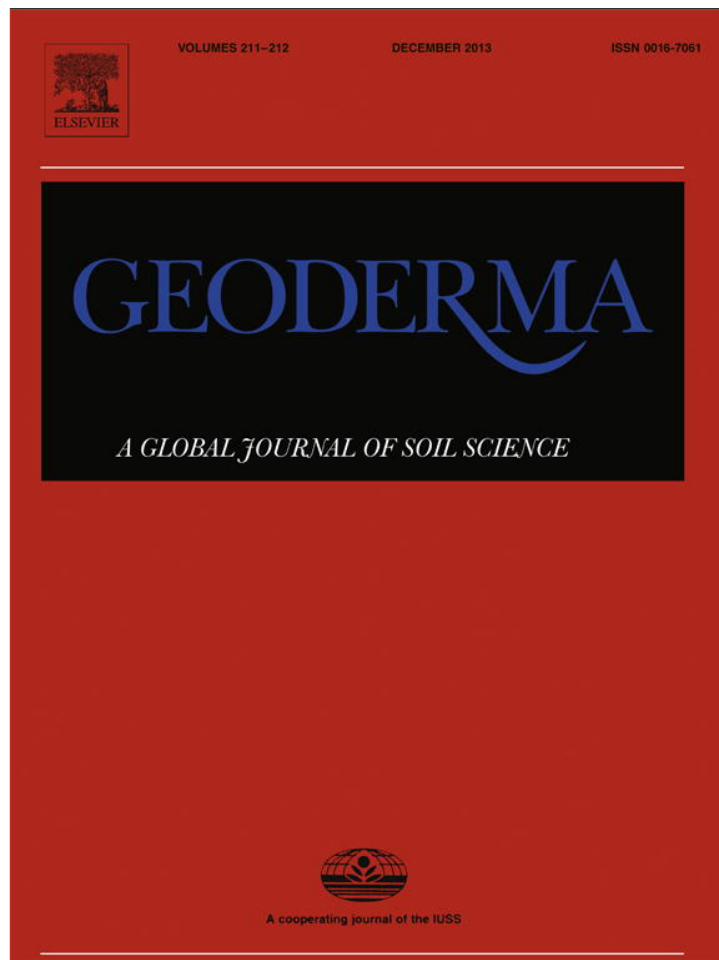


Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/authorsrights>



Contents lists available at ScienceDirect

Geoderma

journal homepage: [www.elsevier.com/locate/geoderma](http://www.elsevier.com/locate/geoderma)

## The influence of soil solution properties on phytotoxicity of soil soluble copper in a wide range of soils



Xiaoqing Zhang<sup>a</sup>, Xuedong Wang<sup>b</sup>, Dongpu Wei<sup>a</sup>, Bo Li<sup>a</sup>, Yibing Ma<sup>a,\*</sup>, Zhanbin Huang<sup>c</sup>

<sup>a</sup> National Soil Fertility and Fertilizer Effects Long-term Monitoring Network, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, 12 Southern Street of Zhongguancun, Beijing 100081, China

<sup>b</sup> College of Resource Environment and Tourism, Capital Normal University, 105 North Road of Xisanhuan, Beijing 100048, China

<sup>c</sup> School of Chemical and Environmental Engineering, China University of Mining and Technology-Beijing, Beijing 100083, China

### ARTICLE INFO

#### Article history:

Received 17 August 2012

Received in revised form 30 June 2013

Accepted 4 July 2013

Available online 30 July 2013

#### Keywords:

Copper

Phytotoxicity

Risk assessment

Soil solution

### ABSTRACT

The bioassay of barley root elongation was performed in 17 Chinese soils with added copper (Cu) to investigate the phytotoxicity of soluble Cu based on soil pore water and 0.01 M CaCl<sub>2</sub> extraction. For soluble Cu in soil pore water, the soil solution property induced variations of effective concentrations that caused 10% (EC10) and 50% (EC50) inhibition in barley root elongation which were 125 and 18 folds, respectively. Similarly for soluble Cu extracted by 0.01 M CaCl<sub>2</sub>, the soil solution property induced variation of EC10 and EC50 values was 14 and 7 folds, respectively, indicating that the concentrations of soluble Cu extracted by 0.01 M CaCl<sub>2</sub> or in soil pore water were largely affected by soil solution chemistry and properties in a wide range of soils. The multiple regression analysis was applied to derive empirical relationships between soil pore water properties (pH, electrical conductivity (EC), K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, S and dissolved organic matter (DOC)) and soluble Cu toxicity thresholds. When incorporating these parameters into the regression equations, the coefficient of determination (*r*<sup>2</sup>) for EC50 or EC10 ranged from 0.78 to 0.87. The results showed that the toxicity thresholds based on both Cu concentrations in soil pore water and 0.01 M CaCl<sub>2</sub> extraction were affinitive to soil pore water chemistry. Meanwhile, DOC was constantly significantly correlated to Cu toxicity thresholds (EC50 and EC10) in the pore water and 0.01 M CaCl<sub>2</sub> extraction, which could explain 70% of the variance of EC50 for Cu in soil pore water.

© 2013 Elsevier B.V. All rights reserved.

### 1. Introduction

Soil properties have definitely influenced the soil heavy metal toxicity/bioavailability. There are a large number of studies about the relationship between the soil properties and phytotoxicity of metals in soils (Li et al., 2010; Rooney et al., 2006; Warne et al., 2008). However, there is still an issue about whether the differences in metal toxicity thresholds could be better explained by soil soluble metal concentrations (Broos et al., 2007; Rooney et al., 2007). For example, the variations of toxicity thresholds based on both added zinc (Zn) and copper (Cu) across the 12 Australian soils were smaller than those on either the soil solution metal concentrations or CaCl<sub>2</sub>-extractable metal concentrations for soil microbial processes (Broos et al., 2007), whereas the EC50 (effective concentrations that caused 50% inhibition) based on the Ni concentration in soil solution varied less among soils than that based on the total added Ni for barley root and tomato shoot growth (Rooney et al., 2007). Even so, further investigation is still needed to evaluate the soil soluble Cu toxicity/bioavailability in soils as a function of soil solution properties.

Metal bioavailability and toxicity are determined by soil water chemistry (Sauvé et al., 2000) and the different speciation distribution of Cu. For instance, free Cu<sup>2+</sup> and CuOH<sup>+</sup> are the most toxic forms while other forms are less toxic or without toxicity for barley root elongation (Guo et al., 2010; Wang et al., 2012a). Soil organic matter (SOM), pH and concentrations of major ions in soil solution are known to influence the speciation of metals and thus affect toxicity of soluble metals to plants. Copper was reported to be preferentially combined with organic matter (Degryse et al., 2009; Luo et al., 2006a,b). Strawn and Baker (2008) mechanically elucidated the Cu speciation using advanced spectroscopic and microscopic methods, indicating that Cu was complexed to organic matter via bidentate inner-sphere coordination with carboxyl or amine ligands. Moreover, Cuss et al. (2010) used the geochemical equilibrium speciation model MINTQA2 to evaluate the impacts of dissolved organic matter (DOC) on copper speciation and their results showed that free Cu concentration decreased with the increase in DOC concentration. Soil solution pH was another important property determining the concentration of soil soluble metal (Salam and Helmke, 1999) and Cu speciation (Wang et al., 2009). More recently it is reported that pH had the significant influence on free Cu concentration (Degryse et al., 2009; Luo et al., 2006a,b) and thus influence soluble Cu toxicity. While whether

\* Corresponding author. Tel.: +86 10 8210 6201; fax: +86 10 8210 6225.  
E-mail address: [ybma@caas.ac.cn](mailto:ybma@caas.ac.cn) (Y. Ma).

the DOC or pH could really affect the soluble Cu toxicity in a wide range of soil solutions is still unclear.

Besides the influences on Cu forms, the solution chemistry parameters, such as  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Na^+$ ,  $K^+$  and  $H^+$ , were supposed to interact with the biotic ligand with Cu and consequently resulted in a decreased phytotoxicity of Cu. This protective effect had been studied on barley, tomato, wheat and lettuce (Lock et al., 2007; Luo et al., 2008; Thakali et al., 2006a,b; Yen Le et al., 2012). Thakali et al. (2006a,b) and Yen Le et al. (2012) revealed that only protons could influence the Cu toxicity on barley, tomato and lettuce, respectively, as competing cation. While Lock et al. (2007) and Luo et al. (2008) reported the ameliorative effects of  $Mg^{2+}$  and  $Ca^{2+}$  on the Cu rhizotoxicity to barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), respectively, but not for  $H^+$ ,  $Na^+$  and  $K^+$  in nutrient solutions. The latter results derived from artificial solutions did not accord with observation of former results using the real soil solution. Since most research focused on the cultural solution rather than the real soil solution, whether these findings derived from artificial solutions are available for the real soil solutions is still unanswered until now.

Therefore, in the present study, bioassays of barley root elongation were performed in 17 Chinese soils with added Cu. The aims of the present study were: (1) to evaluate the phytotoxicity of soil soluble Cu based on soil pore water and 0.01 M  $CaCl_2$  extraction; (2) to use the obtained toxicity thresholds data to derive empirical relationships between soil pore water properties and soluble Cu toxicity and (3) and to determine the main factors in pore water influencing soluble Cu phytotoxicity.

## 2. Materials and methods

### 2.1. Soil samples and treatments

Seventeen soil samples (0–20 cm depth) were used, which are representative of the major types of agricultural soils in China. The soil sampling sites and physical and chemical characteristics of the soils have been described in detail by Li et al. (2010). The ranges of the main soil properties were as follows: pH 4.93–8.90; organic carbon content (OC) 0.60–4.28%; cation exchange capacity (CEC) 6.36–33.59  $cmol^+/kg$  and clay content 10–66%.

The soil samples were air-dried and sieved through a <2 mm screen. Copper was added as  $CuCl_2$  solution at eight concentrations (control plus seven Cu doses) and thoroughly mixed again to produce nominal concentrations of: 0, 12.5, 25, 50, 100, 200, 400 and 800 mg Cu/kg for soils with pH < 5; 0, 25, 50, 100, 200, 400, 800 and 1600 mg Cu/kg for soils with pH 5 to 7; 0, 37.5, 75, 150, 300, 600, 1200 and 2400 mg Cu/kg for soils with pH > 7. All the amended soils at three

replications were incubated for 2 days at 100% maximum water holding capacity (MWHC) (Jenkinson and Powelson, 1976), air-dried, sieved to <2 mm using plastic mesh, then stored at room temperature before bioassay.

The soluble metal concentrations were measured in soil pore water and in soil  $CaCl_2$  extraction. The methods for collecting soil pore water were referred to Thibault and Sheppard (1992). The soil samples which were incubated overnight with addition of deionized water at field capacity at pF 1.7 (log 50 cm water suction) were centrifuged for 45 min at a speed of 3500 r/min and then another 45 min at a higher speed of 15000 r/min. The supernatant was passed through 0.45  $\mu m$  filters and then the properties and chemistry of the filtrates were measured immediately. The Cu extraction, using 0.01 M  $CaCl_2$  solution, was undertaken using an air-dried soil in a ratio of soil to solution 1:5 (m/V). The soil suspension with 0.01 M  $CaCl_2$  solution was shaken for 30 min and centrifuged for 15 min at a speed of 3500 r/min. The supernatants were passed through 0.45  $\mu m$  filters. The basic parameters of the soil pore water were measured, such as DOC, pH, electrical conductivity (EC),  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$  and S. The pH and EC of the pore water were measured using a microelectrode pH and EC meter (Thermo Fisher Scientific Inc., New York, USA) and the concentration of DOC in the solutions was determined using a Formacs SERIES TOC/TN Analyser (Skalar Ltd., Breda, The Netherlands). The concentrations of a number of cations ( $K^+$ ,  $Ca^{2+}$ ,  $Na^+$ ,  $Mg^{2+}$ ,  $Cu^{2+}$ ) and S of soil pore water and the soluble Cu concentration in the 0.01 M  $CaCl_2$  extraction were measured either by inductively coupled plasma-atomic emission spectrometry (Spectro flame Modula, Spectro, Boschstr, Kleve, Germany) or inductively coupled plasma-mass spectrometry (Spectro flame Modula, Spectro, Boschstr, Kleve, Germany) depending on their concentrations in the solutions (Zarcinas et al., 1996). The main pore water properties of soil samples are presented in Table 1.

### 2.2. Barley root elongation bioassay

The barley root elongation bioassay was performed based on the International Organization for Standardization (ISO) 11269-1 (1993). All the detailed procedures for the barley root elongation bioassay have been described by Li et al. (2010). The percentage difference in barley root elongation compared to the corresponding control samples (RE, %) in a test medium was calculated using the equation:

$$RE = \frac{RE_t}{RE_c} \times 100 \quad (1)$$

**Table 1**  
Initial properties of pore water samples in soils without Cu addition.

Soil no.	Location	pH	EC (mS/cm)	DOC (mg/L)	Ca (mg/L)	K (mg/L)	Mg (mg/L)	Na (mg/L)	S (mg/L)
S1	Beijing	7.53	1.03	623	219	31.4	38.3	6.15	47.1
S2	Chongqing	7.73	0.532	207	134	2.44	11.4	13.4	52.6
S3	Gansu	8.4	2.087	243	132	8.9	42.7	54.2	28.7
S4	Guangzhou	7.76	1.196	259	1359	31.5	16.1	47.1	187
S5	Hailun	6.97	0.847	308	168	3.14	41.4	20.1	58.7
S6	Hainan	4.94	1.063	153	50.4	50.8	16.8	18.8	3.45
S7	Hangzhou	6.86	2.13	286	438	34.2	78.3	135	237
S8	Hunan	4.9	1.084	152	206	12	26.1	26.8	63.4
S9	Jiaying	7.21	2.137	117	353	7.2	81.5	154	119
S10	Jilin	8	1.271	158	239	5.79	22.1	12	51.6
S11	Langfang	8.16	0.854	153	155	22.5	22.1	23.3	42.2
S12	Neimeng	7.59	11.36	337	354	16.4	483	2128	721
S13	Shandong	8.18	2.31	138	248	1.6	95.1	248	123
S14	Shanxi	8.2	1.139	53	236	11.8	18.9	19.3	50.7
S15	Shijiazhuang	7.96	2.835	207	730	8.11	104	69	424
S16	Xinjiang	8.29	3.303	294	444	51.5	90.3	605	446
S17	Zhengzhou	8.31	1.121	166	169	4	38.6	65.4	81.8

where  $RE_t$  is the root length in the test soil samples and  $RE_c$  is the root length in the control samples without spiked Cu.

### 2.3. Data statistical analysis

The EC10 and EC50 values were performed on the log-logistic dose–response curves based on the measured soluble Cu concentrations in both the  $CaCl_2$  extraction solutions and in the pore water samples. The 95% confidence intervals of EC10 and EC50 values were also obtained from the fitted curve parameters according to Haanstra et al. (1985).

The toxicity thresholds (EC10 and EC50) and the properties of soil pore water were regressed using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA). Relationships were deemed significant at  $p \leq 0.05$ .

## 3. Results

### 3.1. Toxicity thresholds for soil soluble Cu

Barley root elongation exhibited a significant response to Cu additions in all soils. Compared to the unamended control, the inhibition on barley root elongation due to the addition of Cu was observed and the data fitted reasonably well to the log-logistic dose–response curve (Figs. 1 and 2). When considering the toxicity thresholds for Cu in soil pore water, it was found that the EC10 ranged from 0.01 (S6) to 0.97 mg/L (S1) and EC50 ranged from 0.14 (S14) to 2.50 mg/L (S1), representing 125 and 18 fold differences, respectively (Table 2). Similarly, the toxicity thresholds for Cu extracted by 0.01 M  $CaCl_2$  showed that the EC10 values varied from 0.08 (S6) to 1.21 mg/kg (S1) and the EC50 varied from 0.52 (S14) to 3.60 mg/kg (S1), representing 14 and 7 fold differences, respectively. In the same set of soils, Li et al. (2010) reported that the EC10 values of the added Cu ranged from 31 to 444 mg/kg (14.3 fold differences) and EC50 ranged from 67 to 1129 mg/kg (16.8 fold differences). These results showed that the differences between the toxicity thresholds expressed by Cu extracted by 0.01 M  $CaCl_2$  were only smaller than the toxicity thresholds of the added Cu for EC50 and those expressed by soluble Cu in soil pore water were greater than those of expressed by added Cu. Similarly, soluble Cu in soil pore water did not narrow the soil to soil variation

in the toxicity thresholds to barley in 18 European soils (Zhao et al., 2006). Therefore, all the results indicated that the toxicity of either Cu extracted by 0.01 M  $CaCl_2$  or Cu in soil pore water is largely affected by soil solution chemistry and properties in a wide range of soils.

### 3.2. Effects of soil solution on the soluble Cu toxicity

The multiple regression analysis considered the important water chemistry that affected Cu toxicity, including pH, EC,  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ , S and DOC. Since not all soil pore water parameters are statistically significant in all equations, the regression equations with the specific significant parameters ( $p < 0.05$ ) are presented in Table 3. The regression results showed that DOC constantly significantly correlated to Cu toxicity thresholds (EC50 and EC10) in the pore water and 0.01 M  $CaCl_2$  extraction for 17 soil samples (Table 3). For example, single DOC parameter could explain 55% and 70% of the variance of EC10 and EC50 for Cu in soil pore water. Meanwhile, the DOC was significantly positive with the toxicity thresholds, indicating that the toxicity threshold increased as the DOC concentration increased.

Except DOC, the second important parameter was soil solution pH. For instance, including pH, the coefficient of determination ( $r^2$ ) of EC10 increased from 0.55 to 0.68 in the pore water (Table 3). In addition,  $Na^+$  was found to be a significant parameter of toxicity thresholds (EC10) of Cu in soil pore water, which increased  $r^2$  from 0.68 to 0.78 (Table 3).

When incorporating initial soil pore water chemistry (pH, EC,  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ , S and DOC) of soil pore water samples without addition of Cu into the regression models, together with corresponding toxicity thresholds for Cu from the pore waters and the 0.01 M  $CaCl_2$  extractions, the  $r^2$  for EC50 or EC10 ranged from 0.78 to 0.87 (Appendix Tables 1 and 2), indicating that more factors could further improve predictions. When comparing the  $r^2$  values for EC50 and EC10 for Cu from pore water and the 0.01 M  $CaCl_2$  extraction samples, the values were a little higher in pore water than that in 0.01 M  $CaCl_2$  extraction (Appendix Tables 1 and 2). The results suggested that the toxicity thresholds based on Cu concentrations in soil pore water and 0.01 M  $CaCl_2$  extraction were both affinitive to soil pore water chemistry, even better for Cu toxicity thresholds based pore water.

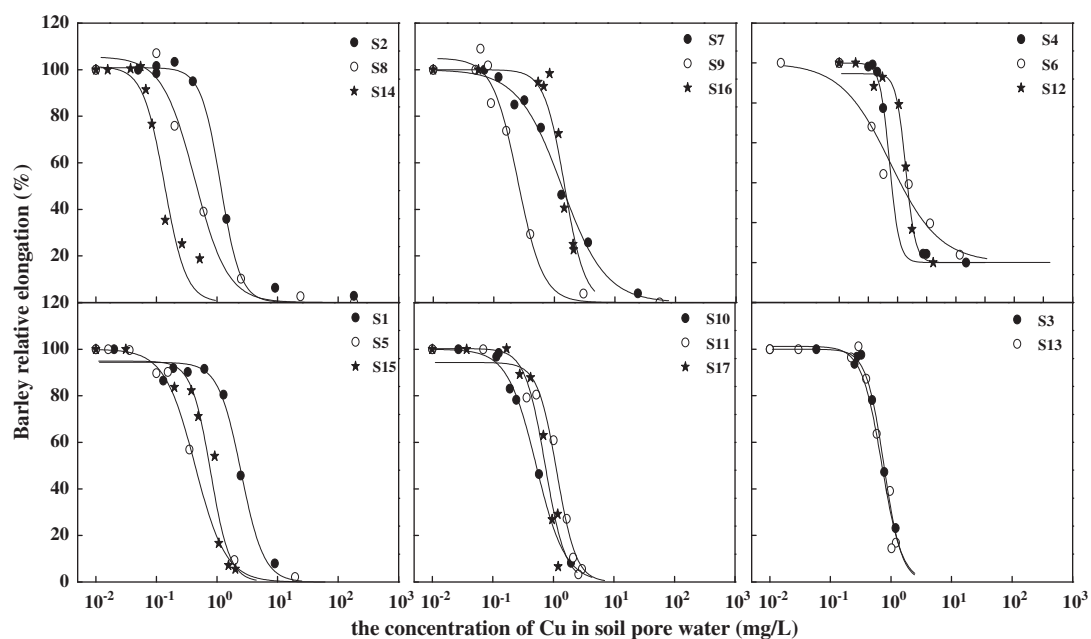


Fig. 1. Dose–response curves for barley root elongation as a function of the concentrations of Cu in soil pore waters in 17 soils. Symbols represent all replicated data points and lines are the fitted log-logistic curves.

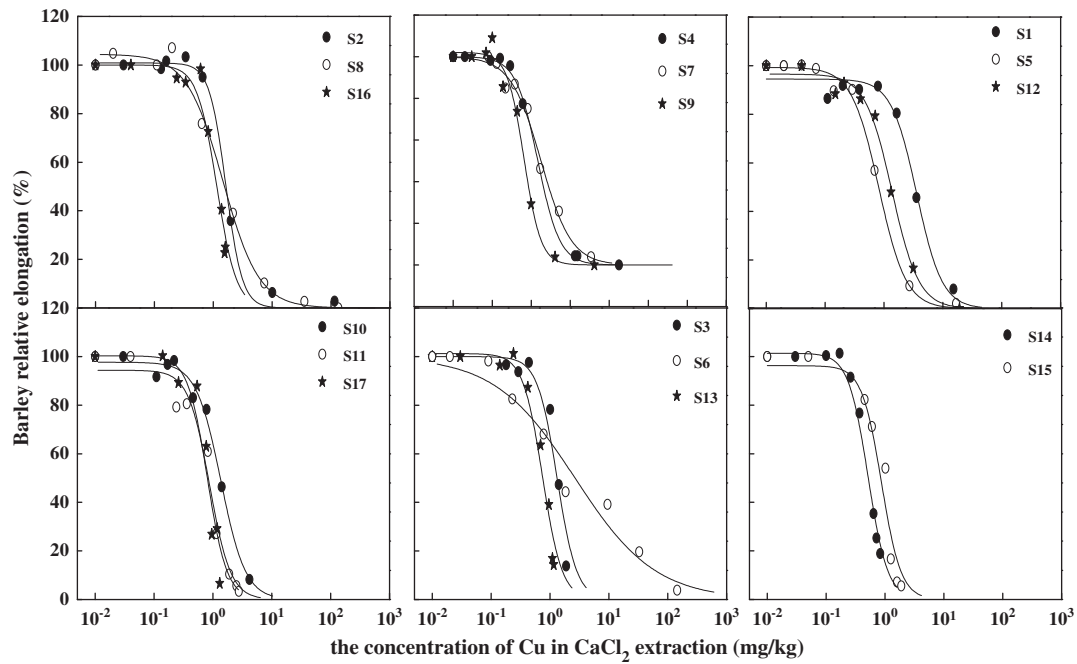


Fig. 2. Dose-response curves for barley root elongation as a function of the concentrations of Cu in the 0.01 M CaCl<sub>2</sub> extraction in 17 soils. Symbols represent all replicated data points and lines are the fitted log-logistic curves.

4. Discussion

4.1. Comparison of soluble Cu toxicity based on pore water and 0.01 M CaCl<sub>2</sub> extraction

In the present study, the toxicity of soluble Cu based on 0.01 M CaCl<sub>2</sub> extraction had smaller variation in wide range of soils and with slightly lower coefficient of determination in the regression equations, comparing with the toxicity based on Cu in soil pore water. Moreover, the discrepancy of the significant factors in determining the soluble Cu toxicity was also observed. For instance, except DOC and pH, the soluble Cu toxicity thresholds in 0.01 M CaCl<sub>2</sub> extraction showed more dependency on K<sup>+</sup> content, whereas that in pore water did not. The 0.01 M CaCl<sub>2</sub> extraction always replaced soil

pore water as a simple way to estimate the soluble metal toxicity. However, there was a query about its accuracy for Cu. In most soil solutions, Cu was predominately complexed with dissolved organic matter (DOM) and thereby the Cu concentration in solution was largely decided by the DOM concentration (Strawn and Baker, 2008). The DOM concentration was lower in 0.01 M CaCl<sub>2</sub> extraction than that in soil pore water because it undergone the aggregation, dilution and sorption of Ca–DOM complexes on the solid phase in the process of CaCl<sub>2</sub> extraction. Therefore, the concentrations of Cu and speciation distributions in CaCl<sub>2</sub> extraction would differ from those in the soil pore water. In the present soil samples, the ratio of the soluble Cu concentrations in pore water and 0.01 M CaCl<sub>2</sub> extraction varied from 0.81 to 4.0 at highest dosage of Cu addition in the same unit of mg/kg (Fig. 3), indicating that these differences directly caused the different dosage response curve results and then influenced the toxicity thresholds. Although the extents of toxicity threshold variations were smaller for soluble Cu based on 0.01 M CaCl<sub>2</sub> extraction than pore water, its toxicity threshold variations were still larger than 7 times and thus the soluble Cu extracted by

Table 2  
Toxicity thresholds measured by barley root elongation for soluble Cu for 17 Chinese soils.

Soil no.	Pore water Cu (mg/L)				CaCl <sub>2</sub> -extractable Cu (mg/kg)			
	EC10	95% C. I. <sup>a</sup>	EC50	95% C. I.	EC10	95% C. I.	EC50	95% C. I.
S1	0.97	0.54–1.74	2.50	1.98–3.16	1.21	0.65–2.24	3.60	2.78–4.66
S2	0.50	0.31–0.83	1.15	0.98–1.34	0.81	0.53–1.23	1.64	1.44–1.87
S3	0.37	0.30–0.44	0.77	0.71–0.83	0.57	0.27–1.19	1.32	1.05–1.66
S4	0.23	0.15–0.34	0.53	0.27–1.03	0.36	0.27–0.46	1.48	1.08–2.02
S5	0.12	0.08–0.18	0.44	0.36–0.53	0.25	0.18–0.36	0.82	0.70–0.96
S6	0.01	0.00–0.24	0.56	0.11–2.91	0.08	0.01–0.52	2.66	1.10–6.46
S7	0.21	0.12–0.37	1.33	1.05–1.67	0.25	0.12–0.51	1.78	1.33–2.38
S8	0.11	0.04–0.30	0.43	0.27–0.70	0.31	0.18–0.55	1.46	1.12–1.91
S9	0.09	0.05–0.15	0.25	0.19–0.33	0.22	0.12–0.41	0.65	0.52–0.83
S10	0.16	0.12–0.21	0.52	0.45–0.60	0.47	0.32–0.70	1.37	1.16–1.61
S11	0.51	0.24–1.11	1.14	0.84–1.55	0.35	0.12–1.02	0.88	0.61–1.27
S12	0.82	0.52–1.29	1.90	1.62–2.24	0.43	0.28–0.68	1.37	1.15–1.63
S13	0.30	0.20–0.46	0.70	0.58–0.85	0.32	0.20–0.52	0.75	0.62–0.89
S14	0.06	0.03–0.10	0.14	0.09–0.20	0.22	0.17–0.29	0.52	0.46–0.57
S15	0.35	0.15–0.84	0.78	0.55–1.11	0.38	0.18–0.78	0.87	0.65–1.18
S16	0.63	0.40–1.01	1.48	1.20–1.81	0.49	0.32–0.78	1.15	0.97–1.37
S17	0.32	0.18–0.55	0.73	0.59–0.91	0.35	0.15–0.82	0.80	0.60–1.08

<sup>a</sup> Ranges given as 95% confidence intervals (C. I.).

Table 3  
Simple and multiple linear regressions between soluble Cu toxicity thresholds and pore water chemistry (n = 17).

Regression equation	r <sup>2</sup>	p		
<i>Toxicity thresholds based on soil pore water</i>				
1 EC10 = -0.65 + 0.085pH + 0.0002Na + 0.001DOC	0.78	0.0026	0.034	<0.001
2 EC10 = -0.70 + 0.092pH + 0.002DOC	0.68	0.032	<0.001	-
3 EC10 = -0.019 + 0.002DOC	0.55	<0.001	-	-
4 EC50 = -0.014 + 0.004DOC	0.70	<0.001	-	-
<i>Toxicity thresholds based on CaCl<sub>2</sub> extraction</i>				
5 EC10 = 0.074 + 0.002DOC	0.54	<0.001	-	-
6 EC50 = 2.8-0.32pH + 0.004DOC	0.68	0.011	<0.001	-
7 EC50 = 0.40 + 0.004DOC	0.48	0.002	-	-

r<sup>2</sup>: coefficient of determination (percentage of variance accounted for by the regression model); p: significant level; EC10: effective concentrations that caused 10% inhibition and EC50: effective concentrations that caused 50% inhibition.



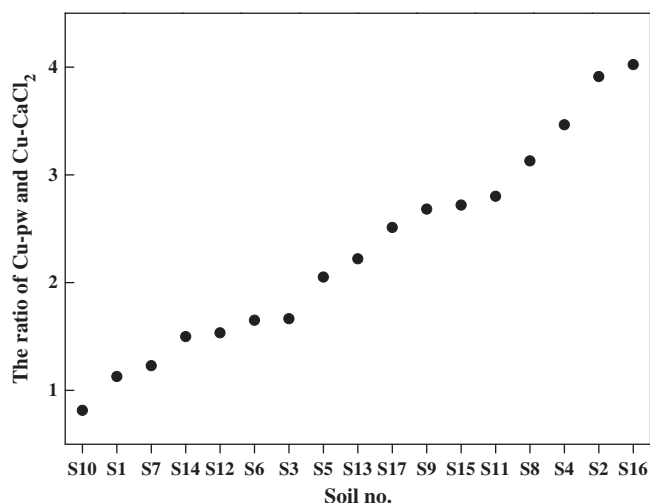


Fig. 3. The ratio of soluble Cu concentrations in the soil pore water (expressed as Cu-pw, mg/kg) and 0.01 M CaCl<sub>2</sub> extraction (expressed as, Cu-CaCl<sub>2</sub> mg/kg) at highest dosage of Cu addition.

0.01 M CaCl<sub>2</sub> should also not be deemed as a precise predictor of toxicity to barley root elongation.

#### 4.2. The effect of soil solution properties on soluble Cu toxicity

In the present study, DOC was the most important soil solution properties, which significantly alleviates the phytotoxicity of soil soluble Cu in wide range of soils. For toxicity thresholds based on soil pore water, the EC<sub>10</sub> increased 0.2 mg/L, and EC<sub>50</sub> increased 0.4 mg/L, respectively, as DOC in the soil solution increased 100 mg/L according to the regression Eqs. (3) and (4) in Table 3. It was more obvious that the EC<sub>x</sub> ( $x = 10, 50$ ) based on soluble Cu in pore water was influenced by DOC in the same trend, however the DOC exhibited even greater impacts on EC<sub>50</sub> (Fig. 4). In the 17 tested soils, the EC<sub>50</sub> values were more than 2 folds higher than corresponding EC<sub>10</sub> values. Thus, more soluble Cu existed in the soil solution system and a large number of free Cu<sup>2+</sup> was more readily to be complexed with DOC, which resulted in the reduction of free Cu<sup>2+</sup> concentration and thus reduced Cu toxicity (Qiu et al., 2006; Temminghoff et al., 1997). Therefore, when the DOC increased, the EC<sub>50</sub> values were increased more than EC<sub>10</sub>. The DOC

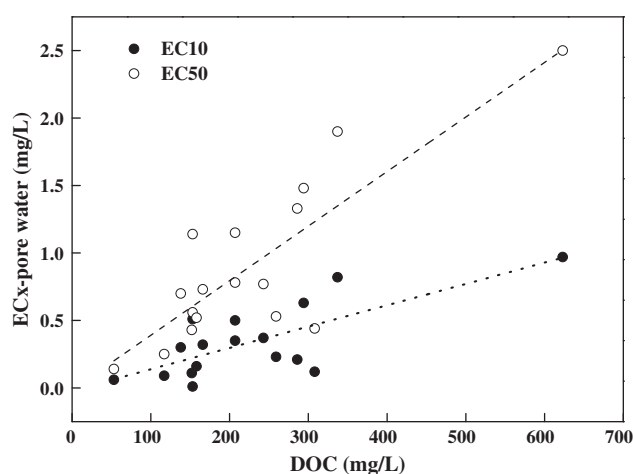


Fig. 4. Relationships between soil dissolved organic carbon (DOC) and toxicity threshold of Cu in soil pore water expressed as EC<sub>x</sub>-pore water (EC<sub>10</sub> and EC<sub>50</sub> which were 10% and 50% effective concentrations for barley root elongation, respectively).

is present in both terrestrial and aquatic ecosystem. Although the chemical structures and molecular weights may be different between soil solution and natural water, they share similar functional active groups (acidic (primarily carboxylic and phenolic), carboxyl, hydroxyl and other bridging units), capable of binding with Cu (Gardea-Torresdey et al., 1996). In water, Arnold et al. (2010) previously found that only DOC was significantly related to the median lethal concentration (LC<sub>50</sub>) to rotifer *Brachionus plicatilis* in 48-h static acute copper toxicity experiments, while pH and salinity were not statistically significant. Unanimous result was also observed in the culture solution (Guo et al., 2010; Linbo et al., 2009), where it was demonstrated that Cu toxicity can be decreased by the presence of organic ligands. Therefore, organic carbon could greatly influence the Cu toxicity not only in water system and culture solution but also the soil solution system.

It is not recommended to estimate the relationship between the soluble Cu toxicity thresholds in 0.01 M CaCl<sub>2</sub> extraction and cations because of dilution of DOC and colloids and also the variation of soil properties. Therefore, it is proper to focus on the soluble Cu toxicity threshold in pore water. Compared to DOC, pH was the second important, significant in Eqs. (1) and (2) (Table 3). The EC<sub>10</sub> was positively correlated with soil solution pH in the multiple regression analysis, while the linear relationship between 1/EC<sub>x</sub> and OH<sup>-</sup>, lgEC<sub>x</sub> and pH or EC<sub>x</sub> and H<sup>+</sup> was not found. It indicated that the effect of pH on soluble Cu toxicity thresholds in the complicated soil solution was greatly influenced by other parameters in the soils. In culture solution, the significant linear relationship between pH and EC<sub>50</sub> was reported, in which the variation of speciation distribution of Cu and other toxic forms of soluble Cu besides free Cu<sup>2+</sup> in the soil solution was considered (Wang et al., 2009). Previous study has revealed that the CuOH<sup>+</sup> was toxic besides free Cu<sup>2+</sup> and could improve the accuracy of evaluating the effect of soluble Cu toxicity in a wide range of pH conditions (Wang et al., 2009, 2012a). The soluble Cu toxicity increased with the CuOH<sup>+</sup> concentration increased. From the other hand, the soil organic carbon would partition more to soil solution as pH increased, which resulted in more free Cu<sup>2+</sup> complexed with DOC (Temminghoff et al., 1997). These Cu-DOC compounds were less toxic than free Cu<sup>2+</sup> (Guo et al., 2010) and thus the soluble Cu toxicity reduced as pH increased. For the pH < 8, the concentration of CuOH<sup>+</sup> steadily increased which contributed to the increase of soluble Cu toxicity. For the pH > 8, the concentrations of free Cu and CuOH<sup>+</sup> decreased and increased the DOC-Cu, which lead to the reduction of soluble Cu toxicity. Similarly, Wang et al. (2012a) reported that there were no significant variations on EC<sub>50</sub> at solution pH less than 6.5 and apparent reduction on EC<sub>50</sub> with increasing pH at solution pH of 7–8. The discrepancy of pH effect existed between EC<sub>10</sub> and EC<sub>50</sub>, for which the EC<sub>50</sub> was not significant in multiple regression analysis, indicating that the EC<sub>50</sub> was influenced by other soil solution properties simultaneously. In our bioassay, most soluble Cu may bind with DOC when added dosage Cu was small, while more Cu was added to cause root growth inhibition from 10% and 50%, which not only resulted the concentration of other speciation (such as free Cu<sup>2+</sup>, CuOH<sup>+</sup>) to increase, but also more free Cu<sup>2+</sup> would compete for the limited absorption site of DOC. Therefore, the complicated factors showed more influence on the EC<sub>50</sub> values in common with that on EC<sub>10</sub>.

The effects of cations were relatively small in the present study, which was supposed to alleviate the soluble Cu toxicity (Brown et al., 2012; Cheng and Allen, 2001; De Schamphelaere and Janssen, 2004; Kinraide et al., 2004). The K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup> did not significantly affect soluble Cu<sup>2+</sup> toxicity in the regression results. Na<sup>+</sup> more closely related with EC<sub>10</sub> and the protective ability of Mg<sup>2+</sup> against Cu toxicity was only found in Eq. (12) (Appendix Table 1). In general, these cations were not obviously linearly correlated to the EC<sub>50</sub> or EC<sub>10</sub>, indicating that competition for binding sites between Cu<sup>2+</sup> and cations such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup> was not an

important factor in determining soluble Cu<sup>2+</sup> phytotoxicity in common soil solutions. In the similar concentration ranges of Ca<sup>2+</sup>, Na<sup>+</sup> and Mg<sup>2+</sup> of 10–250 mg/L, 10–250 mg/L, 12.5–100 mg/L, respectively, Schwartz and Vigneault (2007) studied chronic Cu toxicity to *Ceriodaphnia dubia* and found that there was no effect of cationic competition using increasing Ca<sup>2+</sup> and Na<sup>+</sup> concentrations in the exposures and a small protective effect for Mg<sup>2+</sup>. Meanwhile, De Schampheleere and Janssen (2004) also reported that a competition effect of Na<sup>+</sup> was observed in chronic Cu toxicity to *Daphnia magna* not for Mg<sup>2+</sup> and Ca<sup>2+</sup>, in which the Ca<sup>2+</sup>, Na<sup>+</sup> and Mg<sup>2+</sup> activities were  $1.24 \times 10^{-4}$ – $2.07 \times 10^{-3}$ ,  $1.42 \times 10^{-3}$ – $1.85 \times 10^{-2}$  and  $3.08 \times 10^{-5}$ – $5.14 \times 10^{-4}$ , respectively. Some studies indicated that besides the competitive effect between the cation and Cu<sup>2+</sup> (Thakali et al., 2006a,b), the coexistent cations can be attributable to the changes in the enhancement factor of Cu<sup>2+</sup> activity at the outer surface of the plasma membrane (PM) and the electrical driving force across PMs (Wang et al., 2012b). These dual effects might be more adequate to explain the no apparent effects of cation on soluble Cu toxicity, rather than the sole cation competition effects.

In addition, S did not significantly relate to the soluble Cu toxicity in multiple regression equation. The S was often less significant in predicting copper toxicity compared with other important parameters, such as DOC and pH. The EC of the soil pore water was a reliable indicator of the solute (cation or anion) concentrations. Since the cation showed different influence on the soluble Cu toxicity, EC did not exhibit as an important predictor. Therefore, in the present study, DOC was the most important factor influencing the soluble Cu toxicity

thresholds based on pore water, followed by the pH, S, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and/or EC which were the least important factors.

### 5. Conclusions

Based on soluble Cu in the 0.01 M CaCl<sub>2</sub> extraction and soil pore water, the toxicity thresholds to barley root elongation varied widely with ≥7 folds in a wide range of Chinese soils and largely affected by soil solution chemistry and properties. The regression results showed that DOC was the most important factor in determining the soluble Cu toxicity, which decreased with increasing concentration of DOC in soils. Soil solution pH was less important but more complicated than DOC in influencing Cu toxicity. Other soil properties such as cations have inconsistent significant effect on soluble Cu toxicity, while they improve the r<sup>2</sup> in multiple regression analysis. We recommend to use these empirical relationships deriving from the common soil pore water chemistry for risk assessments of soluble Cu in a wide range of soils.

### Acknowledgments

This work was financially supported by the Natural Science Foundation of China (project no. 21007042 and 40971262), and the Special Fund for Public Industry in China (Agriculture, project no. 200903015). The authors also thank Cathy Fiebiger and Gillian Cozens from CSIRO for their technical assistance.

## Appendix A

Appendix Table 1

Simple and multiple linear regressions between Cu toxicity thresholds based on Cu concentrations in soil pore water (mg/L) and soil pore water chemistry (n = 17).

Regression equation	r <sup>2</sup>	p										Significant factors
1 EC10 = -0.62 + 0.094pH + 0.001 K - 0.0002Ca - 0.059EC + 0.0001 Mg + 0.0004Na + 0.0003S + 0.001DOC	0.82	0.086	0.87	0.28	0.73	1.0	0.42	0.59	0.008	DOC, pH, Na		
2 EC10 = -0.63 + 0.095pH + 0.001 K - 0.0002Ca - 0.064EC + 0.0004Na + 0.0003S + 0.001DOC	0.82	0.045	0.80	0.25	0.51	0.38	0.561	0.002	-	-		
3 EC10 = -0.58 + 0.090pH - 0.0002Ca - 0.073EC + 0.0004Na + 0.0004S + 0.001DOC	0.82	0.028	0.24	0.39	0.29	0.47	<0.001	-	-	-		
4 EC10 = -0.62 + 0.093pH - 0.0001Ca - 0.049EC + 0.0004Na + 0.001DOC	0.81	0.02	0.33	0.52	0.28	<0.001	-	-	-	-		
5 EC10 = -0.66 + 0.092pH - 0.0001Ca + 0.0002Na + 0.001DOC	0.80	0.017	0.23	0.031	<0.001	-	-	-	-	-		
6 EC10 = -0.65 + 0.085pH + 0.0002Na + 0.001DOC	0.78	0.026	0.034	<0.001	-	-	-	-	-	-		
7 EC10 = -0.70 + 0.092pH + 0.002DOC	0.68	0.032	<0.001	-	-	-	-	-	-	-		
8 EC10 = -0.019 + 0.002DOC	0.55	<0.001	-	-	-	-	-	-	-	-		
9 EC50 = -0.96 + 0.15pH + 0.012 K - 0.001Ca - 0.27EC + 0.006 Mg + 0.0001Na + 0.001S + 0.003DOC	0.87	0.14	0.17	0.12	0.43	0.43	0.88	0.54	0.003	DOC, Mg		
10 EC50 = -1.0 + 0.15pH + 0.013 K - 0.001Ca - 0.25EC + 0.006 Mg + 0.001S + 0.003DOC	0.87	0.11	0.11	0.088	0.4	0.37	0.52	0.002	-	-		
11 EC50 = -1.1 + 0.17pH + 0.014 K - 0.0005Ca - 0.23EC + 0.007 Mg + 0.003DOC	0.87	0.065	0.057	0.084	0.42	0.31	0.001	-	-	-		
12 EC50 = -1.0 + 0.14pH + 0.011 K - 0.0005Ca + 0.002 Mg + 0.003DOC	0.86	0.081	0.059	0.064	0.041	<0.001	-	-	-	-		
13 EC50 = -0.014 + 0.004DOC	0.70	<0.001	-	-	-	-	-	-	-	-		

r<sup>2</sup>: coefficient of determination (percentage of variance accounted for by the regression model); p: significant level; EC10: effective concentrations that caused 10% inhibition and EC50: effective concentrations that caused 50% inhibition; DOC: dissolved organic carbon; EC: electrical conductivity.

Appendix Table 2

Simple and multiple linear regressions between Cu toxicity thresholds based on Cu concentrations in 0.01 M CaCl<sub>2</sub> extraction (mg/kg) and soil pore water chemistry (n = 17).

Regression equation	r <sup>2</sup>	p										Significant factors
1 EC10 = -0.14 + 0.037pH - 0.007 K - 0.0001Ca + 0.14EC - 0.005 Mg + 0.0003Na + 0.0001S + 0.002DOC	0.78	0.50	0.17	0.48	0.45	0.21	0.51	0.91	0.002	DOC, K		
2 EC10 = -0.16 + 0.038pH - 0.007 K - 0.0001Ca + 0.14EC - 0.005 Mg + 0.0003Na + 0.002DOC	0.78	0.44	0.13	0.42	0.41	0.18	0.49	<0.001	-	-		
3 EC10 = -0.26 + 0.044pH - 0.006 K - 0.0002Ca + 0.18EC - 0.005 Mg + 0.002DOC	0.76	0.35	0.16	0.28	0.26	0.20	<0.001	-	-	-		
4 EC10 = 0.041 - 0.007 K - 0.0001Ca + 0.24EC - 0.006 Mg + 0.002DOC	0.74	0.036	0.35	0.11	0.084	<0.001	-	-	-	-		
5 EC10 = 0.024 - 0.008 K + 0.21EC - 0.005 Mg + 0.002DOC	0.72	0.031	0.14	0.11	<0.001	-	-	-	-	-		

Appendix Table 2 (continued)

Regression equation	$r^2$	p		Significant factors							
6 EC10 = 0.13 – 0.005 K – 0.001 Mg + 0.002DOC	0.66	0.097	0.19	<0.001	–	–	–	–	–	–	–
7 EC10 = 0.11 – 0.004 K + 0.002DOC	0.61	0.13	<0.001	–	–	–	–	–	–	–	
8 EC10 = 0.074 + 0.002DOC	0.54	<0.001	–	–	–	–	–	–	–	–	
9 EC50 = 1.52 – 0.20pH + 0.016 K – 0.0001Ca + 0.40EC – 0.003 Mg – 0.001Na – 0.002S + 0.004DOC	0.83	0.18	0.20	0.92	0.41	0.81	0.49	0.17	0.005	DOC, pH, K	
10 EC50 = 1.52 – 0.20pH + 0.016 K + 0.39EC – 0.002 Mg – 0.001Na – 0.003S + 0.004DOC	0.83	0.16	0.17	0.39	0.80	0.47	0.079	0.003	–	–	
11 EC50 = 1.4 – 0.18pH + 0.018 K + 0.30EC – 0.001Na – 0.003S + 0.004DOC	0.83	0.12	0.043	0.25	0.39	0.06	<0.001	–	–	–	
12 EC50 = 1.74 – 0.19pH + 0.016 K + 0.095EC – 0.002S + 0.004DOC	0.82	0.1	0.053	0.30	0.073	<0.001	–	–	–	–	
13 EC50 = 2.0 – 0.22pH + 0.013 K – 0.001S + 0.004DOC	0.78	0.059	0.082	0.051	<0.001	–	–	–	–	–	
14 EC50 = 2.8 – 0.32pH + 0.004DOC	0.68	0.011	<0.001	–	–	–	–	–	–	–	
15 EC50 = 0.40 + 0.004DOC	0.48	0.002	–	–	–	–	–	–	–	–	

$r^2$ : coefficient of determination (percentage of variance accounted for by the regression model); p: significant level; EC10: effective concentrations that caused 10% inhibition and EC50: effective concentrations that caused 50% inhibition; DOC: dissolved organic carbon; EC: electrical conductivity.

## References

- Arnold, W.R., Diamond, R.L., Smith, D.S., 2010. The effects of salinity, pH, and Dissolved organic matter on acute copper toxicity to the rotifer, *Brachionus plicatilis* ("L" strain). *Archives of Environmental Contamination and Toxicology* 59 (2), 225–234.
- Broos, K., Warne, M.S.T.J., Heemsbergen, D., Stevens, D., Barnes, M.B., Correll, R.L., McLaughlin, M.J., 2007. Soil factors controlling the toxicity of copper and zinc to microbial processes in Australian soils. *Environmental Toxicology and Chemistry* 26 (4), 583–590.
- Brown, M.G., Dobbs, E.K., Snodgrass, J.W., Ownby, D.R., 2012. Ameliorative effects of sodium chloride on acute copper toxicity among Cope's gray tree frog (*Hyla chrysoscelis*) and green frog (*Rana clamitans*) embryos. *Environmental Toxicology and Chemistry* 31 (4), 836–842.
- Cheng, T., Allen, H.E., 2001. Prediction of uptake of copper from solution by lettuce (*Lactuca sativa* Romance). *Environmental Toxicology and Chemistry* 20 (11), 2544–2551.
- Cuss, C.W., Guéguen, C., Hill, E., Dillon, P.J., 2010. Spatio-temporal variation in the characteristics of dissolved organic matter in the streams of boreal forests: impacts on modelled copper speciation. *Chemosphere* 80 (7), 764–770.
- De Schampelaere, K.A.C., Janssen, C.R., 2004. Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. *Environmental Toxicology and Chemistry* 23 (6), 1365–1375.
- Degryse, F., Smolders, E., Parker, D.R., 2009. Partitioning of metals (Cd, Co, Cu, Ni, Pb, Zn) in soils: concepts, methodologies, prediction and applications – a review. *European Journal of Soil Science* 60 (4), 590–612.
- Gardea-Torresdey, J.L., Tang, L., Salvador, J.M., 1996. Copper adsorption by esterified and unesterified fractions of Sphagnum peat moss and its different humic substances. *Journal of Hazardous Materials* 48 (1–3), 191–206.
- Guo, X.Y., Ma, Y.B., Wang, X.D., Chen, S.B., 2010. Re-evaluating the effects of organic ligands on copper toxicity to barley root elongation in culture solution. *Chemical Speciation and Bioavailability* 22 (1), 51–59.
- Haanstra, L., Doelman, P., Voshaar, J.H.O., 1985. The use of sigmoidal dose response curves in soil ecotoxicological research. *Plant and Soil* 84, 293–297.
- Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil-V: a method for measuring soil biomass. *Soil Biology and Biochemistry* 8 (3), 209–213.
- Kinraide, T.B., Pedler, J.F., Parker, D.R., 2004. Relative effectiveness of calcium and magnesium in the alleviation of rhizotoxicity in wheat induced by copper, zinc, aluminum, sodium and low pH. *Plant and Soil* 259 (1–2), 201–208.
- Li, B., Ma, Y.B., McLaughlin, M.J., Kirby, J.K., Cozens, G., Liu, J.F., 2010. Influences of soil properties and leaching on copper toxicity to barley root elongation. *Environmental Toxicology and Chemistry* 29 (4), 835–842.
- Linbo, T.L., Baldwin, D.H., McIntyre, J.K., Scholz, N.L., 2009. Effects of water hardness, alkalinity, and dissolved organic carbon on the toxicity of copper to the lateral line of developing fish. *Environmental Toxicology and Chemistry* 28 (7), 1455–1461.
- Lock, K., Criel, P., De Schampelaere, K.A.C., Van Eeckhout, H., Janssen, C.R., 2007. Influence of calcium, magnesium, sodium, potassium and pH on copper toxicity to barley (*Hordeum vulgare*). *Ecotoxicology and Environmental Safety* 68 (2), 299–304.
- Luo, X.S., Zhou, D.M., Wang, Y.J., 2006a. Solid/solution partitioning and speciation of heavy metals in the contaminated agricultural soils around a copper mine in eastern Nanjing city, China. *Journal of Hazardous Materials* 131 (1–3), 19–27.
- Luo, X.S., Zhou, D.M., Wang, Y.J., 2006b. Free cupric ions in contaminated agricultural soils around a copper mine in eastern Nanjing City, China. *Journal of Environmental Sciences* 18 (5), 927–931.
- Luo, X.S., Li, L.Z., Zhou, D.M., 2008. Effect of cations on copper toxicity to wheat root: Implications for the biotic ligand model. *Chemosphere* 73 (3), 401–406.
- Warne, M.S.T.J., Heemsbergen, D., Stevens, D., McLaughlin, M., Cozens, G., Whatmuff, M., Broos, K., Barry, G., Bell, M., Nash, D., Pritchard, D., Penney, N., 2008. Modeling the toxicity of copper and zinc salts to wheat in 14 soils. *Environmental Toxicology and Chemistry* 27 (4), 786–792.
- Qiu, J.W., Tang, X., Zheng, C.B., Li, Y., Huang, Y.L., 2006. Copper complexation by fulvic acid effects copper toxicity to the larvae of the polychaete *Hydroides elegans*. *Marine Environmental Research* 64 (5), 563–573.
- Rooney, C.P., Zhao, F.J., McGrath, S.P., 2006. Soil factors controlling the expression of copper toxicity to plants in a wide range of European soils. *Environmental Toxicology and Chemistry* 25 (3), 726–732.
- Rooney, C.P., Zhao, F.J., McGrath, S.P., 2007. Phytotoxicity of nickel in a range of European soils: influence of soil properties, Ni solubility and speciation. *Environmental Pollution* 145 (2), 596–605.
- Salam, A.K., Helmke, P.A., 1999. The pH dependence of free ionic activities and total dissolved concentrations of copper and cadmium in soil solution. *Geoderma* 83 (3–4), 281–291.
- Sauvé, S., Hendershot, W., Allen, H.E., 2000. Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. *Environmental Science and Technology* 34 (7), 1125–1131.
- Schwartz, M.L., Vigneault, B., 2007. Development and validation of a chronic copper biotic ligand model for *Ceriodaphnia dubia*. *Aquatic Toxicology* 84 (2), 247–254.
- Strawn, D.G., Baker, L.L., 2008. Speciation of Cu in a contaminated agricultural soil measured by XAFS,  $\mu$ -XAFS, and  $\mu$ -XRF. *Environmental Science & Technology* 42 (1), 37–42.
- Temminghoff, E.J.M., Van der Zee, S.E.A.T.M., de Haan, F.A.M., 1997. Copper mobility in a copper-contaminated sandy soil as affected by pH and solid and dissolved organic matter. *Environmental Science & Technology* 31 (4), 1109–1115.
- Thakali, S., Allen, H.E., Di Toro, D.M., Ponizovsky, A.A., Rooney, C.P., Zhao, F.J., McGrath, S.P., 2006a. A terrestrial biotic ligand model. 1. Development and application to Cu and Ni toxicities to barley root elongation in soils. *Environmental Science & Technology* 40 (22), 7085–7093.
- Thakali, S., Allen, H.E., Di Toro, D.M., Ponizovsky, A.A., Rooney, C.P., Zhao, F.J., McGrath, S.P., Criel, P., Van Eeckhout, H., Janssen, C.R., Oorts, K., Smolders, E., 2006b. Terrestrial biotic ligand model. 2. Application to Ni and Cu toxicities to plants, invertebrates, and microbes in soil. *Environmental Science & Technology* 40 (22), 7094–7100.
- Thibault, D.H., Sheppard, M.L., 1992. A disposable system for soil pore-water extraction by centrifugation. *Communications in Soil Science and Plant Analysis* 23 (13–14), 1629–1641.
- Wang, X.D., Ma, Y.B., Hua, L., McLaughlin, M.J., 2009. Identification of hydroxyl copper toxicity to barley (*Hordeum vulgare*) root elongation in solution culture. *Environmental Toxicology and Chemistry* 28 (3), 662–667.
- Wang, X.D., Hua, L., Ma, Y.B., 2012a. A biotic ligand model predicting acute copper toxicity for barley (*Hordeum vulgare*): influence of calcium, magnesium, sodium, potassium and pH. *Chemosphere* 89 (1), 89–95.
- Wang, P., De Schampelaere, K.A.C., Kopittke, P.M., Zhou, D.M., Peijnenburg, W.J.G.M., Lock, K., 2012b. Development of an electrostatic model predicting copper toxicity to plants. *Journal of Experimental Botany* 63 (2), 659–668.
- Yen Le, T.T., Peijnenburg, W.J.G.M., Jan Hendriks, A., Vijver, M.G., 2012. Predicting effects of cations on copper toxicity to lettuce (*Lactuca sativa*) by the biotic ligand model. *Environmental Toxicology and Chemistry* 31 (2), 355–359.
- Zarcinas, B.A., McLaughlin, M.J., Smart, M.K., 1996. The effect of acid digestion technique on the performance of nebulisation systems used in inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis* 27 (5–8), 1331–1354.
- Zhao, F.J., Rooney, C.P., Zhang, H., McGrath, S.P., 2006. Comparison of soil solution speciation and diffusive gradients in thin-films measurement as an indicator of copper bioavailability to plants. *Environmental Toxicology and Chemistry* 25 (3), 733–742.