fractions. However, aging had little effect on Cu bioavailability to wheat and earthworm. Comparing the soil being incubated for 3 d and 56 d, Cu concentration in wheat roots decreased from 14.5 to 12.8 mg/kg, and no significant changes in Cu concentration were observed in both wheat shoots and earthworms. The Cu concentration was around 2.0 and 50 mg/kg for wheat shoots and earthworms, respectively, irrespective of soil incubation time. The $CaCl_2$-extractable Cu had a linear relationship with Cu concentration in wheat roots ($R^2 = 0.65$, $P < 0.05$), but no linear relationship can be found for wheat shoots and earthworms. Biological control may be more crucial for Cu accumulation in organism than the changes in soil Cu fraction caused by aging.

Key words: aging effect; copper; mobility; bioavailability
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Introduction

The importance of metal mobility and bioavailability in the environmental risk assessment of contaminated soils is widely recognized (Samsoe-Petersen *et al*., 2002). Aging is expected to have a significant influence on the mobility and bioavailability of heavy metals. Contaminants in field soils are often found to be less toxic than would be expected using laboratory tests with freshly spiked soils (Alexander, 2000). Although it is often assumed that long residence time leads to a decrease in metal mobility and bioavailability, few investigations have been attempted to determine whether and how residence time influence the fractionation and bioavailability of metals in soil (Kjœr *et al*., 1998; Pedersen *et al*., 2000; Ehlers and Luthy, 2003; Martínez *et al*., 2003) .

Mobility and bioavailability of heavy metals in soil depends on their speciation or fractionation. Direct methods are generally insufficient sensitivity for metal speciation analysis or require very specialized equipments such as synchrotron X-ray absorption spectroscopy (Kersten, 2002; Pueyo *et al*., 2003). Despite some disadvantages such as non-selectivity of extractants and redistribution of heavy metals among phases during extraction, sequential extraction methods are still commonly used to determine metal fractionation in soil to provide an understanding of mobility and bioavailability of metals (Tessier *et al*., 1979; Hullebusch *et al*., 2005). The Community Bureau of Reference (BCR) method has been widely used in metal fractionation for both soil and sediment (Quevauiller *et al*., 1993; Fernández *et al*., 2004). According to the method, metal is divided into acid soluble/exchangeable, reducible, oxidisable and residual fractions. The exchangeable fraction is considered readily mobile and bioavailable, whereas the residual fraction appears to incorporate into crystalline lattice of soil and becomes relatively inactive. Reducible and oxidisable fractions could be relatively active depending on soil properties. Unlike sequential extraction, single extraction method only focuses on the metal bioavailable fraction. Among various extractants, electrolyte solutions such as $CaCl_2$ and $Ca(NO_3)_2$ solutions have shown promise for the measurement of bioavailability of metals in soil (Conder and Lanno, 2000; Sauvé, 2003).

Plant tests have been developed to evaluate bioavailability or toxicity of metals in soil (Chaignon and Hinsinger, 2003). Besides plant experiments, earthworm has also been considered as a convenient bioindicator of metal bioavailability or toxicity (Kennette *et al*., 2002; Lanno *et al*., 2004; Ma, 2005). The direct measurement of heavy

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metal concentration in organism tissues can provide means of assessing environmental pollution, given the demonstrated correlation between soil contamination and metal bioaccumulation (Motalib, 1997).

The aim of the present study was to evaluate the aging effect on the mobility and bioavailability of metal in soil with a focus on Cu. Soil samples after being incubated for different periods were extracted by a modified BCR method to provide the information on metal mobility. Wheat (*Troticum aestivum* L.) and earthworm (*Eisenia fetida*) were used to test the changes of Cu bioavailability. Through such experiments, we hope to give a comprehensive appreciation of the aging effect on mobility and bioavailability of Cu in soil.

1 Materials and methods

1.1 Sample Collection

A loamy soil was collected from the top layer (0–20 cm) of a farmland near Beijing, China. The soil was air-dried, grounded and passed through a 2.0-mm nylon sieve and homogenized. Soil properties were determined following the standard methods. Soil pH was measured using a soil-to-water ratio of 1:1 (*W/V*) (Hendershot *et al*., 1993a), organic matter (OM) was measured by $K_2Cr_2O_7$ digestion (Nelson and Sommers, 1996), cation exchange capacity (CEC) was determined using a 0.1 mol/L $BaCl_2$ displacement (Hendershot *et al*., 1993b), and the particle size was analyzed by the hydrometer method (Sheldrick and Wang, 1993). The Cu concentration in soil was determined by inductively coupled plasma-mass spectrometry (ICP-MS) (VG PlasnaQuard 3, Fisons Instruments, UK) under optimized operating conditions after digestion with HNO_3-HF-$HClO_4$ (1:1:1, *V/V/V*) mixture under high-pressure conditions (Zhang and Shan, 1997). The soil properties are presented in Table 1.

Table 1 Selected properties of the tested soil

Parameter	Value
pH	6.90
OM (%)	4.35
CEC (cmol/kg)	15.7
Particle size distribution (%)	
Clay	10.8
Silt	51.6
Sand	37.6
Cu concentration (mg/kg)	21.6

1.2 Soil incubation

Copper was added into soil as nitrate salt ($Cu(NO_3)_2{\cdot}3H_2O$) in aqueous solution (19.5 mmol/L $Cu(NO_3)_2$) and then mixed thoroughly. The application rate of Cu was 500 mg/kg soil. An extra pot containing soil without Cu addition was prepared as the blank sample. A 500 g of each incubated soil was placed in a plastic pot. Incubation was carried out in a controlled-environment incubator. The day/night temperature regime was 25°C/20°C and the relative humidity was maintained at 70% by weighing the pots and adding distilled water daily. The soil incubation was started at different times to attain a different aging period. The incubation periods were 3, 7, 14, 21, 28, 42, and 56 d, respectively.

1.3 Soil analysis

Three soil subsamples were collected from each pot for analysis. A modified BCR method was applied for Cu fractionation (Mossop *et al*., 2003; Hullebusch *et al*., 2005). The reagents, extraction conditions and the corresponding fractions are listed in Table 2. Between each successive extraction, supernatants were obtained by centrifuging (15 min, 6000 r/min), decanting and filtering. The $CaCl_2$-extractable Cu was obtained by shaking 1.0 g soil in 10 mL of 0.01 mol/L $CaCl_2$ solution for 20 h, and subsequently centrifuging, decanting and filtering. The Cu concentration in the supernatants was measured by ICP-MS.

1.4 Plant experiments

Wheat seeds were obtained from the Chinese Academy of Agricultural Sciences, Beijing, China. Seeds were surface sterilized in a 10% (*V/V*) solution of hydrogen peroxide for 10 min, rinsed with sterile distilled water and pregerminated on moist filter in the dark at 20°C. After 24 h, uniformly germinated seeds with radical emerged were selected and sown in the soils for different incubation periods. Fifteen seeds were sown per pot and then subsequently thinned to 10 plants. Plants were grown under the greenhouse conditions with temperature ranging from 20 to 25°C during the day and 8 to 15°C at the night. Soil water content was kept around 15% by adding deionized water daily. Plants were harvested on day 56 after germination. The aboveground parts of the plants were first harvested as shoots and then roots were separated from soil. Roots were carefully washed with tap water to remove any adhering soil particles. Roots and shoots were then carefully rinsed with deionized water thoroughly

Table 2 Modified sequential extraction procedure and the corresponding fractions

Step	Fraction	Nominal target phase	Operational definition
1	Exchangeable, water and acid soluble (F1)	Soluble, carbonates, cation exchange	40 mL CH_3COOH (0.11 mol/L, pH 7), shaking 16 h at 20°C
2	Reducible (F2)	Iron and manganese oxyhydroxides	40 mL NH_2OH-HCl (0.5 mol/L, pH 1.5), shaking 16 h at 20°C
3	Oxidisable (F3)	Organic matter and sulfides	20 mL H_2O_2 (30%, pH 2) shaking 1and 2 h at 20 and 85°C, respectively, and then 50 mL CH_3COONH_4 (1 mol/L, pH 2), shaking 16 h at 20°C
4	Residual (RES)		10 mL aqua regia (HCl/HNO_3, 3:1, *V/V*) under high pressure at 160°C for 6 h

and dried at 60°C for 48 h. The dried samples were weighted and finely ground for metal analysis. Total Cu concentrations in shoots and roots were determined by ICP-MS after samples being decomposed with 4 mL of the HNO_3-$HClO_4$ mixture (1:1, *V/V*).

1.5 Earthworm experiments

Adult earthworms purchased from China Agricultural University (Beijing, China) were acclimated to laboratory conditions (20°C, 12 h dark/ 12 h light) for 7 d prior to experiment. They were placed on the moist filter paper to depurate culture bedding from their gastrointestinal tracts for 24 h before exposure to the test soil. Ten earthworms about 4.0 to 4.5 cm long were selected and cultivated in each container with 500 g incubated soil. Water contents were monitored daily. Room temperature was kept at (20 ± 1)°C in 12 h light : 12 h dark regime. At the end of 4 weeks, surviving worms were removed, counted, depurated, freeze-dried, weighted, and then digested with HNO_3, diluted and analyzed for Cu concentration by ICP-MS.

1.6 Data analysis

All the experiments were performed in triplicate and data are presented as means with standard errors. Statistical analysis was performed using Excel_XP (Microsoft, Inc) and SPSS for Win 11.0 (SPSS, Inc).

2 Results and discussion

2.1 Aging effect on Cu fractionation in soil

The distribution of Cu in different soil fractions during experimental period is shown in Fig. 1. For clarification, the distributions of Cu fractionation in the blank soil and spiked soils incubated for 3 d and 56 d are given in Table 3.

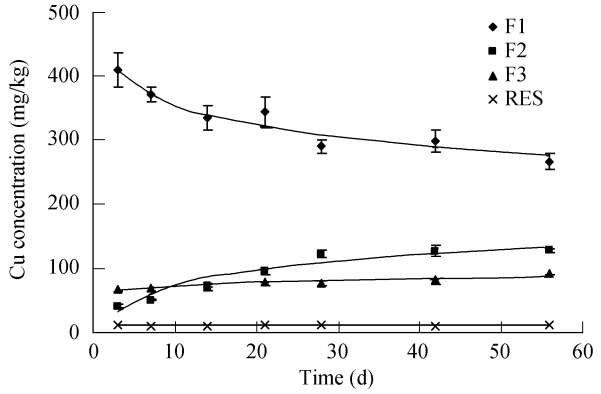

Fig. 1 Cu concentration in different fractions of the incubated soils aged from 3 to 56 d.

As a good check on the reliability of sequential extraction procedure, the recovery (the sum of Cu in each fraction to the total Cu concentration in soil) of all the tested soils was considered acceptable, ranging from 93% to 104%. The sum of Cu in each fraction is generally lower than the total Cu concentration detected, which was considered mainly due to small cumulative losses of material during fractionation.

As we can see from Table 3 that Cu was found predominantly in the residual fraction (RES) in the blank soil with 47.7% of the total content, indicating that Cu is mainly retained in the crystalline lattice constitutes. Another important observation is that there was no significant difference in Cu concentration in the residual fraction between the blank and the incubated soils, which suggests the Cu ions hardly enter the soil crystalline lattice within 56 d of incubation.

The newly added Cu mainly existed as F1 fraction (Fig. 1). After 3 d of incubation, the concentration of Cu in F1 fraction was 410 mg/kg and it decreased gradually as the incubation time. At the end of 56 d period, only 267 mg/kg of Cu remained in this fraction. About 143 mg/kg Cu in soil slowly transformed from the loosely bound fraction (F1 fraction) into the more stable fractions such as F2 and F3 fractions. The Cu concentration in F2 and F3 fractions consistently increased with the increase of incubation time, from 40.9 to 127 mg/kg for F2 fraction and from 67.1 to 91.9 mg/kg for F3 fraction. This result suggests that Cu mobility estimated on the basis of exchangeable, water and acid soluble fraction (F1) decreases with aging. Such a decrease was found to be statistically significant ($P < 0.01$).

The decrease of Cu in the exchangeable fraction (F1) could be simulated as the following Eq. (1):

$$C_m = 391 \exp(-0.0072 \times T) \quad R^2 = 0.86, \ P < 0.01 \quad (1)$$

where, C_m is the concentration of Cu in F1 fraction, T is time. It is one of the solutions for the diffusion equation (Eq. (2)):

$$\frac{\partial C}{\partial T} = D\left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right) \quad (2)$$

where, C is Cu concentration, r is soil particle radius and D is diffusion coefficient. This solution indicates that the decrease of Cu in exchangeable fraction mainly attributes to the diffusive processes of Cu through soil micro- and mesopores possibly (Spark, 2001).

During 56 d incubation, Cu was incorporated from the active fractions into the relative stable soil fractions, and only a little change was observed in the residual fraction. However, as can be seen in Fig. 1, Cu transformation among soil fractions did not reach equilibrium within 56 d although the rate decreased gradually.

Table 3 Cu concentration in different soil fraction (mg/kg)

	F1	F2	F3	RES	Sum of each fraction	Detected total concentration
Blank soil	6.35	3.10	1.18	10.3	20.9	21.6
Soil aged for 3 d	410	40.9	67.1	11.2	529	517
Soil aged for 56 d	267	127	91.9	10.9	497	524

2.2 Aging effect on copper bioavailability to wheat and earthworm

The dry biomass of both wheat and earthworm was affected by soil incubation time (Fig. 2). The dry shoot weight harvested from the aged soils ranged from 31.6% to 45.0% of that from the blank soil; the relevant value ranged from 39.7% to 66.8% for roots, and from 60.7% to 77.7% for earthworms. We can observe from Fig. 2 that the dry biomass of wheat or earthworms increased with increasing incubation time, indicating that prolonged soil incubation would reduce Cu toxicity.

Figure 3 presents Cu accumulation in both wheat and earthworms. Compared with the blank samples, Cu concentration in wheat and earthworms collected from the incubated soils increased obviously. There was a consistent decrease from 14.5 to 12.8 mg/kg in Cu concentration in wheat roots with the increase of incubation time from 3 to 56 d. But the changes in Cu concentrations in wheat shoots and earthworms were insignificant ($P > 0.05$). Soil bioprocess, such as root exudatation has effects on metal speciation in soil, and thereby may influence the bioavailability of metals in soil.

Metal extracted by 0.01 mol/L $CaCl_2$ is usually considered as the phyto-bioavailable fraction in soil. $CaCl_2$-extraction was also applied as a surrogate method for measuring metal bioavailability to earthworm (Peijnenburg, 1999). The amount of 0.01 mol/L $CaCl_2$-extractable Cu was affected by aging, decreased from 127 to 98 mg/kg (see Fig. 4).

Significant correlation coefficient ($R^2 = 0.65$, $P < 0.05$, $n = 7$) was obtained between the Cu concentrations extracted with 0.01 mol/L $CaCl_2$ and the Cu contents in wheat roots.

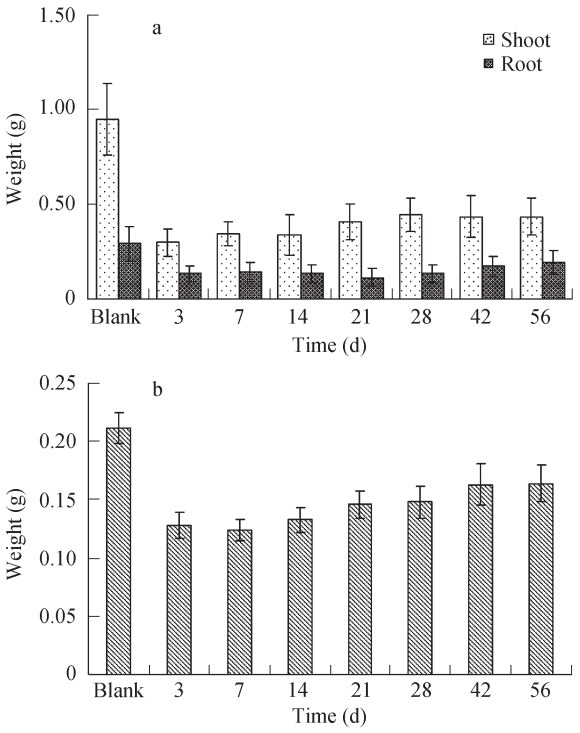

Fig. 2 Dry biomass of wheat shoots and roots (a) and earthworms (b) in the blank and the incubated soils aged from 3 to 56 d.

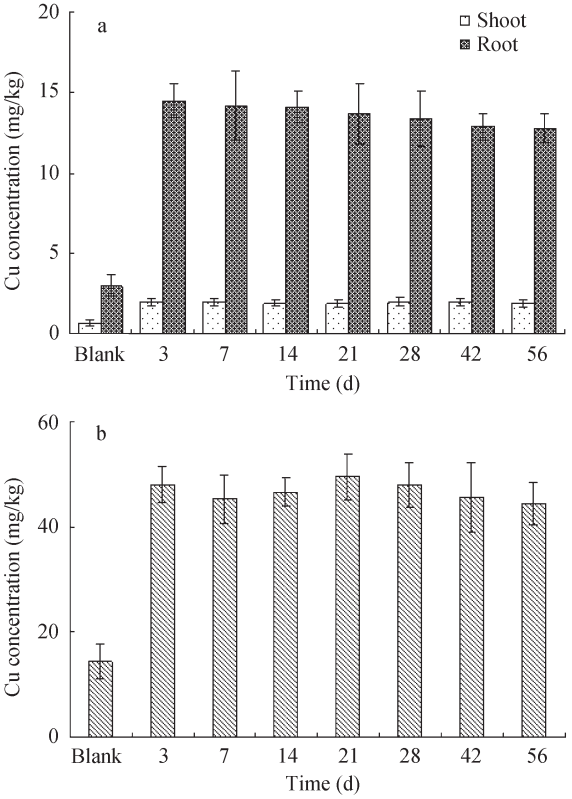

Fig. 3 Cu concentration in wheat (a) and earthworm (b) in the blank and the incubated soils aged from 3 d to 56 d.

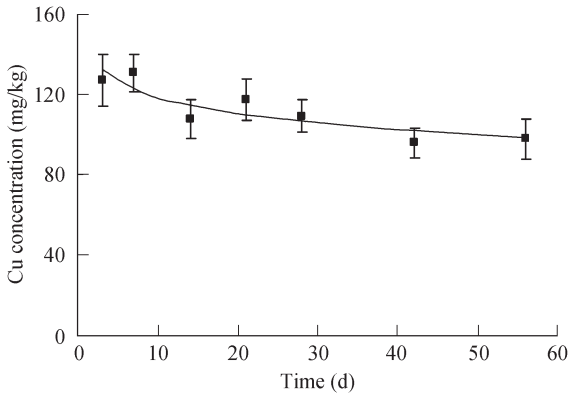

Fig. 4 Concentration of 0.01 mol/L $CaCl_2$-extractable Cu in soils aged from 3 to 56 d.

However, for Cu concentrations in shoots and earthworms, no significant liner relationships could be obtained (R^2 = 0.12, and 0.14 for wheat shoot and earthworms, respectively) (Figs. 3 and 4).

The Cu accumulation in roots was much higher than in shoots (Fig. 3). Studies have confirmed that wheat could resist metal-contaminated soil by immobilizing metal outside or in roots, thereby preventing excess translocation into shoots (Brun, 2001). Due to the positive charge, metal ions were more easily bound to root cell wall (Lasat, 2002). This was the possible reason for the significant linear relationship between the concentration of $CaCl_2$-extractable Cu and accumulated Cu in roots. Bioavailability of Cu to wheat roots reduced with aging time due to the fact that more Cu in soil was incorporated into the more stable

fractions. Once Cu entered wheat roots, metal-complex peptides (phytochelatins) might be induced, which could reduce the concentration of free Cu ions in the cytosol. A similar function might be played by vacuoles in root. Control of metal transportation to different parts might keep them away from more sensitive parts such as plant shoots (Macnicol and Beckett, 1985). These regulatory mechanisms effectively limit metal toxicity to a certain level.

Earthworm was considered to have two uptake pathways for heavy metal: dermal and intestinal. According to Lanno *et al*. (2004), earthworm could take up metals from soil either through direct dermal contact with them in soil solution or by ingestion of bulk soil or specific soil fractions. Therefore, whether Cu in soil is bioavailable to earthworm depends on the physiology and behavior of the worms. Distribution of metals among soil fractions was also considered to be important for their toxicity and bioavailability to earthworm (Becquer *et al*., 2005). Conder and Lanno (2000) have demonstrated that concentrations of Cd, Pb and Zn in weak-electrolyte extractants such as $CaCl_2$ or $Ca(NO_3)_2$ are distantly related to their accumulation in earthworms exposed to metal-spiked soils. However in our experiment, $CaCl_2$-extractable Cu did not relate to Cu concentrations in earthworms, and no aging effect was found on Cu accumulation in earthworms. Scott-Fordsmand *et al*. (2000) have also observed that Cu accumulation was nearly the same in earthworm whether in the filed soil which is contaminated for 70 years or in the soil which is newly spiked with Cu salts. They attributed such an observation to the rapid binding of Cu to soil particles. Therefore, effects of aging on metal toxicity and bioavailability could not be considered only to metal fractionation in soil.

The Cu concentration was found to be around 2.0 and 50 mg/kg (Fig. 3) in wheat shoots and earthworms, respectively, irrespective of soil incubation time. Similar Cu concentration in earthworm in all the incubated soils might be attributed to earthworm homeostatic control. Aging affected the concentration of $CaCl_2$-extractable Cu in soil, but not the concentration in earthworms. Same phenomenon was observed by Davies *et al*. (2003), in which Pb accumulation in earthworms reached to 3000 mg/kg and then resulted in earthworm mortality. With regard to Cu, body concentration may continue to increase until reach the corresponding exposure intensity level, where the influence of homeostasis would be no longer valid (Bogomolov *et al*., 1996). Furthermore, the uptake of metals by organism from soil is a dynamic process and such character is difficult to be included in establishment of the relationship between metal accumulation in organism and its concentration in soil.

3 Conclusions

The present study demonstrates that aging has a significant effect on the fraction distribution of Cu in soil. Cu tends to incorporate from exchangeable, water and acid soluble fraction into reducible and oxidisable fractions. However, 56 d incubation does not reduce Cu accumulation in wheat and earthworms. Biological control may be more crucial for Cu accumulation in organism than the changes in soil Cu fraction caused by aging. Therefore, further research works are necessary to understand the complicated metal uptake mechanisms for different organisms.

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