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CHEMICAL FRACTIONATION OF CU AND ZN AND THEIR IMPACTS ON MICROBIAL PROPERTIES IN SLIGHTLY CONTAMINATED SOILS

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Abstract

Chemical fractionation of Cu and Zn in bulk soil and its effects on soil microbial properties were determined in Cu and Zn contaminated soils (Cu: 35.57~46.37 mg·kg^{-1}, Zn: 74.33~127.20 mg·kg^{-1}) sampled from an agricultural field in outskirts of Zibo, China during the month of September, 2011. A sequential extraction technique (SET) was used for metals chemical fractionation analysis in soils and a correlation analysis was applied to determinate the effects of metal on soil microbial properties. Chemical speciation showed that Cu and Zn were mostly present in the residual fraction and their concentrations in the most labile fraction (acid soluble fraction) were the lowest in the investigated soils. However, the correlation analysis indicated that the labile forms of Cu/Zn, such as its acid soluble, reducible or oxidizable fractions, were usually significantly negatively correlated with the tested microbial activities at 0.05 or 0.01 probability levels. These results indicate that the metal labile fractions could exert an inhibitory effect on the soil microbial parameters even in the minor contaminated soils.

Keywords: Chemical Fractionation, Heavy Metals, Microbial Biomass, Soil Enzymes

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Introduction

Soil microbial biomass and enzyme activities are considered to be good bio-indicators reflecting natural and anthropogenic disturbances, and evaluating soil enzyme activities is one of the cheapest and easiest techniques that can be used to evaluate soil pollution (Hinojosa *et al.,* 2004; Khan *et al.,* 2007). A considerable body of information has been accumulated in the last century on the inhibition of heavy metals on soil microbial activities and the factors that affect this inhibition (Karaca *et al.*, 2010). Nevertheless, an enormous disparity of results is evident from the studies, in particular such as the concentration to which metal is toxic (Ge *et al.,* 2000; Li *et al.,* 2001). These discrepancies are due to factors, which modify the toxicity of the metals and to the differences in sensitivity of soil enzymes (Giller *et al.,* 2009; Karaca *et al.*, 2010).

Beside the environmental factors, microbial toxicity of heavy metals had been attributed to the two factors: metal concentration and bioavailability. The available forms of a metal are significant when attempting to understand metal toxicity, and its available forms are related to its chemical forms in the soil (Wang *et al.,* 2007a, b). Several workers studied the correlation between metal forms and microbial activities in long-term contaminated soils (Wang *et al.,* 2007a, b; Bhattacharyya *et al.,* 2008), but not much information is available on the relationship of different metal fractions and soil microbial activities in slightly contaminated soils with trace metals.

Present work aimed to study chemical fractionation of Cu and Zn in bulk soil and its effects on soil microbial biomass and enzymes activities in minor Cu and Zn contaminated soils.

Materials and Methods

Study area and samples collection

The study sites are situated at the agricultural fields grown with wheat and corn in the outskirts of Zibo, China (36°48′ N, 118°03′E). Four sites were selected, of which the site 1, 2 and 4 lie in district of Zibo city, the site 3 is near to industrial area.

Three surface soil samples from each of the 4 selected study sites were collected during the month of September, 2011. Altogether 12 soil samples were brought to the laboratory in the sealed polythene bags. The soils were passed through a 2 mm sieve and dispersed well. A portion of the soil samples was air-dried for chemical analysis. The others were store at 4°C for microbial analysis, which was completed within two weeks.

Physico-chemical analysis of soils

The pH was determined in soil: water @ 1:2.5 suspensions. Organic carbon (OC) and total metal contents were determined by the method of Lu (1999). Soil texture was determined with a laser particle size analyzer (Model: Rise-2028, China).

Fractionations of metals

Chemical fractionation of trace metal Cu and Zn in soil was carried out following a sequential extraction technique (Quevauviller *et al.,* 1997). To explain the method briefly, 1.0 g of soil samples (in dry weight) were sequentially extracted with 0.11 M CH_3COOH (16 h shaking, 120 rpm) for acid soluble fraction, with 0.1 M $NH_3{\cdot}HCl$ (pH = 1.5, 16 h shaking, 120 rpm) for reducible fraction, with 8.8 M H_2O_2 (pH=2) at room temperature (1 h, shaking, 120 rpm) and 85 (1 h, shaking,120 rpm), and then added 1M NH_4COOH (pH = 2, 16 h shaking, 120 rpm) for oxidizable fraction, and finally with concentrated HNO_3 at 105°C for residual faction. As concentrations in the extracts were measured by ICP-MS (7500ce, Agilent, USA) and the results were expressed on a moisture-free basis.

Microbiological analysis

Microbial biomass carbon (MBC) content was determined by the fumigation extraction method (Gregorich *et al.,* 1990) using K_{EC} (K_{EC} is the calibration factor) factor of 0.38 (Joergensen, 1995). Urease, Dehydrogenase and arylsulphatase activities of soils were estimated following the methods suggested by Tabatabai and Bremner (1970, 1972); Trevors (1984); García *et al.* (1993) respectively. These measurements were performed using the field moist soils. However, the parameters were expressed on a moisture-free basis.

Statistical analysis

The significance of differences among the parameters was tested by two-way analysis of variance (ANOVA) followed by Tukey's test. Pearson correlation analysis and partial correlation analysis were performed to investigate the forms of metals suppressing the soil microbial activities in the soil. All statistical analyses were carried out with the program SPPS 17.0 for Windows.

Results and Discussion

Physico-chemical properties of soil

Table 1 shows the properties of the sampled soils. The soils were slightly alkaline in nature as the pH values ranged from 7.39 to 7.54. Organic carbon contents of the soil samples were ranged from 7.76% to 8.70%, which are somewhat lower than or comparable with other agriculture soils in China (Meng *et al.,* 1996; Dai *et al.,* 2009) and the clay contents were ranged from 45.53% to 48.87%. No differences were found between the investigated sites on the tested soil properties.

Table 1. Soil characteristics and heavy metals contents at each sampling sites

Sample sites	pH	OC (g/kg)	Clay (%)	Cu ($mg{\cdot}kg^{-1}$)	Zn ($mg{\cdot}kg^{-1}$)
1	7.52±0.06a*	8.30±0.11a	45.53±2.30a	41.23±1.50a	83.33±1.53a
2	7.54±0.09a	8.02±0.14a	48.87±1.89a	35.17±2.76b	121.01±5.31b
3	7.44±0.01a	8.50±0.10a	57.73±3.02a	46.37±0.95c	127.20±4.58b
4	7.39±0.03a	8.76±0.59a	46.90±1.31a	42.20±0.95a	74.33±3.61c
Background values[b]	--	--	--	20.4±10.05d	60.6±18.88d

Mean values followed by the same letter are not significantly different according to ANOVA and multiple comparisons with Tukey test ($p < 0.05$); [b] the background values of soils (Luo, 1994).

Total amount of Cu and Zn in soils ranged from 35.17 to 46.37 $mg{\cdot}kg^{-1}$ and from 74.33 to 130.67 $mg{\cdot}kg^{-1}$, respectively, and both were highest in the site 3. However, in the present study, soil concentrations of Cu and Zn are generally lower than those reported in other Cu/Zn-contaminated sites (Li *et al.,* 2008), although they are significantly higher than their soil background values.

Chemical speciations of Cu and Zn in soils

Soil Cu and Zn were present in various forms from easily leachable to recalcitrant ones due to interactions with various soil components. Consequently, the total concentrations cannot provide a precise index representing the influence of Cu and Zn on soil microorganisms and enzyme activities (Kunito *et al.,* 2001; Sun *et al.,* 2006). Distribution of Cu and Zn forms in the investigated soils is given in Fig. 1.

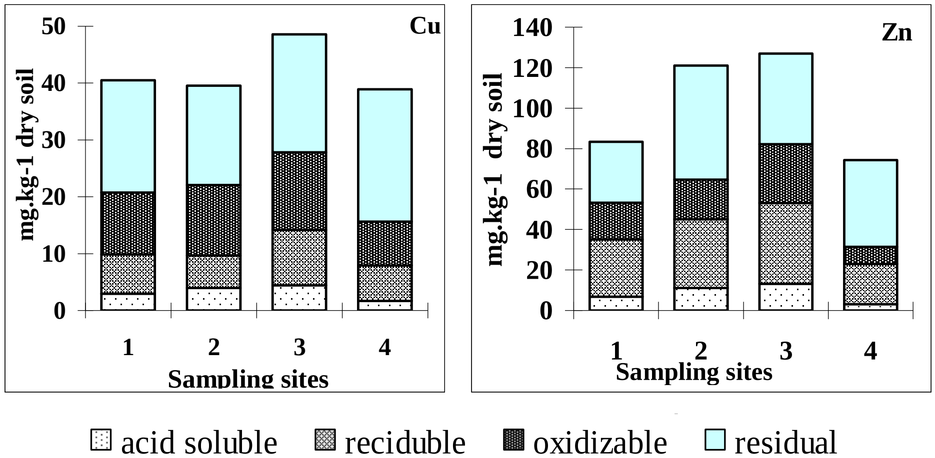

Fig. 1. Form patterns of Cu and Zn in the studied soils

It was shown that Cu and Zn were mainly present residual fractions in the investigated soils, and their concentrations in the most labile fraction (acid soluble fraction) were the lowest. In addition, the oxidizable Cu fraction and the reducible Zn fraction possessed the highest level among their three bioavailable fractions (acid soluble-, reducible- and oxidizable fraction), respectively. These are consistent with those of other reports (Bhattacharyya *et al.*, 2008, Kunito *et al.*, 2001; Saeki and Okazaki, 1993; Saeki *et al.*, 1993). The high percentages of reducible-Zn and oxidizable-Cu might indicate that the labile fractions of soil Cu or Zn would potentially increase in the future by reductive dissolution of Fe (III)/Mn (IV) oxides and mineralization of organic moieties under an appropriate condition in the investigated area (Lagomarsino *et al.,* 2011). Finally, in all the investigated sites, the sum of three bioavailable fractions of Cu/Zn, was highest in the site 3 and the lowest in the site 4. These trends are consistent with that of their total amount (Table 1). Some studies also reveal that total metal encompasses all metal forms including water soluble and exchangeable ones in soils (Bhattacharyya *et al.,* 2008).

Soil microbial activities

Soil enzymes and microbial biomass carbon are used to estimate the adverse effects of various pollutants on soil quality (Dick, 1997). Some significant variations in soil mcirobial activities between the soils were indicated in this study (Table 2).

Table 2. Microbial biomass carbon and enzyme activities in each soil

Parameter	1	2	3	4
MBC ($mg\cdot kg^{-1}$)	17.32±0.75a*	16.82±0.26a	14.85±0.33c	19.18±0.39d
Urease (mmol NH4$kg^{-1}\cdot 5h^{-1}$)	1.84±0.04a	1.94±0.08b	1.05±0.08c	1.99±0.02b
Dehydrogenase (mmol INTF $kg^{-1}\cdot 2h^{-1}$)	7.83±0.21a	7.41±0.20a	7.05±0.14b	7.86±0.15a
Arylsulfatase (mmol p-nitrophenol $kg^{-1}\cdot h^{-1}$)	43.21±1.60a	37.35±1.21b	36.03±0.90b	41.71±0.92a

*Mean values followed by the same letter are not significantly different according to ANOVA and multiple comparisons with Tukey test ($p < 0.05$)

The MBC in the tested soils ranged from 104.85 to 119.18 $mg\cdot kg^{-1}$, being lowest in the site 3 (Table 2). The tested soils enzymes also showed the significantly lower activities in soils from the site 3 than that from other sites. This indicated the inhibitory effect of metals in the contaminated soils on the production of the enzymes, which were conformed by the Pearson-correlation analysis results showed in Table 3. Others, activities of the studied soil enzymes and soil MBC were all almost significantly and positively correlated with soil pH and OM at 0.05 or 0.01 level of statistical significance (Table 3). Therefore, beside of Cu and Zn, soil organic matter and pH are also key factors for microbial growth and enzyme synthesis in the investigated soils (Karaca *et al.*, 2010).

Table 3. Pearson-correlation analysis results between studied parameters

	Urease	Dehydrogenase	Arylsulphatase	MBC
A1	-.919**	-.873**	-.741**	-.959**
B1	-.365	-.628*	-.664*	-.445
C1	-.903**	-.890**	-.776**	-.968**
Cu	-.647*	-.838**	-.806**	-.735**
A2	-.808**	-.739**	-.652*	-.904**
B2	-.676*	-.659*	-.594*	-.870**
C2	-.410	-.404	-.374	-.132
Zn	-.455	-.674*	-.657*	-.606*
pH	0.839**	0.820**	0.809**	0.670*
OM	0.760**	0.889**	0.807**	0.880**

*,** Correlation is significant at 0.05 and 0.01 levels, respectively; the same below

Influence of metals on soil microbial parameters

In the present study, partial correlations analysis, under the control of pH and OM, were conducted to distinguish the effects of trace metals on the microbial parameters from the soil properties. The total metal amount and its bioavailable values (acid soluble-, reducible- and oxidizable-fractions) were used for the analysis. The results obtained were showed in Table 4.

Table 4. Partial correlations of metals forms and microbial properties under the control variables of pH and OM

	Urease	Dehydrogenase	Arylsulphatase	MBC
A1	-.871**	-.857**	-.341	-.926**
B1	.522	.156	-.602*	.378
C1	-.858**	-.883**	-.436	-.935**
Cu	-.257	-.517	-.540	-.552
A2	-.815**	-.844**	-.479	-.926**
B2	-.799*	-.805**	-.547	-.946**
C2	.389	.503	.470	.634*
Zn	-.436	-.714*	-.911**	-.638*

As shown in Table 4, the labile Cu/Zn forms, such as its acid soluble, reducible and oxidizable fractions, were significantly negatively correlated with the studied enzyme activities at 0.05 or 0.01 probability levels. According to Bhattacharyya *et al.* (2008), the MBC and soil enzymes activities were significantly and negatively correlated with the metal labile forms as well as with its total metal concentrations due to the colinearity effect because total metal encompasses all the metal forms. However, no significant inhibition of the total Cu amounts on MBC and enzyme activities except of Arysulfatase were found in the partial correlation analysis (Table 4), although the adverse effects of the total Zn amounts on dehydrogenase, arysulfatase and MBC is increased in the partial correlation analysis (Table 4). These results indicate that labile metal forms are the most important factors regulating MBC and enzyme activities in heavy metal contaminated soils (Karaca *et al.*, 2010).

The Cu and Zn concentrations in the present study (Table 1) were quietly lower than the lowest observed adverse effect concentrations (LOAECs) of Cu (150 $mg \cdot kg^{-1}$) and Zn at Ludington (McGrath *et al.*, 1995). However, the significant inhibition of their forms on the tested microbial parameters was also found in the investigated soils. This may be related to that there are wide variations in the concentrations because various soil properties may also influence the LOAECs (Baath, 1989; McGrath *et al.*, 1995).

Conclusions

The trace elements Cu and Zn were slightly enriched in the investigated soils according to the soil back ground values, and they were mostly present in the residual fractions in bulk soil. Moreover, the correlation analysis between chemical speciation and soil microbial parameters also revealed that the labile forms of Cu and Zn could exert an inhibitory effect on soil Urease, Dehydrogenase and soil microbial biomass in the investigated field. The results signify the important role of the labile metal fractions exerting inhibitory effect on the microbial parameters even though they occupy only a small part of the total metal concentration.

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