Free cupric ions in contaminated agricultural soils around a copper mine in eastern Nanjing City, China

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Abstract: To determine the environmental free metal ion activity was a recent hot issue. A method to measure low-level free cupric ion activity in soil solution extracted with 0.01 mol/L KNO_3 was developed by using cupric ion-selective electrode (ISE) and calibrating with Cu-buffer solution. Three copper buffers including iminodiacetic acid (IDA), ethylenediamine (EN), and glycine (Gly) were compared for calibrating the Cu-ISE curves in the range of free cupric ions (pCu^{2+}) 7—13. The Cu-EN buffer showed the best electrode response and thus was applied as the calibration buffer. The pCu^{2+} of 39 contaminated agricultural soils around a copper mine was measured, ranging from 5.03 to 9.20. Most Cu in the soil solutions was found to be complexed with dissolved soil organic matters, averaging 98.1%. The proportion of free Cu^{2+} ions in the soil solutions decreased with the increasing of solution pH. Soluble Cu and free Cu^{2+} ions concentrations were analyzed by multiple linear regressions to evaluate the effects of soil properties on metal levels and speciation. The results showed that soil solution pH was the most significant factor influencing pCu^{2+} (with R^2 value of 0.76), while not important for the soluble Cu concentration.

Keywords: copper ion selective electrode (Cu-ISE); free metal ion activity; soil solution; metal speciation; bioavailability

Introduction

It was widely accepted that total soil heavy metal concentration alone was not a good measure for its short-term bioavailability and also was not a very useful tool to determine its potential environmental and human health risks from soil contamination (Houba et al., 1996; Belotti, 1998; Shan et al., 2003). In fact, it is well-known that metals exist in a number of different soluble and particulate forms, which influence their reactivity and hence their mobility and bioavailability (Das et al., 1995; Ge et al., 2005; Luo et al., 2006). In the past 20 years, a large number of publications have dealt with the study of the fractionation of heavy metals in soil solid phase (Tessier et al., 1979; Kaasalainen and Yli-Halla, 2003). However, metal speciation in the solution phase, which is one of the key factors that regulate metal uptake by plants as well as its toxicity for soil and aquatic organisms (Prokop et al., 2003), has not been deeply studied because of its low concentration in soil solution.

There have been several models predicting metal bioavailability/toxicity to aquatic ecosystems (Janssen et al., 2003). Free-ion activity model (FIAM) postulated that the biological effects of metals were best predicted by the activity of free metal ions (Brown and Markich, 2000). It has been effective in explaining the central role of free-ion activity as a regulator of interactions (uptake, toxicity) between metals and aquatic organisms (Hare and Tessier, 1996). For instance, free copper activity has been shown to be the main bioavailable species of copper in

aquatic systems (Markich et al., 2003). The ecotoxicological studies have shown the similar results in soil-plant system also (Minnich et al., 1987; Sauvé et al., 1996; Vulkan et al., 2000; McBride, 2001). Therefore, the measurement of free metal ions in soil solution became a hot issue.

Metal activity can be measured or estimated by various techniques: ion exchange resins (Chakrabarti et al., 1993), cation-exchange membrane technique (Gustafesson et al., 2003), Donnan dialysis (Temminghoff et al., 2000), competitive chelation (Catlett et al., 2002), and ion-selective electrodes (ISEs) (Luider et al., 2004). Among the various analytical methods, only potentiometry with ISEs can directly determine the free metal ions in situ without perturbing the natural metal speciation. Cu-ISEs have been far and wide used in both the toxicological studies and environmental monitoring of aquatic systems (Eriksen et al., 2001). Whereas, the conventional calibration of an ISE in the range below 10^{-5}—10^{-6} mol/L with solutions obtained from metal salts or by serial dilutions of more concentrated standard metal solutions is undesirable. For the following reasons, it leads to serious errors or is even completely inadmissible: the preparation of very dilute solutions of accurately known concentration is neither simple nor accurate; loss or contamination of dilute solutions may give rise to serious, positive or negative errors in electrode response; and various processes at the electrode-solution interface (such as adsorption, interaction with interstitial ions, redox processes, etc.) may affect the electrode response (Hulanicki et al., 1991). However, the use of metal buffers which can

regulate metal ion concentrations at extremely low levels ($<10^{-9}$ mol/L) avoids these difficulties and may give many additional advantages. A metal buffer solution can be defined as a solution for which the pM-value is only slightly affected by the addition of the metal ion (M) or the ligand (L) which complexes with the metal ion (Hulanicki *et al.*, 1991). It has been successfully used for the ISE analysis of free Cu^{2+}, Cd^{2+}, and Pb^{2+} activity as low as pM 20 (Buck and Coşofreţ, 1993).

International Union of Pure and Applied Chemistry (IUPAC) recommended the metal buffer procedure for calibration of ISEs in the low metal activity range (Buck and Coşofreţ, 1993). Specifically, for a cupric ion buffer with a relatively high total Cu concentration, adjusting the pH to control the complex degree between the ligands and Cu^{2+} can give different free Cu^{2+} concentrations. Calculating the pCu^{2+} of different pH based on the chemical equilibriums, recording corresponding electrode response E/mV values, and then plotting the E values against pCu^{2+} will produce the calibration curves of low free Cu^{2+} concentrations.

Since ISEs were very sensitive in metal buffers, and most of soil-solution systems probably contain enough ligands such as fulvic acids to buffer the free copper, they should permit the determination of pCu^{2+} in most soil solutions. Sauvé *et al.* (1995) measured the free Cu^{2+} in 0.01 mol/L $CaCl_2$ soil extracts using a Cu-ISE calibrated with Cu-IDA iminodiacetic acid buffer, down to pCu^{2+} 13. However, the calibration curve in the neutral pH range of the buffer had some problems, and Cl^- as a complex ligand might disturb the speciation equilibrium of Cu^{2+} in soil solution. Thus it was necessary to validate the usefulness of otherid metal buffers and sustaining electrolytes in determining the free metal ions in soil solutions.

The objectives of this study were to develop the ISE calibration curves of free cupric ions in the low activity range, determine the concentration of free cupric ions in soil solutions, and evaluate the influencing factors for soil Cu speciation.

1 Materials and methods

1.1 Cu-ISE calibrations

Three complex ligands (L) including imino-diacetic acid (IDA), ethylenediamine (EN), and glycine (Gly) were chosen to make the Cu buffers. The calibration buffers (Cu-L) were prepared with 5.0 mg/L Cu and 1.0 mmol/L ligands, and 0.01 mol/L KNO_3 as the supporting electrolyte. The copper standard salt solutions were prepared by serial dilution of standard stock solution (500 mg/L Cu, China EPA) in 0.01 mol/L KNO_3 with the Cu concentrations of 10, 1.0, and 0.1 mg/L, respectively. All the water used has been deionized and distilled (DD-water).

For a measurement, 10 ml of Cu buffer was used, the pH of which was adjusted with HNO_3/NaOH for controlling the free Cu^{2+} activity. After the electrode response became steady, the E/mV value was recorded, and the buffer pH was accurately measured by an Orion CHN060 combined pH electrode at the same time. The calculations of the free Cu^{2+} concentrations in the buffers with different pH values were done via a chemical equilibrium computer program, MINEQL+ (Schecher and McAvoy, 1994). Because MINEQL+ did not include the IDA species, the appropriate stability constants were took from the JESS thermodynamics database (May and Murray, 2000), and were added into the MINEQL+ database. The E-pCu^{2+} calibration curves were plotted respectively, and the most excellent calibration buffer was selected. When practically used in the soil determination, the Cu-ISE was calibrated daily.

1.2 Collection and analysis of soil samples

Total 39 agricultural soil samples (yellow brown soil) were collected from the surface horizon (0—15 cm) of a copper mine in eastern Nanjing City, China. The samples were air-dried, grounded, and passed through a 2-mm sieve. Total soil Cu was analyzed by digesting the soil with a mixture of HF, $HClO_4$ and HNO_3 with a ratio of 3:1:1 at 240℃, and determined by the atomic absorption spectrophotometer (AAS, Hitachi 180-80, Japan). Soil organic carbon (SOC) was analyzed by titration with $FeSO_4$ after oxidized by $K_2Cr_2O_7 + H_2SO_4$ at 180℃. And the clay content was determined by a LS 230 laser diffraction particle analyzer (Beckman Coulter).

1.3 ISE determination of pCu^{2+}

Soil solutions were extracted by 0.01 mol/L KNO_3 with a soil/solution ratio of 1:2, shaken for 2 h at a constant temperature. The solution pH, electrical conductivity (EC), pH, dissolved Cu, and the free cupric ions (pCu^{2+}) were measured.

The pCu^{2+} was determined with an Orion 868 pH/ISE meter, a 306 model Cu-ISE (Jiangsu Electroanalytical Instrument Factory, China), and an 801C double junction saturated calomel electrode (SCE) as the reference electrode. The outer filling solution (0.01 mol/L KNO_3) of the reference electrode was changed daily. Each time before use, the Cu-ISE was polished with 3 μm aluminum oxide powder on the nylon cloth, activated in 10^{-3} mol/L $Cu(NO_3)_2$, and then the electrodes were soaked in 0.025 mol/L H_2SO_4 and 0.1 mol/L EDTA sequentially each for 5 min and rinsed with DD-water.

The ISE measurements were performed under a constant temperature (23 ± 1)℃ and reduced lighting conditions. The soil solutions (10 ml) were transferred into glass sample cups, and stirred by magnetic force stirrer. When the potential stayed within 0.3 mV/3 min, the Cu electrode was considered to be

equilibrated, and the E/mV value was recorded. Between samples, both electrodes were rinsed with DD-water, soaked 2 min in DD-water, and then wiped dry with filter paper.

2 Results and discussion

2.1 Selecting the Cu-ISE calibration buffers

Reliability of the metal buffer method mainly depended on the determination of buffer pH and the accuracy of the stability constants selected. For the Cu-ISE determination, the pH of Cu buffer was accurately measured in this study. As the Cu-IDA data is not included in MINEQL+ program, we took the appropriate stability constants of corresponding temperature and ionic strength from the thermodynamics database. Calibration effects of the three buffers were shown as follows.

Cu-Gly: pCu^{2+} was controlled between 5.71—13.32 by adjusting the buffer pH between 4.07—10.0, and the ISE curve slope of the portion of $pCu^{2+}<7$ was -40 mV/pCu^{2+}. Thus, when practically applied, the curve combining the data points of Cu buffer ($pCu^{2+}>7$) and standard salt solutions ($pCu^{2+}<7$) was adopted as the calibration curve. Fig.1a illustrats the Cu-ISE response in mV vs pCu^{2+} at 20℃ combining 6 Cu buffer points of twice calibrations and 2 points of copper salt solutions (0.1 and 1.0 mg/L). The electrode response was linear even at a pCu^{2+} of 13 and was near-Nernstian with a response slope of -27.4 mV/pCu^{2+} (theoretic value 29.1).

Cu-IDA: Adjusting the buffer pH between 3.65—9.24 gave a pCu^{2+} range of 5.92—13.77. The

calibration curve of 9 buffer points of twice calibrations at 20℃ was linear also, as shown in Fig. 1b. But the response slope was -23.0 mV/pCu^{2+}, considerably discrepant to the theoretic value of 29.1 mV/pCu^{2+}. And the potential response was unsteady while using this buffer. Sauvé *et al.* (1995) had reported similar phenomenon that the data points in the neutral pH range (6.5—7.5) were variable and thus the calibration curve was obtained using acidic and alkaline IDA solutions. So we recommend that this buffer should not be suitable for Cu-ISE calibration.

Cu-EN: Adjusting the buffer pH between 3.67—6.78 resulted in a pCu^{2+} range of 4.12—10.38. The linearity of the calibration curves of the buffers alone was very good, with $R^2>0.999$ each time. And we had a good linear regression for all of the calibration curves accumulated through a month. When practically applied, the calibration curve could either adopt the curve combining the data of Cu buffer and standard salt solutions, or use the calibration buffer alone which has covered the high free Cu^{2+} activity. Fig.1c illustrats the calibration curve combining 18 buffer points of thrice calibrations in 3 d and the points of 0.1, 1.0, and 10 mg/L copper salt solutions at (23±1)℃. The electrode response was linear even at a pCu^{2+} of 11 and was near-Nernstian with a response slope of -27.6 mV/pCu^{2+} (theoretic value of 23℃ was 29.4).

In view of the above comparison, Cu-ISE showed steady response and good linearity in Cu-EN buffer, which could be used for calibrating free Cu^{2+} activity accurately in low Cu concentration range. Moreover, the adjusted pH range was fit for the working range of the Cu-ISE. So Cu-EN buffer calibration-ISE determination was selected to measure the free Cu^{2+} activity in the soil solutions in this study.

2.2 Determination of free Cu^{2+} in the soil solutions

The soil samples were extracted with 0.01 mol/L KNO_3 solution, and soluble metal was assumed to correspond to the amount of metal measured in the extracts. Measured result of the electric conductivity indicated that 0.01 mol/L KNO_3 extracts of soil could blank out the effects of other salts on total solution ionic strength. Sauvé *et al.* (1997) had reported that ionic strength between 0.01 and 0.10 showed only very small effects on the electrode potential. Using the experiential relation IS=0.13 EC (Griffin and Jurinak, 1973), the EC measured in the extracts 0.12—0.26 S/m (average 0.16 S/m) was equal to IS of 0.015—0.033 (average 0.02). Therefore negligible effects from the IS variations could be assumed when measuring the Cu-ISE potential of 0.01 mol/L KNO_3 extracts. Because of very low activities of Cu^{2+} in the soil solutions, the electrode equilibration was very slow, over 30 min in most of the samples. When the pH was lower than 5.5, the measured pCu^{2+} was
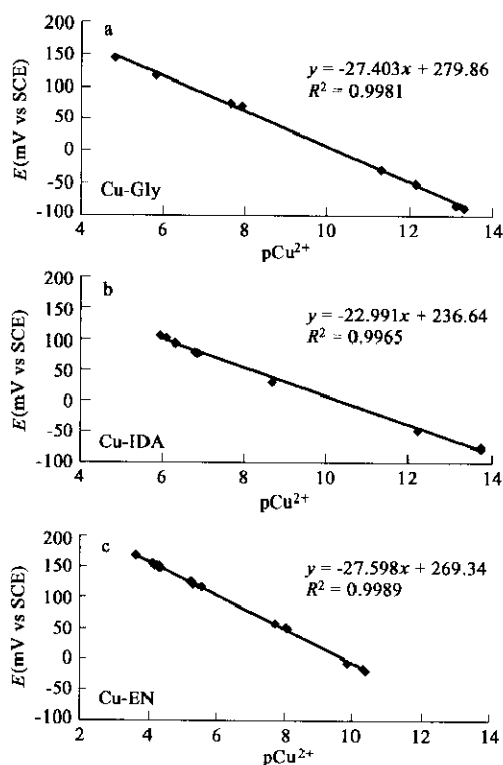

Fig. 1 Calibration curves of Cu-ISE in different Cu buffers

overestimated, which might be caused by the electrode responses of other increased metal ions such as Zn^{2+}. The free Cu^{2+} ions (pCu^{2+}) of 39 contaminated soil samples ranged from 5.03 to 9.20.

2.3 Copper speciation in contaminated soils

Soil solution pH, SOC, clay content, and Cu concentrations (total soil Cu, dissolved Cu, and free Cu^{2+}) are shown in Table 1. Total soil Cu concentration varied between 280 and 1752 mg/kg, all of which were higher than the second grade value of China Environmental Quality Standards for Soils (China EPA, GB 15618-1995) and some were even higher than the third grade value (400 mg/kg).

Table 1 Physical and chemical properties of the soils tested (*n*= 39)

Parameter	Mean	SD	CV	Median	Min.	Max.
Solution pH	6.98	0.57	0.08	7.16	5.39	7.61
SOC, %	2.2	0.38	0.17	2.2	1.3	3.1
Clay, %	13.6	1.79	0.13	13.3	9.3	17.3
Soil Cu, mg/kg	1039	302	0.29	1085	280	1752
Dissolved Cu, μg/L	370	157	0.42	326	105	814
pCu^{2+}	8.04	0.96	0.12	8.38	5.03	9.20
Free/dissolved^a, %	1.9	0.07	3.51	0.1	0.01	39.3

Notes: ^{a} a sample with an overestimated pCu^{2+} value because of its low pH was not included

There was not any good relationship between pCu^{2+} and total soil Cu. And the soil sample with the highest free Cu^{2+} activity was not the one that had the highest soil Cu concentration. Some samples had high pCu^{2+} because of their low soil pH, while their total soil Cu concentrations were relatively low. It showed the influence of environmental conditions on the metal speciation, and hence on its bioavailability. Earlier it was demonstrated that Cu was almost exclusively present in organically bound form in soil solutions (Vulkan *et al.*, 2000; Krishnamurti and Naidu, 2002; Nolan *et al.*, 2003). In this study, the Cu complexes were found to be the most dominant species. Only a very small fraction of the soluble Cu appeared as free ion species in most of the soil samples, ranging from 0.01% to 39.3% and averaging 1.9%. The complexed proportion decreased with the decline in pH, as shown
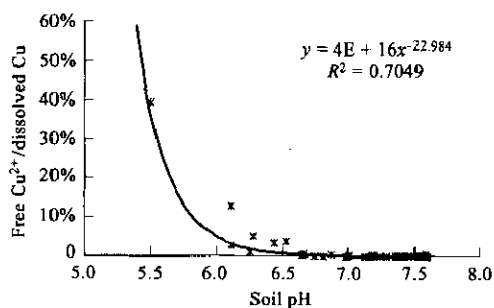
$$y = 4E + 16x^{-22.984}$$
$$R^2 = 0.7049$$

Fig.2 Relation of the percentage of free Cu^{2+} ions in solution with pH

in Fig.2, which was similar with the result of McBride and Bouldin (1984).

Multiple linear regression analysis by statistical software SAS relating lg(dissolved Cu) and pCu^{2+} respectively with the soil physical-chemical properties, including lg(total soil Cu), soil pH, lgSOC, and lg(clay content) were shown in the following equations.

$$lg(\text{dissolved Cu}) = 2.08(\pm 0.11) + 1.33(\pm 0.32)\,lg\,SOC\ (R^2=0.32, n=39)$$

$$lg(\text{dissolved Cu}) = 1.21(\pm 0.45) + 1.08(\pm 0.33)\,lgSOC + 0.32\,(\pm 0.16)\,lg(\text{total Cu})\ (R^2=0.38, n=39)$$

$$pCu^{2+} = -2.24(\pm 0.96) + 1.47(\pm 0.14)\,pH\ (R^2=0.76, n=39)$$

For dissolved soil Cu, only SOC and total soil Cu among those soil parameters met the 0.1 significance level for entry into the model. While solution pH was not found to correlate well with it, resembling a previous report (Sauvé *et al.*, 1997). For free cupric ions, however, its concentration was mainly influenced by solution pH, as shown in Fig.3, similar to other reports (Salam and Helmke, 1998; Nolan *et al.*, 2003). And pH alone could explain 76% variability in pCu^{2+}. Adding other variables did not improve the regressions significantly. It also indicated the important influence of pH on the bioavailability/toxicity of soil Cu. Some studies have shown the increasing phytotoxicities of Cu with increasing pH (Song *et al.*, 2004). Such increase was possibly caused by a reduction of proton competition or modification in the organic ligands.
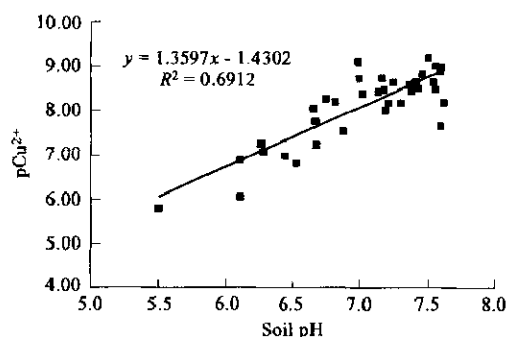
$$y = 1.3597x - 1.4302$$
$$R^2 = 0.6912$$

Fig.3 Free cupric ions concentration (pCu^{2+}) as a function of soil solution pH

Copper toxicity depended directly on the free Cu^{2+} in aquatic ecosystems. The threshold values varied between pCu^{2+} 6—11, depending on the organisms studied and the methodology utilized (Giesy *et al.*, 1983; Verweij *et al.*, 1992). If Cu toxicity to soil organisms also depended on the free Cu^{2+} activity in the soil solution, the potential risks of the contaminated soils could be evaluated. Compared with the toxicity threshold of pCu^{2+} 10 which was the median value found in aquatic studies, all the soil samples of this study had a potentially toxic free Cu

activity.

3 Conclusions

The free Cu^{2+} activity in soil solutions could be measured by Cu-ISE, which could be calibrated effectively by Cu buffers in low activity range. Environmental quality standards for soils were not only based on total metal concentration, but also depended on metal speciation. Since most of the Cu^{2+} ions in soil solutions were complexed by DOM, the free Cu^{2+} should have a stronger toxicity to organisms, and would be a more sensitive toxicity determination than soluble Cu. It was possible that in soils, Cu^{2+} activity might determine the intensity of Cu toxicity, while the ecotoxicological effects of Cu would be actually dependent on the capacity of the chemical and physical processes controlling the rates of replenishment of free Cu^{2+}. Therefore, the links between the free Cu^{2+} replenishment rate and Cu bioavailability to soil organisms need more detail and in-depth investigation in the future.

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