



# Copper fractionation and extractability in two contaminated variable charge soils

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## Abstract

The impact of heavy metal contamination on crop growth and water quality has become a public concern in southern China where variable charge soils are widespread. A laboratory experiment was designed with contaminated levels of copper (Cu) to estimate the distribution of anthropogenic Cu in different constituents of two variable charge soils using a sequential extraction procedure (SEP) and to evaluate Cu extractability with a single extraction procedure. Soil Cu was chemically fractionated into water-soluble, exchangeable, weakly specifically adsorbed, Fe/Mn oxide-bound, organically bound, and residual fractions using the SEP. One mole  $\text{NH}_4\text{Ac l}^{-1}$  (ammonium acetate, pH 5.0), 0.1 mol  $\text{HCl l}^{-1}$ , and Mehlich 3 were employed as the single extractant to evaluate the mobility or availability of anthropogenic Cu added in the variable charge soils. The two soils were an inceptisol (clayey mixed siliceous thermic Dystrachrept) and an ultisol (clayey kaolinitic thermic Plinthudults), and the Cu levels were treated at 250, 500, 1000 or 1500 mg Cu  $\text{kg}^{-1}$  soil as  $\text{Cu}(\text{NO}_3)_2$ . During a 6-week incubation, most of the anthropogenic Cu was associated with the mobile fractions, i.e. water-soluble, exchangeable, and weakly specifically adsorbed fractions in the inceptisol, whereas in the ultisol the mobile fractions became dominant only at the Cu amendments of 1000 and 1500 mg Cu  $\text{kg}^{-1}$  soil. The Fe/Mn oxide-bound fractions that are relatively stable prevailed in both soils except for the high Cu level treatments. Soil organic matter had a minimal effect on the anthropogenic Cu distribution in the soils because of its low concentration range. Transformations between the mobile fractions and Fe/Mn oxide-bound fraction occurred during the 6-week incubation in both soils. The 1 mol  $\text{NH}_4\text{Ac l}^{-1}$  (pH 5.0) and Mehlich 3 extractable Cu in both Cu-amended soils were statistically associated with the mobile fractions according to the stepwise multiple regressions. However, the 0.1 mol  $\text{HCl l}^{-1}$  extractions resulted in an overestimation of Cu availability in these soils due to the dissolution of Fe/Mn oxides, which released part of the adsorbed Cu that may not be available to plants under normal soil conditions.

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## 1. Introduction

Under intensifying pressure of industrialization in both developing and developed countries, more anthropogenic copper and other heavy metals enter

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agricultural fields and contaminate the food-chain of human beings. Dry and wet deposition around mining and smelting sites (Karczewska 1995; Grazebisz et al., 1997; Derome and Lindroos, 1998), wastewater irrigation (Tam and Wong, 1996; Cao et al., 2000; Luo et al., 2003), compost application, including municipal waste, sewage sludge or their combination (Pichtel and Anderson, 1997; Nyamangara, 1998; Planquart et al., 1999; Walter and Cuevas, 1999; Baldwin and Shelton, 1999; Veeken and Hamelers, 2002; Soumaré et al., 2003; Zheljzakov and Warman, 2003), and spraying of heavy metal-containing pesticides or herbicides (Besnard et al., 2001; Balasoju et al., 2001) have been reported to contribute to the input of anthropogenic copper and other heavy metals into agricultural soils.

Excess copper and heavy metals in soils are toxic to plants and soil organisms. Plant yield reduction and growth retardation (Moreno et al., 1997) and structural community changes of soil microorganisms and nematodes (Ellis et al., 2001) were reported to occur in heavy metal contaminated soils. More effect-based limits have been legislated by several nations or organizations, such as China, the Commission of European Communities, France, New Zealand, and the USA, to limit the use of heavy metal-contaminated wastes or composts in agricultural land. A series of permissible concentrations were established for each metal mainly based on total metal content. However, phytotoxicities or toxicities to soil organisms are often not determined by total metal contents in soils but by metal's 'bioavailable' concentrations (Qian et al., 1996). Moreover, the risk assessments of heavy metals in soils for surface and ground water contamination or other environmental issues are based on their chemical 'lability' in soils rather than on their total contents. 'Bioavailability' or chemical 'lability' of heavy metals strongly depends on their specific physicochemical features in soils, i.e. chemical fraction or speciation (Kabata-Pendias, 1993) and, consequently, on soil physicochemical characteristics (Planquart et al., 1999).

Fractionation studies of copper in soils using a sequential extraction procedure (SEP) can provide an understanding of its chemical fractions and potential bioavailability (Grazebisz et al., 1997), although the SEP is more operationally than theoretically defined because few of the extractants are specific enough to

isolate a well-defined phase (Schramel et al., 2000). Routinely, the SEP fractionates soil copper into water soluble (WS), exchangeable (EX), weakly specifically adsorbed (SP), Fe/Mn oxides-bound (OX), organically bound (OR), and residual (RE) fractions using specific extractants by successive extractions (Tessier et al., 1979; Planquart et al., 1999; Schramel et al., 2000).

Heavy metals associated with different fractions have different impacts on the environment (Tam and Wong, 1996). Copper in soils is mainly associated with clay minerals and organo-clay associations (<2 µm fractions) and particulate organic matter according to the analyses of the physical fractionation scheme (Besnard et al., 2001). Results from the SEP studies showed that in Cu-contaminated soils, fractionation of copper was determined by organic matter and Fe, Al and Mn oxides. Up to 57% of total soil fractionated Cu was bound to organic constituents in contaminated soils around a Poland copper smelter within a 4-km-diameter area (Grazebisz et al., 1997). However, in unpolluted soils, most Cu was found in the residual fraction (Kuo et al., 1983; Levesque and Mathur, 1986; Grazebisz et al., 1997; Nyamangara, 1998). Agbenin and Olojo (2004) reported that the removal of amorphous hydrous oxides resulted in a greater decrease in Cu adsorption in an alfisol than the removal of organic matter. Therefore, for variable charge soils, especially those with low organic matter content, oxides of Fe, Mn, and Al are believed to play a key role in adsorption of anthropogenic Cu.

In southern China, there is a large range of variable charge soils, including oxisols, ultisols, inceptisols, and alfisols. These soils are usually acidic and poor in plant nutrients but rich in Fe and Al oxides. Since the 1980s, with a rapid economic development in this region, vast citrus groves and vineyards have been planted, and many small Cu smelters have also been established to traditionally recycle used copper products. Copper contaminations from application of Cu-containing herbicides and municipal wastes in fruit gardens, and from dust emission, and solid and liquid wastes produced by small Cu smelters have resulted in severe environmental problems, such as reduced crop growth and degraded water quality. Risk assessment of Cu contamination and remediation of Cu-contaminated soils are of great importance in this region. Although Cu adsorption–desorption has been studied

for these variable charge soils (Yu et al., 2002), the relationships between Cu bioavailability/lability and soil Cu fractions remain to be evaluated. Information on the fates of anthropogenic Cu and its transformation dynamics in these acid variable charge soils is needed for improving soil and environmental quality.

This study was designed to investigate dynamic changes of Cu fraction and extractability as affected by loads of anthropogenic Cu under contaminated levels in two representative variable charge soils from southern China, and to understand the fate of anthropogenic Cu in these variable charge soils and the potential risks of Cu contamination to crop growth and environmental quality.

## 2. Method and materials

### 2.1. Soils

Two variable charge soils (an ultisol and an inceptisol) were sampled at the depth of 0–20 cm from two citrus groves in Longyou County, Zhejiang Province, China (E 119°02′–120°20′, N 28°44′–29°17′). Longyou County is located in a semitropical monsoon zone with an annual average temperature of 17 °C, an annual precipitation of 1400 mm, and an annual nonfrost period of up to 249 days. According to USDA soil taxonomy (USDA, 1988), the clayey loamy soil that developed on the Arenaceous rock (a sedimentary sandstone) is an inceptisol (clayey mixed siliceous thermic typic Dystrochrept), and the other clayey soil derived from the highly weathered Quaternary red earths is an ultisol (clayey kaolinitic thermic Plinthudults). Soils were air-dried and ground to <2 mm for an incubation experiment, and subsamples were ground to <0.25 and <0.125 mm, respectively, for physical and chemical property analyses. Subsamples of each soil were analyzed for (1) particle-size distribution by a hydrometer method (Liu et al., 1996); (2) mineralogical composition of the clay fraction by X-ray diffraction (Siemens D500 X-ray diffractometer with CuK $\alpha$  at 40 kV and 40 mA); (3) soil pH at a soil/solution ratio of 1:2.5 in both deionized water and 1 mol KCl l<sup>-1</sup> using a pH meter (Mettler-Toledo MP120, Schwerzenbach, Switzerland); (4) soil organic carbon by a modified Tinsley method (Lu, 2000); (5) total exchangeable acidity and

exchangeable-Al<sup>3+</sup> and -H<sup>+</sup> by the 1 mol KCl l<sup>-1</sup> extraction–titration method (Liu et al., 1996); (6) cation exchange capacity (CEC) and exchangeable bases extracted by the 1 mol NH<sub>4</sub>Cl l<sup>-1</sup> (pH 7.0) as described by Lu (2000); (7) exchangeable Cu extracted by the 0.1 mol HCl l<sup>-1</sup> at a soil/solution ratio of 1:5 (Lu, 2000) and total Cu using the HF-HClO<sub>4</sub> digestion method (Tessier et al., 1979), with the concentrations of Cu in the extracts or digests

Table 1  
Selected physiochemical properties of the variable charge soils

Soil properties	Soils	
	The inceptisol	The ultisol
Particle size (g kg <sup>-1</sup> )		
Clay (<0.002 mm)	421.0	483.4
Silt (>0.002 and <0.05 mm)	350.1	261.9
Sand (>0.05 mm)	228.8	254.7
Textural class (American textural grade)	clayey loam	clay
Main clay minerals <sup>a</sup>	K, O, Chl, F	K, O
PH (H <sub>2</sub> O)/(1 mol KCl l <sup>-1</sup> )	4.86 (0.01) <sup>b</sup> / 3.96 (0.00)	5.39 (0.01)/ 4.33 (0.01)
Total organic carbon (g C kg <sup>-1</sup> )	6.65 (0.20)	9.03 (0.29)
Exchangeable acidity (mmol H <sup>+</sup> kg <sup>-1</sup> )		
Total	41.09 (0.65)	15.20 (0.00)
H <sup>+</sup>	4.15 (0.65)	0.95 (0.00)
Al <sup>3+</sup>	12.31 (0.00)	4.75 (0.00)
CEC <sub>pH 7.0</sub> (cmol kg <sup>-1</sup> )	12.12 (0.17)	15.99 (0.36)
Exchangeable bases (mmol kg <sup>-1</sup> )		
Total	77.32 (1.11)	120.04 (0.44)
Ca <sup>2+</sup>	33.94 (0.47)	50.98 (0.26)
Mg <sup>2+</sup>	3.40 (0.07)	7.72 (0.01)
K <sup>+</sup>	2.64 (0.09)	2.64 (0.09)
Na <sup>+</sup>	nd <sup>c</sup>	nd
Background levels of Cu (mg kg <sup>-1</sup> )		
Total Cu	26.39 (0.53)	80.45 (1.57)
Exchangeable Cu (0.1 mol l <sup>-1</sup> HCl, 1:5)	1.83 (0.02)	32.34 (0.19)
Oxides of Fe and Al (g kg <sup>-1</sup> )		
Free Fe oxide (Fe <sub>2</sub> O <sub>3</sub> )	60.36 (5.65)	52.45 (1.89)
Free Al oxide (Al <sub>2</sub> O <sub>3</sub> )	24.64 (1.39)	35.57 (0.66)
Amorphous Fe oxide (Fe <sub>2</sub> O <sub>3</sub> )	0.99 (0.11)	1.47 (0.06)
Amorphous Al oxide (Al <sub>2</sub> O <sub>3</sub> )	1.19 (0.06)	1.53 (0.19)

<sup>a</sup> K, kaolinite; O, Fe and Al oxides; Chl, chlorite; F, feldspar.

<sup>b</sup> Mean (standard deviation, SD).

<sup>c</sup> nd means 'not detected'.

determined using an atomic absorption spectrometry (AAS) with acetylene/air flame (AA6800, Shimadzu, Kyoto, Japan); (8) the contents of free Fe and Al oxides by the citrate–bicarbonate–dithionite method, and amorphous Fe and Al oxides by the ammonium oxalate method (pH 3.2) (Lu, 2000), with the concentrations of Fe and Al in the extracts determined by the colorimetric method proposed by Xu and Chen (1980). Selected properties of the soils mentioned above are presented in Table 1.

### 2.2. Incubation experiment

Portions of soils (1.0 kg) were treated respectively with 0, 250, 500, 1000 or 1500 mg Cu kg<sup>-1</sup> as Cu(NO<sub>3</sub>)<sub>2</sub>. Each treatment was thoroughly mixed and triplicated. The treated soils were placed in small plastic buckets and incubated in a greenhouse with a random arrangement. Soil moisture was adjusted to 45 ± 5% of water holding capacity (WHC) and main-

tained by daily addition of deionized water. Subsamples of the incubated soils were collected at weeks 1, 2, 4 and 6 of the incubation. The samples were air-dried and ground to <0.25 mm prior to use.

### 2.3. Sequential extraction of copper from soils

The sequential extraction procedure (SEP) of Tessier et al. (1979) was modified and used for sequential extraction as outlined in Table 2. The water soluble fraction step was added before the extraction of exchangeable fraction. Two grams of soil was used for extraction and a water-washing step was placed between the two successive extractions to prevent carryover of Cu<sup>2+</sup> in the trapped solution. Copper concentration in the filtrates of each extract was determined using the flame AAS. In order to evaluate Cu recovery of the SEP, a separate soil sample was simultaneously digested as the residual fraction digestion of the SEP.

Table 2  
Detailed procedure for the sequential extraction of copper from the soils

Fraction	Extractant	Soil/ solution ratio (W/V)	Extraction procedure
Water soluble	Deionized water	1:8	The suspension is shaken at 200 rpm for 2 h at 25 °C, then centrifuged at 2500 × g for 15 min and the supernatant is filtered through a Whatman #42 filter paper.
Exchangeable	1 mol MgCl <sub>2</sub> l <sup>-1</sup> (pH 7.0)	1:8	
Weakly specifically adsorbed	1 mol NaOAc l <sup>-1</sup> (pH 5.0)	1:8	
Fe/Mn oxide-bound	0.04 mol NH <sub>2</sub> OH·HCl l <sup>-1</sup> in 25% HAc	1:20	The extraction is conducted in a water bath at 96 ± 3 °C for 6 h with occasional stirring, and the suspension centrifuged at 2500 × g for 15 min. The supernatant is filtered through a Whatman #42 filter paper.
Organically bound	3 ml of 0.02 mol l <sup>-1</sup> HNO <sub>3</sub> and 5 ml of 30% H <sub>2</sub> O <sub>2</sub> (pH 2.0, adjusted by conc. HNO <sub>3</sub> ) 3 ml of 30% H <sub>2</sub> O <sub>2</sub> (pH 2.0) 5 ml of 3.2 mol NH <sub>4</sub> Ac l <sup>-1</sup> in 20% HNO <sub>3</sub> , diluted up to 20 ml by deionized water		The extraction is conducted in a water bath at 85 ± 2 °C for 2 h with occasional stirring.  The suspension is shaken at 200 rpm for 2 h at 25 °C, and centrifuged at 2500 × g for 15 min. The supernatant is filtered through a Whatman #42 filter paper.
Residual	The residue is resuspended into 5 ml ethanol and quantitatively transferred to a polytetrafluoroethylene crucible. The suspension was dried by evaporation and digested as described by Tessier et al. (1979)		

#### 2.4. Single extraction for available copper evaluation

The extractants of 0.1 mol HCl l<sup>-1</sup>, 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0), and Mehlich 3 (Mehlich, 1984) were employed to estimate the amounts of soil available copper in soil samples collected at the end of incubation. Ratios of soil vs. solution were 1:20, 1:10 and 1:8 for 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0), 0.1 mol HCl l<sup>-1</sup>, and Mehlich 3, respectively. All extractions were subjected to a 30-min shaking at 200 rpm and a 15-min stand. The suspensions were filtered through a Whatman #42 filter paper, and Cu concentrations in the filtrates were determined using the flame AAS.

#### 2.5. Statistical analysis

All data were processed by Microsoft Excel 2002, and the linear regression and the stepwise linear regressions were conducted using the programs of SAS System for Windows Release 8.02 (SAS Institute, 1999).

### 3. Results and discussion

#### 3.1. The soils

The ultisol was rich in kaolinite and Fe and Al oxides according to results of the X-ray diffraction of clay minerals (<0.002 mm) (Table 1). The inceptisol contains some chlorite and feldspar as well as kaolinite and Fe and Al oxides (Table 1). Both soils were limed for better citrus production resulting in a high content of exchangeable Ca for both the soils (Table 1). Compared with the inceptisol, the ultisol pH was approximately 0.5 units higher and the organic carbon content was 50% higher, but exchangeable acidity was lower (Table 1). Results from soil analyses (including the cation exchange capacity (CEC), exchangeable bases, and organic carbon and clay contents) and Cu adsorption experiments indicate that the ultisol had a larger Cu adsorption capacity than the inceptisol. The Cu monolayer adsorption maximum was 25.9 mmol Cu<sup>2+</sup> kg<sup>-1</sup> soil for the ultisol and 20.2 mmol Cu<sup>2+</sup> kg<sup>-1</sup> soil for the inceptisol, as obtained from the Langmuir model (Yu et al., 2002). These two variable charge soils had a Cu adsorption capacity 13–50 times higher than the four Portugal soils, two Fluvi-

sols, one Luvisol, and one Podzol tested by Mesquita and Vieira e Silva (2002), of which maximum Cu adsorption ranged from 0.5 to 1.5 mmol Cu<sup>2+</sup> kg<sup>-1</sup> soil.

Total Cu content in the original soils was higher in the ultisol than in the inceptisol, 80.5 and 26.4 mg kg<sup>-1</sup> soil, respectively. The ultisol from a mature citrus grove had received some anthropogenic Cu by application of municipal wastes, whereas the total Cu content in the inceptisol was mainly derived from the parent material, for the average soil total Cu in southern China is approximately 26.0 mg kg<sup>-1</sup> soil (Xie, 1996). More than 67.1% of the total soil Cu in the inceptisol was in the residual fraction, the lithogenic form, whereas the ultisol had only 32.9% of total soil Cu in this fraction (Table 3). Moreover, the ultisol had more Cu in the Fe/Mn oxide-bound fraction than in the organically bound fraction, 40.5% and 15.3% of the total soil Cu, respectively and, the sum of mobile fractions (water soluble, exchangeable and weakly specifically adsorbed fractions) accounted for only 11% of the total soil Cu (Table 3). The inceptisol had approximately 11% of the total soil Cu bound to the Fe/Mn oxides or organic matter, and a similar percentage of the mobile fractions to the ultisol (Table 3). Unlike other soils with high organic matter contents, the organically bound Cu did not account for a

Table 3  
Fractionation of Cu in the variable charge soils without Cu amendment and the recovery of Cu by the SEP

Fractions	The ultisol		The inceptisol	
	mg Cu kg <sup>-1</sup>	%	mg Cu kg <sup>-1</sup>	%
Water soluble	0.50 (0.15) <sup>a</sup>	0.60	0.66 (0.42)	2.32
Exchangeable	1.03 (0.70)	1.25	0.96 (0.10)	3.40
Weakly specifically adsorbed	7.82 (0.56)	9.5	1.41 (0.32)	4.97
Fe/Mn oxide-bound	33.38 (2.98)	40.53	3.19 (0.61)	11.25
Organically bound	12.56 (1.73)	15.25	3.11 (0.94)	10.99
Residual	27.07 (0.50)	32.87	19.00 (0.76)	67.07
Total fractions	82.36	100.00	28.32	100.00
Total Cu <sup>b</sup>	80.45	102.37 <sup>c</sup>	26.39	107.31 <sup>c</sup>

<sup>a</sup> Mean (standard deviation, SD).

<sup>b</sup> Soil total Cu content was measured by HF-HClO<sub>4</sub> digestion.

<sup>c</sup> The recovery rate was calculated as (total Cu fractions/total Cu) × 100.

major part of the soil Cu fractions in both tested soils because of their extremely low organic carbon contents ( $< 1\%$ ) and high concentrations of Fe and Al oxides (Table 1). Tao et al. (2003) reported a permanent charge soil in northern China with  $37.4 \text{ mg organic C kg}^{-1}$  which had 47.4% of total soil Cu (totally  $125.8 \text{ mg Cu kg}^{-1}$  soil) associated with soil organic fraction whereas the oxide-bound Cu only accounted for 15.9% of the total.

### 3.2. The sequential extraction procedure (SEP) of Cu fractionation

The recovery of soil Cu by the modified SEP was from 102% to 107% for the original soils (Table 3) and from 107% to 124% for the soils amended with different levels of Cu. The slight overestimation may be caused by systematic errors involved in the exper-

imental and analytic procedures. These results indicate that this modified SEP scheme should be acceptable for Cu fractionation in both variable charge soils, as it only slightly overestimated Cu fractions even if the soils received anthropogenic Cu.

### 3.3. Soil Cu fractionation and the fate of anthropogenic Cu

With increasing Cu amendment levels, the exchangeable and the water soluble fractions of the inceptisol markedly increased, and the exchangeable fraction became the largest fraction at the Cu amendment levels  $>250 \text{ mg kg}^{-1}$  (Fig. 1). For the ultisol, the exchangeable fraction increased quickly with Cu amendments but the Fe/Mn oxide-bound and the weakly specifically adsorbed fractions dominated soil Cu fractions in the whole range of Cu amendment

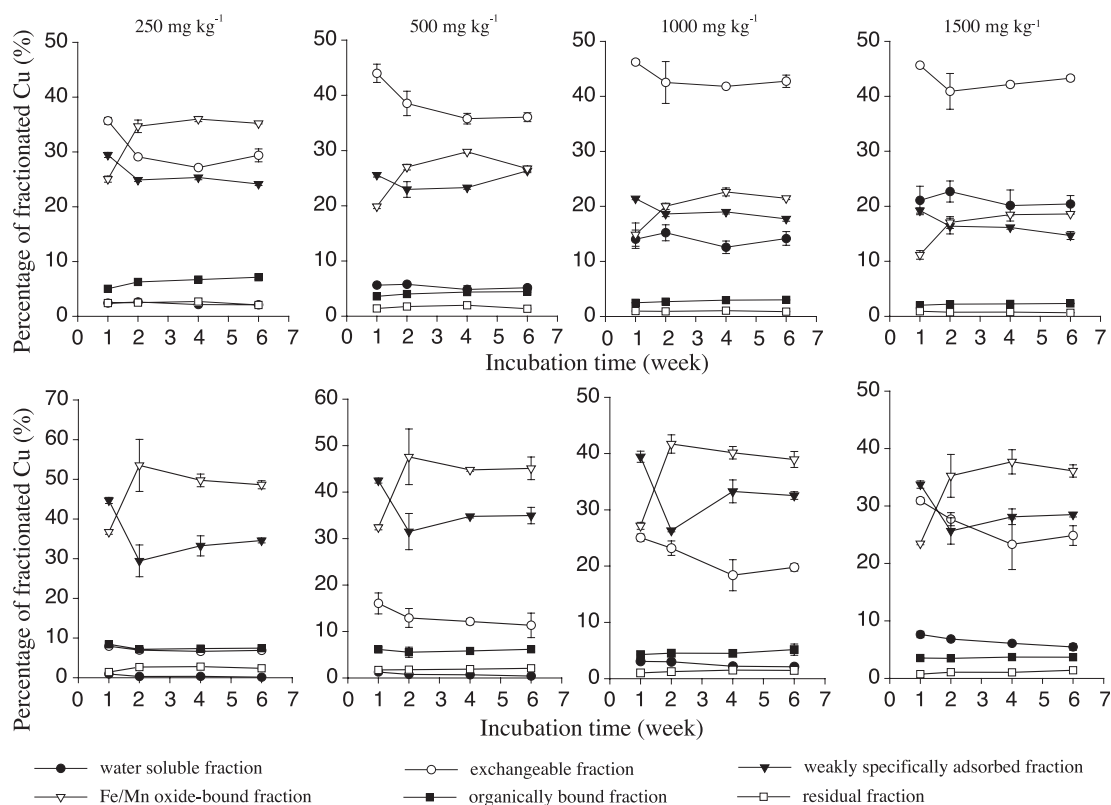


Fig. 1. Dynamic changes of Cu fractions in the two variable charge soils as affected by amended Cu levels (upper, the inceptisol; bottom, the ultisol).

levels (Fig. 1). At the Cu amendment level of 250 mg kg<sup>-1</sup>, the inceptisol had more than 88% of the added Cu associated with the Fe/Mn oxide-bound, the exchangeable, and the weakly specifically adsorbed fractions, whereas for the ultisol, more than 83% of the added Cu was in the Fe/Mn oxide-bound and the weakly specifically adsorbed fractions (Fig. 1). Results from a paddy soil contaminated by Cu-rich wastewater irrigation showed that over 52% of the loaded Cu was associated with exchangeable and oxide-bound fractions (Luo et al., 2003). The organic matter in both soils did not influence the fate of anthropogenic Cu in this study, although it was reported to control Cu chemical behavior in soils with high organic matter contents (Tam and Wong, 1996; Besnard et al., 2001; Impellitteri et al., 2002; Tao et al., 2003). This could be attributed to the low contents of soil organic carbon (<1%) in the variable charge soils. Agbenin and Olojo (2004) found that the removal of amorphous hydrous oxides by oxalate buffer solution (pH 3.0) reduced Cu adsorption more than the organic matter removal by H<sub>2</sub>O<sub>2</sub> in an alfisol (organic carbon content is 3.4 mg kg<sup>-1</sup>). The reduction of the Cu distribution coefficient ( $K_d$ ) in the alfisol by removal of amorphous hydrous oxides was five times more than by the organic matter removal (Agbenin and Olojo, 2004). Therefore, in the variable charge soils, the Fe/Mn oxide-bound fraction appeared to control the mobility and bioavailability of anthropogenic Cu. The contaminated Cu was mainly bound to the exchangeable surfaces and Fe and Mn oxides in the variable charge soils, and had potential to impact plant growth and water quality.

Distribution of soil Cu in different fractions attained equilibrium in both soils after 4–6 weeks of incubation for all levels of the Cu amendment (Figs. 1 and 2). Water-soluble Cu added to soils is rapidly retained by soil colloids, as mobile fractions initially, and transformed into stable fractions with time (Ma and Uren, 1998). After 1 week of incubation, the exchangeable and weakly specifically adsorbed fractions in the inceptisol were transformed into the Fe/Mn oxide-bound fraction. This transformation decreased with increasing Cu amendment levels (Fig. 2). At the Cu amendment of 250 mg Cu kg<sup>-1</sup> soil, the transformation only occurred between the weakly specifically adsorbed and the Fe/Mn oxide-bound fractions in the ultisol. However, at higher Cu amend-

ment levels, the exchangeable fraction was also partially converted into the oxide-bound fraction (Fig. 2). Ma and Uren (1998) also found that the water soluble and exchangeable Cu fractions as well as the EDTA extractable Cu fraction were transformed into the organically bound and Fe oxide-bound Cu fractions when the soil was contaminated by water-soluble anthropogenic Cu. The dose effects on the transformation between mobile (water soluble+exchangeable+weakly specifically adsorbed fractions) and relatively stable fractions (Fe/Mn oxide-bound+organically bound fraction) varied with the nature of the test soils. Compared with the ultisol, the inceptisol had fewer Cu-binding sites because of lower concentrations of organic matter and clay. With increasing Cu<sup>2+</sup> concentrations in soil solution, Fe/Mn oxides and organic matter in the inceptisol could retain Cu<sup>2+</sup> directly from the soil solution with less Cu<sup>2+</sup> transformed from the mobile. Therefore, the transformation between the mobile fractions and the relative stable fractions was less pronounced and declined gradually in the inceptisol. These observations were more meaningful by artificially grouping water soluble, exchangeable, and weakly specifically adsorbed fractions as the mobile fractions, and Fe/Mn oxide- and organically bound fractions as the relatively stable fractions (Fig. 3). The mobile fractions increased linearly with increasing Cu levels amended (the total Cu contents in amended soils determined by HF-HClO<sub>4</sub> digestion minus those in the control soil) with a steeper slope than the relatively stable fractions for the inceptisol, i.e. 1.03 ( $r^2=0.999$ ,  $n=12$ ) for the mobile fractions and 0.20 ( $r^2=0.992$ ,  $n=12$ ) for the relatively stable fractions (Fig. 3). For the ultisol, the relatively stable fractions were slightly higher than the mobile fractions when the Cu amendments were at 250 and 500 mg Cu kg<sup>-1</sup>, but the mobile fractions increased to exceed the relatively stable fractions at the Cu amendments of 1000 and 1500 mg Cu kg<sup>-1</sup> (Fig. 3). The linear slopes of both the mobile (0.74,  $r^2=0.999$ ,  $n=12$ ) and the relatively stable fractions (0.43,  $r^2=0.991$ ,  $n=12$ ) against the Cu levels amended were more comparable for the ultisol than for the inceptisol.

The amount of exchangeable Cu was extremely small, as compared with other fractions, and constituted only 0.06% of the total fractionated Cu (Grazebisz et al., 1997). However, it translated to 234 g Cu ha<sup>-1</sup>

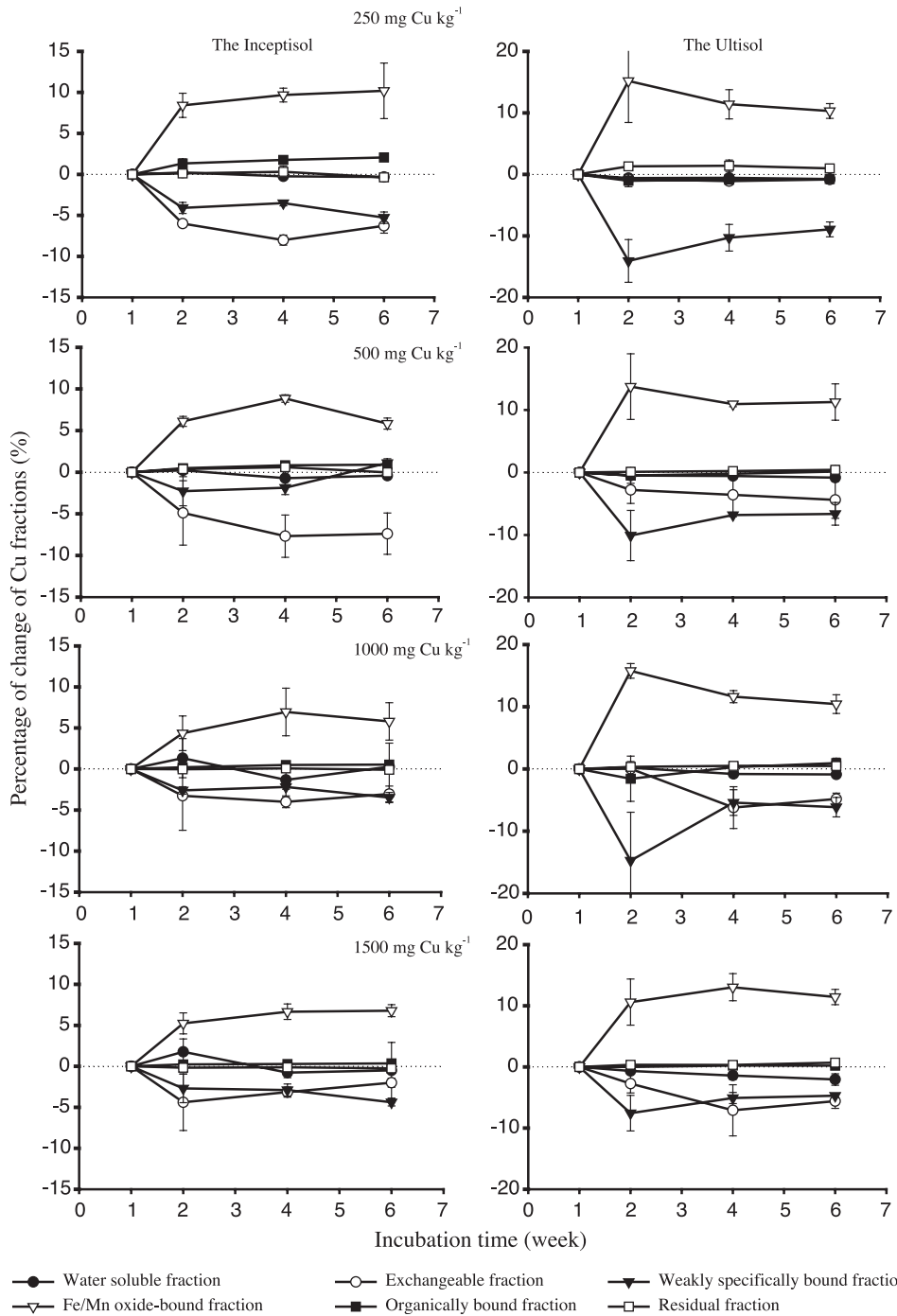


Fig. 2. The percentage change of anthropogenic Cu in different fractions with incubation time in the inceptisol and the ultisol.



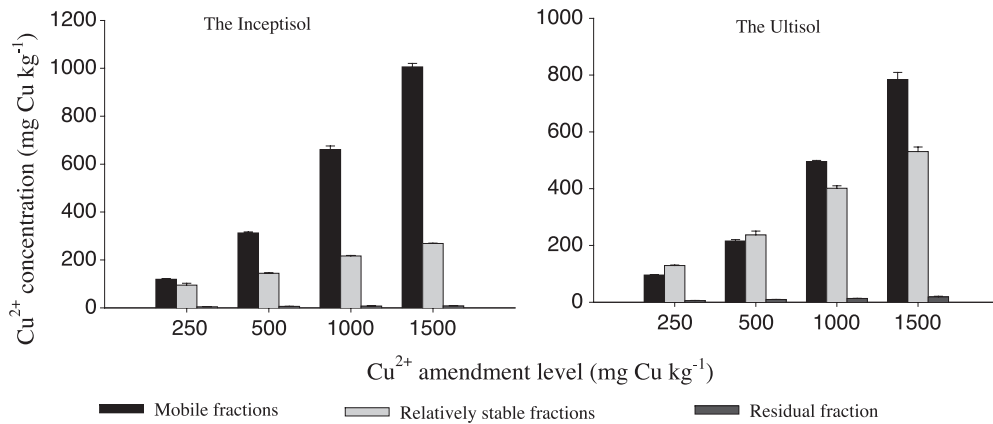


Fig. 3. Distribution of Cu fractions (mobile, relatively stable and residual fractions) in relation to Cu amendment levels in the inceptisol and the ultisol at the 6th week of incubation. (Where the mobile fractions include the water soluble, the exchangeable, and the weakly specifically adsorbed fractions, and the relatively stable fractions include the Fe/Mn oxide-bound and the organically bound fraction.)

in the top soil layer that is potentially available to crops, and only a few crops can take up this amount of Cu during a growing season (Kabata-Pendia et al., 1992).

Moreover, as indicated in Fig. 3, the ultisol adsorbed more Cu than the inceptisol. It is consistent with the result of adsorption isotherm experiment (Yu et al., 2002), probably because the ultisol had 36% more organic carbon, 32% more CEC, 15% more clay,

and higher pH value than the inceptisol (Tam and Wong, 1996; Planquart et al., 1999; Yu et al., 2002).

#### 3.4. Soil extractable Cu by 0.1 mol HCl l<sup>-1</sup>, 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0), and Mehlich 3

The amounts of soil extractable Cu by the 0.1 mol HCl l<sup>-1</sup>, 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0), and Mehlich 3 increased with Cu contaminating levels, and the 0.1

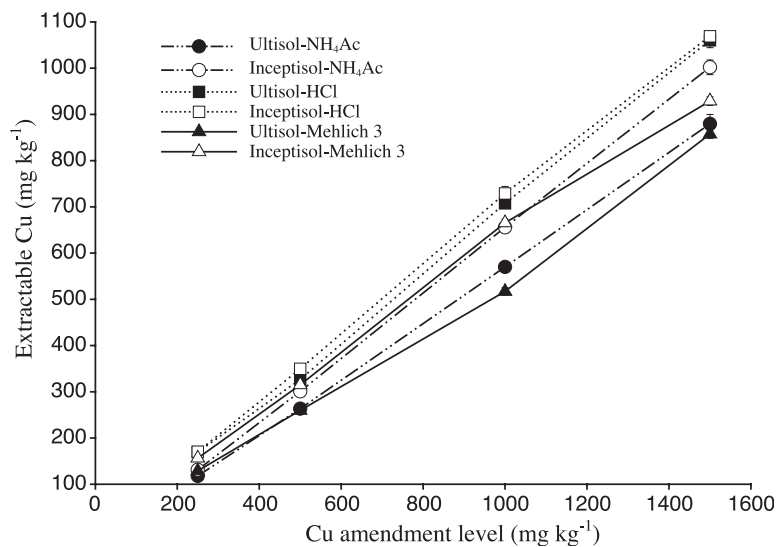


Fig. 4. Soil available Cu as a function of Cu amendment levels, extracted by the 0.1 mol HCl l<sup>-1</sup>, 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0), and Mehlich 3 in the Cu amended soils after a 6-week incubation (the inceptisol and the ultisol).

mol HCl l<sup>-1</sup> extracted slightly more Cu than any of the other two extractants (Fig. 4). The inceptisol released more Cu into the three extractants than the ultisol, which is compatible with the sequential extraction results (Fig. 4). The 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0) and Mehlich 3 extracted similar percentages of amended Cu and the percentages increased with Cu amendment levels, which ranged from 58.4% to 83.1% and 63.9% to 81.1% of amended Cu for the ultisol, and from 69.4% to 89.3% and 82.8% to 88.7% of amended Cu for the inceptisol, respectively (Table 4). However, the 0.1 mol HCl l<sup>-1</sup> extracted about 17.1–25.7% for the ultisol and 6.0–20.7% for the inceptisol more amended Cu than other extractants. In the ultisol at the 1500 mg Cu kg<sup>-1</sup>, the 0.1 mol HCl l<sup>-1</sup> extracted almost all of the amended Cu (Table 4). In a Polish soil contaminated by copper smelter, the amount of extracted Cu by the 1 mol HCl l<sup>-1</sup> was up to four times of that estimated by the Mehlich 3 (Grazebisz et al., 1997). In this laboratory experiment, the extractants extracted higher percentages of amended Cu, as compared with results from fields at the same total Cu concentration. This is due to the fact that Cu contamination in fields is developed in a relatively longer period than in the laboratory experiment, only 6 weeks. Usually, Cu-contaminated soils from fields have a relatively high residual fraction percentage, but in this laboratory experiment the residual fraction had a slight change and its percentage decreased with increasing Cu amendment levels, compared with all other fractions except for the organically bound fraction (Fig. 1).

Extractable Cu in the inceptisol by the three extractants was related to the mobile Cu fractions, and the exchangeable fraction accounted for more than 98% of the variability in the extractable Cu in

relation to the stepwise linear regressions (Table 5). For the ultisol, soil extractable Cu by the 1 mol l<sup>-1</sup> NH<sub>4</sub>Ac (pH 5.0) or Mehlich 3 was mainly associated with the exchangeable fraction as in the inceptisol, but the amount extracted by the 0.1 mol l<sup>-1</sup> HCl was correlated to the Fe/Mn oxide-bound fraction ( $R^2 = 0.9873$ ), and the exchangeable fraction accounted for only 0.93% of the variability (Table 5). This is possibly due to the nature of HCl, which could dissolve the Fe/Mn oxides and release their associated Cu.

Selection of extractant for measuring availability or bioavailability of copper in soils usually depends on the relationship between the extracted Cu amount and Cu uptake by plants (Grazebisz et al., 1997; Brun et al., 1998; Schramel et al., 2000; Brun et al., 2001). Generally, the extracted Cu that provides an estimate of soil available Cu should derive from mobile fractions which are ready to be taken up by plants or utilized by soil organism. The 0.1 mol NaNO<sub>3</sub> l<sup>-1</sup> and Mehlich 3 (Grazebisz et al., 1997), and 0.05 mol EDTA l<sup>-1</sup> and 0.43 mol HAc l<sup>-1</sup> (Schramel et al., 2000), and DTPA, 0.01 mol Na<sub>2</sub>-EDTA l<sup>-1</sup> + 1 mol NH<sub>4</sub>Ac l<sup>-1</sup>, and 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (Brun et al., 2001) have been used as extractants for assessing soil Cu bioavailability. Although different extractants have different extractability of soil Cu, pH is considered as the most important factor that affects Cu activity in soil-extractant suspension and, subsequently, the extraction of soil Cu. An extractant with lower pH often provides greater amount of soil extractable Cu than the one with higher pH (Martínez and Motto, 2000). Some researchers suggested that soil available Cu contents should be extracted by nonbuffering extractants, for example, 0.01 mol CaCl<sub>2</sub> l<sup>-1</sup>, because of their good relationships with Cu concentration in

Table 4

Percentage of extracted Cu in the total amended Cu by the three available Cu extractants (1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0), 0.1 mol HCl l<sup>-1</sup>, and Mehlich 3) in the two variable charge soils

Treatments (mg Cu kg <sup>-1</sup> )	The ultisol			The inceptisol		
	1 mol NH <sub>4</sub> Ac l <sup>-1</sup> (pH 5.0) (%)	Mehlich 3 (%)	0.1 mol HCl l <sup>-1</sup> (%)	1 mol NH <sub>4</sub> Ac l <sup>-1</sup> (pH 5.0) (%)	Mehlich 3 (%)	0.1 mol HCl l <sup>-1</sup> (%)
250	58.44 (1.16) <sup>a</sup>	63.87 (0.95)	84.07 (1.50)	69.42 (1.84)	82.77 (1.14)	90.15 (1.06)
500	68.06 (2.17)	67.18 (1.06)	88.25 (2.50)	79.46 (0.30)	83.23 (0.64)	92.35 (0.68)
1000	76.90 (0.94)	69.77 (0.98)	95.47 (0.72)	87.38 (1.63)	88.68 (1.08)	97.16 (1.95)
1500	83.14 (1.83)	81.06 (0.81)	100.21 (1.46)	89.28 (1.40)	87.25 (0.82)	95.24 (1.05)

<sup>a</sup> Mean (standard deviation, SD), as a percentage of the net content of the HF-HClO<sub>4</sub> digestion between the control and Cu amendments.

Table 5

Correlations between Cu fractionation and extractable Cu by 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0), 0.1 mol HCl l<sup>-1</sup> or Mehlich 3 in the two variable charge soils using the multiple regression

Extractants	The inceptisol			The ultisol		
	Function	Partial R <sup>2</sup>	p	Function	Partial R <sup>2</sup>	p
1 mol l <sup>-1</sup> NH <sub>4</sub> Ac (pH 5.0) 1:20	Y=22.27+1.06*WS+	EX <sup>a</sup> 0.9973	<0.0001	Y=-13.95+1.24*WS+	EX0.9872	<0.0001
	1.03*EX+0.67*SP	WS0.0017	0.0029	0.86*EX+1.33*SP	SP0.0121	<0.0001
		SP0.0004	0.0359		WS0.0004	0.0098
0.1 mol l <sup>-1</sup> HCl 1:10	Y=11.43+0.57*WS+	EX0.9989	<0.0001	Y=0.83+1.59*WS+	OX0.9873	<0.0001
	1.23*EX+0.93*OX	WS0.0006	0.0127	0.87*EX+1.77*SP-	EX0.0093	0.0008
		OX0.0001	0.1467	0.06*OX	SP0.0016	0.0272
Mehlich 3 1:8	Y=25.57-0.62*WS+	EX0.9823	<0.0001	Y=38.41+3.50*WS+	EX0.9907	<0.0001
	1.91*EX	WS0.0020	0.3125	0.13*EX+1.72*SP-	OX0.0051	0.0089
				0.09*OX-1.64*OR	WS0.0015	0.0678
					SP0.0015	0.0231
					OR0.0005	0.0768

<sup>a</sup> EX, exchangeable fraction; WS, water soluble fraction; SP, weakly specifically adsorbed fraction; OX, Fe/Mn oxide-bound fraction; OR, organically bound fraction.

plants and surface runoffs (Gupta and Aten, 1993; Brun et al., 1998; Zhang et al., 2003), and these extractants do not change soil properties, such as pH during the extraction. They believed that nonbuffering extractants could serve to indicate soil bioavailable Cu under field conditions. However, the extraction efficiency of the 0.01 mol CaCl<sub>2</sub> l<sup>-1</sup> is too low and readsorption of extracted Cu may be unavoidable. However, it is still difficult to accurately assess Cu availability to plant or soil organisms in soil based on a single chemical extraction.

The 0.1 mol HCl l<sup>-1</sup> is a common extractant for assessing heavy metal availability in acid soils in China (Lu, 2000), and the 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> and Mehlich 3 were the most recommended extractants of soil Cu or other heavy metal availability by research-

ers (Grazebisz et al., 1997; Brun et al., 2001). The 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> extractant was modified by pH adjustment to 5.0 using glacial acetic acid in this study because the pH of the two variable charge soils is close to 5.0. This modification attempted to reduce the influence of extractant pH on Cu extraction. The pH values of these extractants increase in the order of 0.1 mol HCl l<sup>-1</sup> (pH 1.0) < Mehlich 3 (pH 2.0) < 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0). The results showed no statistical difference in Cu extraction among the three extractants but the 0.1 mol HCl l<sup>-1</sup> resulted in slightly higher values of extractable Cu than the other two extractants for both soils (Table 6). It suggests that the 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0) has a similar extraction capacity as the HCl and Mehlich 3 but attacks less the oxide-bound Cu fraction in these acid variable charge

Table 6

Total Cu contents and mean of recovery rates of the SEP in the variable charge soils at different levels of Cu amendment<sup>a</sup>

Treatments (mg Cu kg <sup>-1</sup> )	The ultisol			The inceptisol		
	Total Cu <sup>b</sup> (mg kg <sup>-1</sup> )	Total Cu fractions (mg kg <sup>-1</sup> )	Recovery rate <sup>c</sup> (%)	Total Cu (mg kg <sup>-1</sup> )	Total Cu fractions (mg kg <sup>-1</sup> )	Recovery rate (%)
250	188.86	233.49 (3.25) <sup>d</sup>	123.63 (1.72)	201.65	216.30 (9.74)	107.26 (4.83)
500	378.52	453.83 (8.42)	119.90 (2.23)	386.74	447.38 (18.04)	115.68 (4.67)
1000	750.20	882.15 (32.75)	117.59 (4.37)	741.12	886.58 (46.61)	119.63 (6.29)
1500	1122.17	1300.05 (49.72)	115.85 (4.43)	1057.79	1290.00 (33.55)	121.95 (3.17)

<sup>a</sup> All data of Cu fractions and total Cu contents were subtracted from the control values.

<sup>b</sup> Soil total Cu content was measured by HF-HClO<sub>4</sub> digestion.

<sup>c</sup> The recovery rate was calculated as (total Cu fractions/total Cu) × 100.

<sup>d</sup> Mean (standard deviation, SD).

soils. The oxide-bound Cu fraction, just like the organically bound Cu fraction, is a relatively stable fraction, which could be transferred to mobile fractions under certain conditions, but is not a fraction readily available to plants or soil organisms. Dissolution of the oxide-bound Cu fraction might overestimate soil Cu availability/bioavailability. Relationships between the extractable Cu by the three extractants and the fractions measured by the SEP revealed that the 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0) is the best extractant for Cu availability assessment in the acid variable charge soils based on its extracting capacity and efficiency, and the risk of attacking the oxide-bound Cu fraction (Table 5, Fig. 4). The NH<sub>4</sub>Ac extractable Cu is reported to be more closely related to Cu uptake of plants than the CaCl<sub>2</sub> extractable Cu (Sakal et al., 1984) and the -COO functional group of the acetate anion has a strong affinity for Cu<sup>2+</sup> (Stevenson and Fitch, 1981), which forms soluble organic-Cu<sup>2+</sup> complexes to prevent readsorption of released Cu<sup>2+</sup> (Schramel et al., 2000) in the acid variable charge soils with low organic matter contents.

#### 4. Conclusions

Variable charge soils were relatively vulnerable to contamination of anthropogenic Cu because of low organic matter contents and low pH. The sequential extraction procedure provided some useful information on the distribution of anthropogenic Cu in variable charge soils and on the “accurate” assessment of soil Cu availability estimated by the single extraction. The Fe/Mn oxides mainly controlled the distribution and fate of anthropogenic Cu in the two variable charge soils rather than soil organic matter because of its low contents. Although the variable charge soils had higher Cu adsorption capacity than the permanent charges soil because of high Fe/Al oxide contents, most of the anthropogenic Cu was associated with the mobile fractions in these soils. Transformations between the mobile fractions and the relatively stable fractions were observed in both soils during the 6-week incubation, and decreased with increasing Cu amended levels. The 1 mol NH<sub>4</sub>Ac l<sup>-1</sup> (pH 5.0) is useful for chemically evaluating Cu availability in the variable charge soils with similar extraction efficiency as Mehlich 3, whereas the 0.1 mol HCl l<sup>-1</sup> appeared

to overestimate soil available Cu because it releases some Fe/Mn oxide-bound Cu that may not be bioavailable under field conditions.

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