

## Effect of aging on bioavailability of copper on the fluvo aquic soil

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Received 1 July 2010; revised 13 November 2010; accepted 11 March 2011; available online 1 September 2011

**ABSTRACT:** The bioavailability of copper in contaminated soils has received more attention due to the safety concern of food chain. The bioavailability of metals is determined by its fractions which are affected by the soil properties and its aging time. This paper focused on the aging effect on the bioavailability of copper added to the soil. The garden soil (fluvo aquic soil) was treated with 100 mg/kg and 1000 mg/kg of copper(II) sulfate and incubated for 14, 21, 28, 42, 63, 120, 200, 300 and 400 days in the laboratory respectively. The sequential extraction procedure was used to characterize copper bioavailability in the soil. Meanwhile, the barley was cultured on the same soil incubated and its toxicity was assessed according to the guideline of International Organization for Standardization. The findings show that the exchangeable and carbonate-bound copper decreased with the aging time after addition of copper(II) sulfate to soil. Meanwhile, the percentages of Fe-Mn oxides- and organic-bound copper increased. The residual copper was changed little during the aging course. The copper fractions became stable in soils after 60 days. The kinetic equations showed that the Power function and the Elovich equation were well fitted to the experimental data, and the  $r^2$  values ranged from 0.840 to 0.982 and 0.741 to 0.975, respectively. The barley test showed that the barley root length was more sensitive to reflect copper toxicity than the shoot biomass, and the exchangeable and carbonate-bound copper were significantly correlated with the barley root length.

**Keywords:** Aging effect; Bioavailability; Copper; Kinetics; Soil fractions

## INTRODUCTION

In recent years, the impact of heavy metal on soil has been received a particular concern due to its persistency in soil and therefore its relativity to the security of food chain (Chojnacka *et al.*, 2005; Kidd *et al.*, 2007; Ayari *et al.*, 2010). Among the metals, copper in contaminated soils has been received more attentions. Metal fractions in soil is influenced by the soil's physicochemical properties, such as soil pH, clay, organic matter content and content, as well as long-term fixation or aging effects. These soil properties and processes largely determine metal fractions and further its bioavailability in soil environments (Oorts *et al.*, 2006; Jalali *et al.*, 2008; Li *et al.*, 2009; Nouri *et al.*, 2011).

The aging of metals in soils has been demonstrated to be a major factor in determining their availability (Tagami and Uchida 1998; Ma *et al.*, 2006a; Ma *et al.*, 2006b). It has been found that the proportions of the

most labile Cu fractions (exchangeable Cu) were decreased with increasing incubation time, whereas those of Cu bound to crystalline and amorphous materials (residual Cu) exhibited the opposite trend (Lu *et al.*, 2009). It has also been found that a time-dependent decrease in total water extractable and bioavailable Cu during incubation at field capacity for up to 200 days in an *in vitro* assay and suggested that soil moisture and incubation time were the major effects factors on the bioavailability of copper in soil (Nouri, 1980; Petersen *et al.*, 2004). The distribution of Cu fraction is a function of the Cu concentration added to the soil and the incubation time (Arias-Estévez *et al.*, 2007). Although the long-term aging has been recognized as a key process in the risk assessment of metals in field soils, the process still needs more in detail information (Ma *et al.*, 2006; Quazi *et al.*, 2010).

The objective of this study was to examine the effects of aging on the fractionations and the dynamic bioavailability changes of Cu in *fluvo aquic* soils in

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order to assess the ecological risk in the Cu contaminated soils. The effectiveness of aging on bioavailability was assessed by Cu fractions and a plant test. To achieve this end, the aging experiment was carried out from March 2007 to May 2008 at the School of Environmental Science and Engineering, Shanghai Jiao Tong University.

## MATERIALS AND METHODS

### *Soil collection and its physiochemical characterization*

The soil samples (fluvo aquic soil) were collected from the top layer (0-20cm) in a garden of Shanghai city. The values of pH were measured in a solution of 0.01M CaCl<sub>2</sub> (soil: solution = 1: 5). The Total organic carbon (TOC) in soils was calculated from the difference between total carbon and inorganic carbon (Oorts *et al.*, 2006), which were measured by means of Multi N/C 3000 analyzer (Analytik Jena AG, Germany).

The Cation exchange capacity (CEC) was determined by the silver thiourea method at soil pH (Chhabra *et al.*, 1975; Ammann *et al.*, 2005). The texture was analyzed with the hydrometer method. The content of calcium carbonate was determined by treating each soil with HCl and measuring the liberated CO<sub>2</sub>. Total ambient Cu (T-Cu) in the soils was digested with an aqua regia, and analyzed by ICP-AES IRIS Advantage/1000, TJA, USA (McGrath and Cunliffe, 1985). Soil physiochemical properties were characterized in Table 1. Results are presented as the average of three replicates. The recoveries of Cu in this study were 93 %

### *Soil incubation course*

The soil was air-dried, sieved (mesh size, 4 mm) and then spiked with CuSO<sub>4</sub> solution at two concentrations of 100 mg/kg and 1000 mg/kg, respectively. After thoroughly mixed, the soils were air-dried and sieved again through 4 mm mesh. Then the soils were incubated at room temperature. And two portion samples were taken at 14, 21, 28, 42, 63, 120, 200, 300 and 400 days, one for the detection of Cu content and its fractions, another for the

bioavailability assessment below.

### *Bioavailability of Cu in barley*

Two barley bioavailability assays were performed: one with a root elongation assay based on the guideline of International Organization for Standardization (ISO) 11269-1 (ISO, 1993), and another with a shoot biomass assay based on the guideline of ISO 11269-2 (ISO, 1995). Approximately 500 g soil was first mixed thoroughly with deionized water to make the soil grainy and then placed in a plastic pot. 10 seeds of barley were germinated in each plastic pot and irrigated to field capacity with deionized water. Three pots for each treatment were performed as the triplicates and kept in the incubator (SPX-250B-G) with a light intensity of 5000 lux for a duration of 16:8 light:dark photoperiod at the temperature of 15 ± 2 °C and random relocation once a week. The soil moisture of every pot was maintained at 80 % field capacity by the deionized water during the incubation course (Ginocchio *et al.*, 2006). After 21 days, the plants were removed intact and their length of the longest root on each plant was recorded. The root lengths of each treatment were calculated from 30 plants in triplicate pots. Then the fresh plants were air-dried for bioavailability assessment in the laboratory at room temperature in order to record the shoot biomass and to determine Cu content. The same soils without Cu addition were used as controls. Results were expressed in percentage of effect compared to the control.

### *Detection of Cu in soil and plant*

The soil and plant samples in triplication were digested with nitric acid individually and the Cu fractions in soils were extracted according to the process by Tessier *et al.* (1979). All the solutions above were detected by ICP-AES (IRIS Advantage/1000, TJA, USA) (Rooney *et al.*, 2006). The sum of all extraction steps for each metal was 86.6-107.4 % compared with the total Cu contents in the same soil sample. Every digestion batch included a blank sample and a triplicate sample. The standard reference materials (reference

Table 1: Physiochemical properties of the soil

Location	pH CaCl <sub>2</sub> (0.01 M)	CaCO <sub>3</sub> (%)	TOC (g/kg)	CEC (cmol/kg)	Clay (%)	T-Cu (mg/kg)
Shanghai	7.01	2.90	8.87	16.91	17	23.86*

\* The value was lower than 30 mg/kg (WHO)

material serial number GBW-07421; Center for National Standard Reference Materials of China. The content of Cu is 17  $\mu\text{g/g}$  in the reference) was inserted to the analytical process for quality control.

## RESULTS AND DISCUSSION

### *The profile of Cu fractions in soil during the aging course*

During the aging course, the changes of Cu percentage in different fractions of the soils were shown in Fig. 1. Although the external Cu added to the soils were different, i.e. sample A with 100 mg/kg and sample B with 1000 mg/kg, their curve figures of Cu fractions were similar. At the beginning of  $\text{CuSO}_4$  addition into soil, the percentage of exchangeable, carbonate-bound, Fe-Mn oxides-bound, organic-bound and residual fractions in two batches were 50 % and 60 %, 20 % and 21 %, 9 % and 13 %, 8 % and 9 %, 1 % and 10 % respectively. It was well known that the exchangeable

Cu with labile bound could be easily released into the soil solution and further be absorbed by plant, which might cause the hazard to the ecosystem and food chain. With the increase of the incubation time the exchangeable Cu was decreased sharply within the first 60 days after the Cu addition. Then the change became slowly and only 4 % and 5 % of exchangeable Cu was left in the two different soil samples at 400 days.

Similarly, the decreases in the carbonate-bound fraction were also observed during the incubation, but the variation was not as much as the former. It was remained in 6.8 % and 15.3 % respectively in the two soils at 400 days of incubation, which means carbonate fractions with a loosely bound could be changed with environmental conditions (Stone and Marsalek, 1996; Navas and Lindhorfer, 2003), (Fig. 1). In contrast to the fractions above, the proportions of Cu in the Fe-Mn oxides-bound fraction

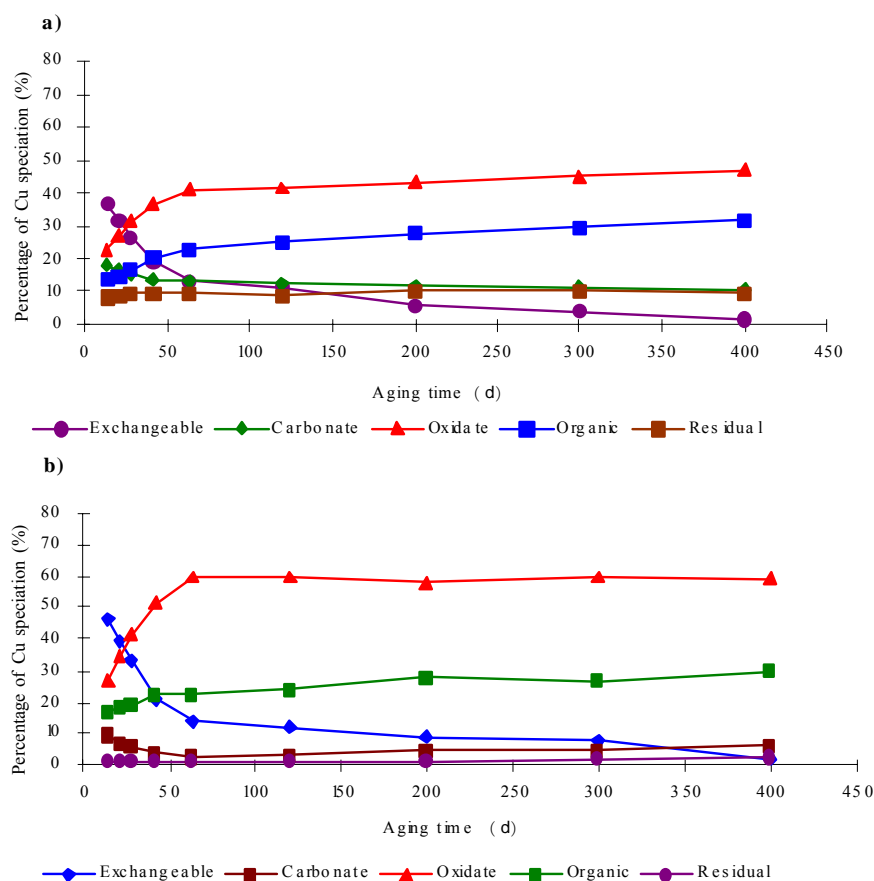


Fig 1: The variation of Cu percentage in different fractions of the soil during time course a) The external Cu addition was 100mg/kg soil; b) The external Cu addition was 1000mg/kg soil

were increased sharply during the incubation in the first 60 days and then became smoothly. At 400 days of incubation it was reached in 44.7 % and 59.2 %, respectively.

The proportions of Cu in the organic-bound fraction were increased slightly during the incubation but the variation was not significant as much as that in Fe-Mn oxides-bound fraction. It was reached in 26.5 % and 30.1 % respectively at the end of the incubation. [Filgueiras et al. \(2002\)](#) found that the metal fractions bound to Fe-Mn oxides- and to organic matter were relatively stable and not easily used by biological paths under normal soil conditions. The residual fraction changed less in this research. The variation rate was less than 1 % and 3 % in sample A and sample B respectively during the aging course. Residual fraction was often considered to be bound within the lattice of minerals and consequently could not be released into the environment ([Balasoju et al., 2001](#)).

In summary, there were clear changes in the proportional distribution of Cu in the two soils during the incubation after Cu addition. The proportions of Cu associated with most weakly bound fractions (exchangeable and carbonate-bound fractions) tended to decrease, with corresponding increases in the other three more strongly binding fractions. The differences in the rates of redistribution are most probably due to the addition Cu into soil.

Results of the present investigation indicate that the metals could be adsorbed on the solid surface by relatively weak electrostatic interaction, and then the sorbed metal fractions may undergo a slow transformation into more stable fractions that were less susceptible to release back into solution and were not readily accessed even by the smallest of

microorganisms and the tissues of higher organisms during the aging course ([Alexander, 2000](#); [Elzinga et al., 2006](#)).

#### The kinetics of Cu fractions turned over during aging course

The kinetics of Cu turned over in different fractions during the aging course could be fitted by the following equations ([Shirvani et al., 2007](#); [Jalali and Khanlari, 2008](#)):

$$\text{Elovich equation: } q_t = a + b \ln t \quad (1)$$

$$\text{Power function } q_t = at^b \quad (2)$$

$$\text{Parabolic diffusion } q_t = a + bt^{1/2} \quad (3)$$

Where  $q_t$  is Cu decreased or increased at time  $t$ ,  $a$  and  $b$  are constants,  $t$  is time (d). The goodness of fit was evaluated based on coefficients of determination ( $r^2$ ) and standard errors of estimate (SEE) calculated as follows:

$$SEE = \left( \frac{\sum (q_t - q'_t)^2}{n - 2} \right)^{1/2}$$

$q_t$  and  $q'_t$  are the measured and predicted Cu decreased or increased, respectively and  $n$  is the number of measurements. The experimental data in the study were matched with the three kinetic equations above and their fits were presented in [Table 2](#).

The values of  $r^2$  were ranged from 0.840 to 0.982 with Power function (PO), from 0.741 to 0.975 with Elovich equation (EL) and from 0.596 to 0.949 with Parabolic diffusion equation (PA). The order of well fitting was  $PO > EL > PA$ . This was accordance with the research of [Xu et al. \(2008\)](#), who observed that the copper and zinc in red soil, paddy soil and cinnamon

Table 2: The  $r^2$  and SEE of the models fit with the different Cu fractions <sup>a</sup>

Soils		Elovich		Power function		Parabolic diffusion	
		$r^2$	SEE	$r^2$	SEE	$r^2$	SEE
A	Exchangeble	0.961	0.771	0.982	1.272	0.832	0.387
	Carbonate	0.933	0.208	0.960	1.307	0.749	0.142
	Fe-Mn xides	0.885	0.843	0.840	2.418	0.685	0.359
	Organic	0.975	0.298	0.944	0.881	0.808	0.164
B	Exchangeble	0.906	1.488	0.977	5.122	0.768	0.456
	Carbonate	0.877	0.226	0.941	2.619	0.786	0.597
	Fe-Mn xides	0.741	2.018	0.879	5.354	0.596	5.501
	Organic	0.958	0.299	0.950	0.781	0.949	0.799

<sup>a</sup> The probability level <0.05

soil fitted better the PO and EI ( $r^2$  were 0.8813 to 0.9359 and 0.8289 to 0.9085), whereas PA has less goodness of fit during the aging processes ( $r^2$  were 0.5409 to 0.7385) (Xu *et al.*, 2008). However, Jalali and Khanlari (2008) found that all three models used above were well fitted to experimental data and the parabolic equation is the best of the equations to describe the experimental data.

By means of the kinetic equation, the concentration of liable Cu in soil could be calculated and further assess the ecological risk.

#### *The bioavailability assessment of Cu during the aging course*

As the indicators of Cu bioavailability, the inhibition rate of barley Root length (RL) and shoot biomass in the soil A and B during aging course was shown in Fig. 2 and Fig. 3 respectively.

Compared to the control, the inhibition rate on barley root length ranged from 14.01 % to 36.07 % in incubation soil A and from 45.86 % to 57.6 % in incubation soil B respectively (Fig. 2). The trend of Cu bioavailability in the two treatments was declined with the aging time, but the bioavailability of Cu in soil A was lower than that in soil B. It was also observed that the inhibition rate of biomass ranged from 56.4 % to 65.3 % in incubation soil A and from 70.1 % to 74.6 % in incubation soil B respectively (Fig. 3). However, the variation of the biomass in two treatments was not as obviously as the RL. It seemed that the indicator with RL was more sensitive than the indicator with shoot biomass. This could be attributed from the different mechanism of uptake by plant. A strong translocation

barrier against Cu has been observed in many plant roots and higher levels of trace metals was accumulated mainly in the root, therefore, the root length is often used as a sensitive test for Cu toxicity (Schat and Ten Bookum, 1992; Nabulo *et al.*, 2008).

The regressions between the Cu bioavailability (the barley RL test and the shoot biomass test) and the Cu fractions (exchangeable, carbonate-bound, Fe-Mn oxides-bound, organic-bound Cu) were estimated to evaluate the Cu toxicity in the two treatments quantitatively (Table 3).

The exchangeable and the carbonate-bound fractions in two soils were significantly correlated with the barley RL ( $r^2=0.867$ ,  $p<0.001$ ;  $r^2=0.835$ ,  $p=0.047$  for incubation A and  $r^2=0.893$ ,  $p=0.001$ ;  $r^2=0.868$ ,  $p=0.035$  for incubation B, respectively). It proved that exchangeable and carbonate-bound Cu in soils are mainly bioavailable fractions, which might cause the hazard on plants (Stone and Marsalek, 1996; Navas and Lindhorfer, 2003). The results also indicated that the exchangeable and carbonate-bound Cu have more effects on the barley root in the incubation B than that in the incubation A. Because the bioavailable fractions of the former was higher than those of the latter.

The regression of Cu bound to the Fe-Mn oxides- and to the organic matter were less significantly correlated with the RL ( $r^2=0.658$ ,  $p=0.061$ ;  $r^2=0.670$ ,  $p=0.124$  for incubation A and  $r^2=0.771$ ,  $p=0.109$ ;  $r^2=0.724$ ,  $p=0.083$  for incubation B, respectively), which indicated the less bioavailability of these two fractions.

The  $r^2$  test from the shoot biomass were generally lower than those test from the barley RL, indicating that the root growth was more directly affected by Cu

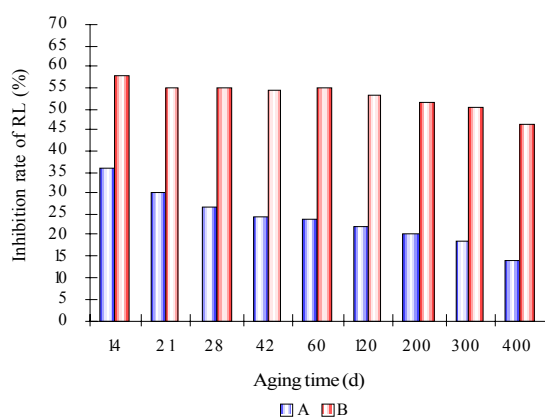


Fig. 2: Inhibition rate of barley RL during the aging course

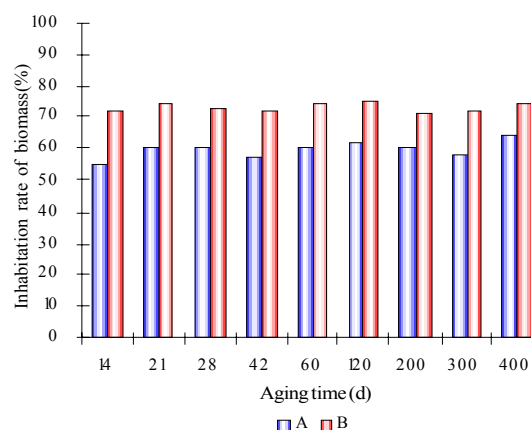


Fig. 3: Inhibition rate of biomass during the aging course

Table 3: The regression of Cu bioavailability against Cu fractions

Bioavailability	Variables	Regression equation	r <sup>2</sup>	P <sup>a</sup>
RL	Exch- Cu	Log(RL) = 1.21 - 0.16log(Exch-Cu)	0.867	<0.001
	Car-Cu	Log (RL) = 1.51 - 0.47log(Car-Cu)	0.835	0.047
	Oxid-Cu	Log (RL) = 0.38 - 0.53log(Oxid-Cu)	0.658	0.061
	Org-Cu	Log (RL) = 1.36 - 0.19log(Org-Cu)	0.670	0.124
B	Exch- Cu	Log(RL) = 1.16 - 0.13log(Exch-Cu)	0.893	<0.001
	Car-Cu	Log (RL) = 1.00 - 0.08log(Car-Cu)	0.868	0.035
	Oxid-Cu	Log (RL) = 0.19 - 0.25log(Oxid-Cu)	0.771	0.109
	Org-Cu	Log (RL) = 0.68 - 0.43log(Org-Cu)	0.724	0.083
Biomass	Exch- Cu	Log(Biomass) = 1.75 + 0.41log(Exch-Cu)	0.638	0.071
	Car-Cu	Log (Biomass) = 1.60 - 0.52log(Car-Cu)	0.702	0.08
	Oxid-Cu	Log (Biomass) = 0.57 - 0.7log(Oxid-Cu)	0.746	0.066
	Org-Cu	Log (Biomass) = 1.43 - 0.82log(Org-Cu)	0.591	0.094
B	Exch- Cu	Log(Biomass) = 0.26 - 1.33log(Exch-Cu)	0.694	0.141
	Car-Cu	Log (Biomass) = 0.86 + 0.15log(Car-Cu)	0.717	0.108
	Oxid-Cu	Log (Biomass) = 2.65 + 0.72log(Oxid-Cu)	0.68	0.077
	Org-Cu	Log (Biomass) = 1.02 + 0.58log(Org-Cu)	0.673	0.082

<sup>a</sup> Significance level ( p) of the independent variables

toxicity than the shoot biomass and the former was more sensitive than the latter. Although sensitivity difference between the two tests was observed, the fractions affected the bioavailability of Cu to the plant in the similar way (Rooney *et al.*, 2007).

## CONCLUSION

In recent years, the metal fractionations are often recognized as an important parameter to assess metal toxicity in soil. The results above confirmed the positive correlations between Cu fractions and its toxicity to the plant.

Cu contents were significantly decreased in exchangeable and the carbonate-bound with the increased time, while the Fe-Mn oxides- and the organic-bound Cu showed the opposite trend. The results indicated that the Cu fractions were varied and their toxicity became less during the aging course.

The aging kinetics can be well fitted with the PO and EI ( $r^2=0.840-0.982$ ;  $r^2=0.741-0.975$ ), which indicated that the aging of added Cu was a slow process. The easily extractable Cu fraction was transformed or transferred from active forms slowly into more stable forms, mostly into the Fe-Mn oxides- and the organic bound Cu which were less available.

The relationship of Cu fractions and their barley bioavailability indicated that the exchangeable and carbonate-bound Cu were the main fractions available to the plant, and its toxicity decreased with time. The other fractions were more stable and difficult to be uptaken by the plant.

In summary, Cu contents in the fractions of exchangeable and carbonate-bound, as well as in total and the aging duration could be as the main factors to assess the ecological risk of soil, especially the soil heavily polluted from an accidental event.

## ACKNOWLEDGEMENTS

The authors are grateful for the financial support from the Key Lab of City Agriculture (South), Ministry of Agriculture, China (09UA004) and from the International Copper Association.

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**How to cite this article: (Harvard style)**

Guo, G. Y.; Yuan, T.; Wang, W. H.; Li, D.; Wang, J., (2011). Effect of aging on bioavailability of copper on the fluvo aquic soil. *Int. J. Environ. Sci. Tech.*, 8 (4), 715-722.