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Accumulation and chemical fractionation of Cu in a paddy soil irrigated with Cu-rich wastewater

Yongming Luo^a, Xianjun Jiang^{a,*}, Longhua Wu^a, Jing Song^a,
Shengchun Wu^a, Ronghui Lu^a, Peter Christie^b

^aLaboratory of Material Cycling in Pedosphere, Institute of Soil Science, Chinese Academy of Sciences,
Nanjing 210008, PR China

^bDepartment of Agricultural and Environmental Science, The Queen's University of Belfast, Newforge Lane,
Belfast BT9 5PX, UK

Abstract

The accumulation, chemical fractionation and availability of copper (Cu) to rice (*Oryza sativa*) in a paddy soil previously irrigated with Cu-enriched wastewater were investigated. Chemical fractions of Cu in soil samples were partitioned using single and sequential extractions. The single extractant solutions used were water, ammonium acetate and EDTA for extraction of water-soluble, exchangeable and complexed Cu fractions in the soil. A three-step sequential extraction procedure (HAOc/NH₂OH.HCl/H₂O₂) was also used to fractionate Cu associated with soil solids into three fractions: weak acid-soluble, reducible Fe and Mn oxides bound and oxidisable organic matter bound. The residual fraction was calculated as the difference between total (*aqua regia* extractable) Cu and the sum of the three sequential fractions.

Copper accumulated mainly in the top layer (10 cm) of the soil profile after wastewater irrigation, increased almost seven-fold (158 mg kg⁻¹) compared with non-irrigated soil (23 mg kg⁻¹) from the same area, and was also elevated significantly in low layer (10–20 cm) below the soil surface. Concentrations of Cu in the water-soluble, exchangeable and complexed fractions increased from 0.02, 0.33 and 14.1 mg kg⁻¹, respectively, in unpolluted soil to 0.12, 6.30 and 98.0 mg kg⁻¹ in polluted soil. Irrigation also led to marked increases in the weak acid-soluble, reducible Fe and Mn oxide bound, oxidisable organic matter bound and residual fractions of Cu. In the contaminated paddy soil, the reducible Fe and Mn oxide bound fraction was the largest (36% of total Cu), followed by the oxidisable fraction, then the residual fraction, and the smallest was the acetic acid-soluble fraction. However, in the unpolluted soil, 53% of total Cu was present in the residual fraction followed by the reducible Fe and Mn oxide bound fraction (22% of total Cu). Accumulation of Cu in the bioavailable fractions resulted in a three-fold increase in Cu concentration (up to 38 mg kg⁻¹) in rice plants in the heavily polluted (HP) soil compared with unpolluted soil. Plant yields decreased

* Corresponding author.

E-mail address: xijiang@issas.ac.cn (X. Jiang).

substantially due to Cu toxicity. Data indicate that the reducible Fe and Mn oxide bound fraction may be more important for controlling the mobility and bioavailability of Cu than the oxidisable organic matter bound fraction in paddy soils.

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1. Introduction

In the last two decades, soil pollution with potentially toxic metals has often been reported, especially in those areas of China experiencing rapid economic development (Chen, 1996). Metal pollution of soils is usually evaluated on the basis of total metal content rather than metal speciation in soils. This provides little information on the mobility and bioavailability of toxic metals in contaminated soils and thus gives poor guidance for the selection of appropriate remediation strategies for contaminated land.

Potentially toxic metals, such as Cu, in soils may be present in several different physicochemical forms with different mobilities and availabilities to living organisms. In the hope of yielding information for assessment of heavy metal mobility and bioavailability in soils, single chemical extraction is often performed using a selective chemical extractant (Beckett, 1989; Lake et al., 1984; Ure, 1996). The single extraction of solid phase metal in soil cannot be considered adequate to obtain a response for long-term risk assessment (Quevauviller et al., 1996) and seems more appropriate for the estimation of short- and medium-term metal risks (Tack and Verloo, 1996). Sequences of different chemical extractants have therefore become more popular for quantification of metals in different forms in soils (Ure et al., 1993b), usually starting with the weakest and least aggressive and ending with the strongest and most aggressive (Beckett, 1989). Use of a sequence of extractants of increasing strength may be of greater value than single extractants to determine the chemical distributions of metals in soils. Sequential chemical extraction techniques fractionate heavy metals into forms of different solubilities and mobilities, and can therefore furnish potentially valuable information for predicting metal availability to plants, metal movement in the soil profile, and transformation between different forms of metals in soils.

Ure et al. (1993a,b) proposed the adoption of a common extraction protocol throughout the Member States of the European Union. It was suggested that a common scheme would allow comparison among the results from different laboratories. A well-designed sequential extraction scheme can give improved selectivity and avoid, or at least minimise, solubilisation of multiple fractions in one extract (Miller et al., 1986) and hence can provide detailed information on the different states of existence of a metal in soils.

In the present work, we partitioned the chemical fractions of Cu in a paddy soil irrigated with Cu-rich wastewater in Jiangsu Province, East China, using single and sequential extractions. The single extractant solutions were water, ammonium acetate and EDTA for extraction of water-soluble, exchangeable and complexed Cu fractions. A three-step sequential extraction procedure recommended by Ure et al. (1993a,b) and subsequently

modified by Luo and Christie (1998) was also used to fractionate soil solid metal into three fractions: weak acid-soluble, reducible Fe and Mn oxide bound, and oxidisable organic matter bound. The residual fraction was calculated as the difference between total (aqua regia extractable) Cu and the sum of the three sequential fractions. Copper accumulation in the soil profile and in rice (*Oryza sativa*) plants was also investigated and their relationship with Cu speciation in the soil is discussed in order to improve our understanding of soil contamination and provide useful information for soil remediation.

2. Materials and methods

The cultivated clay loam soil was developed originally from lacustrine sediments of Lake Tai Hu area, Jiangsu Province, East China. Due to irrigation with Cu-enriched wastewater in a small canal near a family-owned Cu ingot factory in May 1997, an area of 60 ha was polluted and the transplanted rice seedlings died. Soil samples were collected for the present study in July 1998, 1 year after the accidental pollution of the canal. On the basis of rice plant growth (at the tillering stage) in the fields within the polluted area, three soil blocks (each 200 m²) likely to be representative of varying degrees of severity of soil pollution were selected as sampling sites. Composite surface soil samples (0–10 cm), each consisting of 10 subsamples, were collected. Ten samples of rice plant shoots were also collected at the same time. Soil profile samples of different depth categories of 0–10, 10–20, and 20–40 cm were also collected within the sites. The soil samples were categorised into one of three groups as heavily polluted (HP), lightly polluted (LP) and nonpolluted (NP).

The rice plants were cut just above the soil surface. After rinsing with tap water and deionized water, the samples were oven-dried at 80 °C for 24 h, and milled to pass a 0.5-mm stainless steel sieve for chemical analysis. The milled samples were digested in a mixture of HNO₃ and HClO₄ (3:2 v/v).

The soil samples were air-dried to remove stones and coarse plant roots or residues, ground to pass a 2-mm stainless steel sieve, then stored in plastic bags prior to chemical analysis. Subsamples (<2 mm) were used for the determination of soil pH (soil/water ratio: 1:2.5), total carbon and chemical speciation. The milled samples (<0.15 mm) were used for the determination of total Mn and Cu using the aqua regia method (Department of the Environment, 1987). The three types of soils (NP, LP and HP) had a pH of 6.2, 6.2 and 7.0, total C contents of 1.4%, 1.5% and 1.4 %, total Mn concentrations of 360, 278 and 283 mg kg⁻¹, and total Cu concentrations of 23, 90 and 158 mg kg⁻¹, respectively.

2.1. Single chemical extraction

All the following procedures were conducted at ambient room temperature using acid-washed apparatus. Copper was determined on the filtered extracts (including blanks) using a Perkin-Elmer Model 5000 Flame Atomic Absorption Spectrometer. All the chemical extractants were analytical grade reagents.

- (1) H₂O extraction: each sample (40.0 g oven dry basis) of <2 mm air-dried soil was placed in a 250 ml polyethylene centrifuge tube. Distilled water was added to each

centrifuge bottle to give a 1:1 soil/solution ratio (w/v) in order to extract an adequate volume of soil solution for chemical analysis. After shaking at 120 rpm for 30 min and centrifugation at $3000 \times g$ for 30 min using a MISTRAL 3000i Large Capacity Bench Centrifuge, all the supernatants were immediately filtered through Whatman No. 41 filter paper into polycarbonate jars. The filtrates were passed through 0.20- μm pore cellulose acetate filters under negative pressure (1 bar), and then stored at 4 °C prior to analysis. The filtered soil solutions ($<0.20 \mu\text{m}$) were used for measurement of Cu.

- (2) NH_4OAc extraction: each sample (5.0 g oven dry basis) of <2 mm air-dried soil was placed in a 50-ml polyethylene centrifuge tube. Twenty-five milliliters of 1 mol l^{-1} NH_4OAc extractant solution at pH 7.0 was added. The tubes were placed on an orbital shaker (120 rpm) and allowed to equilibrate for 16 h. Following equilibration, the suspensions were centrifuged at $3000 \times g$ for 5 min and the supernatants were filtered through Whatman No. 40 filter paper into 60-ml polycarbonate jars. The filtrates were used directly for the determination of Cu.
- (3) EDTA extraction: each sample (5.0 g oven dry basis) of <2 mm air-dried soil was placed in a 50-ml polyethylene centrifuge tube. Twenty-five milliliters of 0.05 mol l^{-1} EDTA (ethylenediaminetetra-acetic acid di-ammonium salt solution) extractant solution at pH 7.0 was added. The tubes were placed on an orbital shaker (120 rpm) and allowed to equilibrate for 1 h. Following equilibration, all steps were the same as described above for the ammonium acetate extract.

2.2. Sequential chemical extractability

The air-dried subsamples (<2 mm) were also subjected to sequential chemical extraction. A modified three-step sequential extraction procedure (0.11 mol l^{-1} CH_3COOH , 0.1 mol l^{-1} $\text{NH}_2\text{OH} \cdot \text{HCl}$ adjusted to pH 2.0 with HNO_3 , and $30\% \text{H}_2\text{O}_2 + 1 \text{ mol l}^{-1}$ NH_4OAc adjusted to pH 5.0 with HOAc) was used to fractionate soil solid metal into three fractions: weak acid-soluble, reducible Fe and Mn oxide bound, and oxidisable organic matter bound (Luo and Christie, 1998). The residual fraction was taken as the difference between the total (aqua regia extractable) fraction and the sum of the three sequential fractions.

Metal concentrations were adjusted to a soil DM basis. The results were reported as mean values of triplicate analyses. The results of each extract were also expressed as a percentage of the aqua regia-soluble concentration. Data were tested by analysis of variance (Genstat 5 Committee, 1994). Individual means within each variable were compared by least significant difference ($\text{LSD } \alpha=0.05$).

3. Results

3.1. Copper distribution in the soil profiles

Copper accumulated mainly in the top layer (0–10 cm) of the soil profile after wastewater irrigation over 1-year period, and the concentration of Cu increased almost seven-

Fig. 1. Distribution of Cu in the profiles of soils collected from Cu heavily polluted (HP), lightly polluted (LP) and nonpolluted (NP) sites.

fold (158 mg kg^{-1}) compared with that of non-irrigated soil in the same area (23 mg kg^{-1}). Copper in the subsurface layer (10–20 cm) was also significantly elevated (Fig. 1).

3.2. Soil extractable Cu by the single extractions

Table 1 shows the soil extractable Cu fractions using single chemical extractions. The concentrations of Cu in the water-soluble, exchangeable (NH_4OAc -extractable) and complexed (EDTA-extractable) fractions increased rapidly, from 0.02 mg kg^{-1} in unpolluted soil to 0.12 mg kg^{-1} in polluted soil, from 0.33 to 6.30 mg kg^{-1} and from 14.1 to 97.8 mg kg^{-1} , respectively. EDTA-extractable Cu was much higher than NH_4OAc -extractable and water-soluble Cu in the soils.

3.3. Soil extractable Cu by a sequential extraction

Irrigation also led to a marked increase in Cu concentration in the weak acid-soluble (HOAc -extractable), reducible Fe and Mn oxide bound ($\text{NH}_2\text{OH.HCl}$ -extractable), oxidisable organic matter bound (H_2O_2 -extractable) and residual fractions. In the contaminated paddy soil, the reducible Fe and Mn oxide fraction was the largest (36% of total Cu), then the oxidisable followed by the residual, and the smallest was the acetic acid-soluble fraction. However, in the unpolluted soil, 53% of the total was present in the residual

Table 1
Soil extractable Cu by single extractions

Soil ¹	Extractable					
	H_2O (mg kg^{-1})	NH_4OAc (mg kg^{-1})	EDTA (mg kg^{-1})	H_2O_2^2 (%)	NH_4OAc^2 (%)	EDTA ² (%)
NP	0.02c ³	0.33c	14.1c	0.08	1.43	61.3
LP	0.08b	1.63b	60.6b	0.09	1.81	67.3
HP	0.12a	6.30a	97.8a	0.08	3.99	62.0

¹ NP: nonpolluted; LP: lightly polluted; HP: heavily polluted.

² As a percentage of the aqua regia-soluble concentration.

³ Means within columns followed by the same letter are not significant differences by LSD at the 5% level.

Fig. 2. Concentration of Cu in the rice plants grown in Cu heavily (HP) and lightly (LP) polluted and nonpolluted (NP) soil.

fraction. The reducible Fe and Mn oxide fraction was the second largest, representing 22% of total Cu.

3.4. Concentration of Cu in the rice plants grown in the polluted soils

The Cu concentrations in the shoots of the rice grown in the polluted soil (27 mg kg⁻¹ in LP and 38 mg kg⁻¹ in HP) increased greatly, with a magnitude of two- to three-fold than in the unpolluted soil (Fig. 2). Plant yields were observed to be substantially lower in the polluted soil compared to those in the unpolluted soil (data not presented).

4. Discussion

Copper would be expected to accumulate in the top layer of the paddy soil after wastewater irrigation (Fig. 1). This may be attributed to soil sorption as shown by single and sequential chemical extractabilities (Tables 1 and 2). Irrigation substantially increased soil total Cu, with a magnitude seven times than that in the non-irrigated soil, resulting in soil pollution with Cu. A significant elevation in water-soluble, exchangeable and complexed Cu fractions occurred. Sequential chemical extraction also showed an increase in acid-soluble, adsorbed, reducible and oxidisable Cu fractions in the soils. These indicate

Table 2
Extractable soil Cu by a sequential extraction

Soil ¹	Sequentially extractable							
	HOAc ¹ (mg kg ⁻¹)	NH ₂ OH.HCl (mg kg ⁻¹)	H ₂ O ₂ (mg kg ⁻¹)	Residual (mg kg ⁻¹)	HOAc ² (%)	NH ₂ OH. HCl (%)	H ₂ O ₂ ² (%)	Residual ² (%)
NP	1.0	5.0	4.8	12.2	4.3	21.7	20.9	53.0
LP	13.1	28.1	25.4	23.4	14.6	31.2	28.2	26.0
HP	24.3	56.8	40.0	36.9	15.4	35.9	25.3	23.4

¹ NP: nonpolluted; LP: lightly polluted; HP: heavily polluted.

² As a percentage of the aqua regia-soluble concentration.

an increase in mobility and bioavailability of Cu in the polluted paddy soil. The accumulation of Cu in the mobile and bioavailable forms may have increased the Cu concentration (up to 38 mg kg^{-1}) in the rice plants in the contaminated soil, leading to Cu toxicity in the plants. The observation that the concentrations of Cu in rice plants grown in both the lightly and the heavily polluted soils (Fig. 2) were over the critical toxic level of 20 mg kg^{-1} (MacNicol and Beckett, 1985) supports this conclusion.

One unexpected result was that the reducible Fe and Mn oxide bound Cu was much higher than the oxidisable organic matter bound fraction in the polluted soil. A high affinity of Cu for organic matter and its existence mainly in the organic matter bound fraction in upland soils have previously been reported by Luo and Christie (1998). The discrepancy may be attributed to the large quantity of amorphous Fe and Mn hydroxides in the paddy soil in the present study (Ma and Chen, 1999). The amorphous hydroxides have large surface areas and binding capacities for metal sorption (Chen, 1996). The reducible Fe and Mn oxides may be, therefore, more important for controlling mobility and bioavailability of Cu than the oxidisable organic matter in similar paddy soils. Information on metal speciation may be useful for the development of future remediation strategies for metal-contaminated rice paddy soils.

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