Cu fractions, mobility and bioavailability in soil-wheat system after Cu-enriched livestock manure applications

T.X. Guan [a,b], H.B. He [a,*], X.D. Zhang [a], Z. Bai [a]

[a] Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100049, China

ABSTRACT

Fertilization of crops with livestock manure (LM) is a common waste disposal option, but repeated application of LM containing high concentrations of heavy metals such as Cu could lead to crop toxicity and environmental risk. To examine the Cu availability and uptake by wheat in a Mollisol affected by Cu-enriched LM, pot experiments were conducted. LM (376 mg kg^{-1} Cu originally) was spiked with different concentrations of Cu (0, 100, 200, 400, 600 and 800 mg kg^{-1} soil, added as CuSO$_4$) to simulate soil Cu contamination by LM application. The results indicated that Cu was predominately distributed in organic bound fraction, while the most drastic increase was found in reducible fraction. Acid-extractable fraction played a more important role than other fractions in controlling the mobility and bioavailability of Cu. DTPA-extractable Cu may overestimate the Cu bioavailability since DTPA solution could extract soluble and part of stable forms. The application of LM at 1% level significantly decline the Cu mobility, but that at 3% level exhibited the opposite effect.

Although the quantities of Cu in wheat was very low compared with the accumulation in soil, Cu concentrations in roots increased evidently from 12 to 533 mg kg^{-1} and that in aerial parts were in a narrow range from 12.1 to 32.7 mg kg^{-1}, indicating the more sensitivity of roots to the Cu toxicity. The Cu concentrations in grains after 3% manure application did not approach the threshold for Cu toxicity (<20 mg kg^{-1}) even at higher Cu addition rates.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Cu is an essential nutrient for plant growth at low concentrations, but excessive amount are phytotoxic (Michaud et al., 2007) and can bring potential threat to human health (Cattani et al., 2006; Dolgen et al., 2007; Achiba et al., 2009; Soriano-Disla et al., 2010). As a mineral element, the Cu in plant was derived from soil matrix, however, the potential behavior, bioavailability and the transfer dynamics of Cu in soil–plant system can be poorly predicted by the total content (Maiz et al., 2000; Fuentes et al., 2004; He et al., 2009; Achiba et al., 2010). It has been realized that the toxicity of Cu is determined by cupric ion activity; as a result, the mobile Cu is more toxic to the plant growth than strongly complex forms (Sauvé et al., 1998; Meers et al., 2007; Wang et al., 2009). In soil matrix, Cu can be associated with soil components and exist in various chemical physical-forms including soluble and exchangeable Cu, precipitated as Fe and Mn oxides or carbonates, complexed or adsorbed to organic matter and associated to clay mineral in soils (Ahumada et al., 2009). These fractions possess different abilities to retain or release Cu, thus significantly influence the Cu mobility and bioavailability (Kabala and Singh, 2001).

Both single and sequential extraction procedures have been used to estimate the distribution and bioavailability of Cu in soil (Kennedy et al., 1997; Maiz et al., 2000; Chaignon et al., 2003). The single extraction is convenient to perform (Soriano-Disla et al., 2010), and a chelating agent DTPA has been widely used for the diagnosis of plant availability of the metals in soil at regular or even higher concentration (Singh et al., 1998). In most cases, the DTPA-extractable Cu (DTPA-Cu) was found to be correlated with the Cu concentration in crops (Obrador et al., 1997; Fuentes et al., 2004; Cattani et al., 2006). However, the DTPA-Cu sometimes not really represents the available forms due to the varied application times and soil types (Beckett et al., 1983; Bolan et al., 2004). The sequential extraction technique provides more detailed information on Cu fractionation as well as the actual and potential transport among different chemical forms (Tessier et al., 1979; Ure et al., 1993; Nyamangara, 1998; Narwal et al., 1999; Doelsch et al., 2008; Nemati et al., 2009). The acid-extractable and organic bound fractions of Cu in soil have been used to predict plant uptake

---

Abbreviation: DTPA, diethylene triamine penta-acetic acid.
* Corresponding author. Tel./fax: +86 024 83970376.
  E-mail address: hehongbo@iae.ac.cn (H.B. He).

(McBride et al., 2004; Kidd et al., 2007; Ahumada et al., 2009; He et al., 2009), whereas reducible Fe and Mn oxide bound fractions were known to be more important for controlling the mobility and bioavailability of Cu under waterlogged reduced conditions (Bhattacharyya et al., 2006). Considering the complicated manner in soil chemical processes which would influence the plant uptake of metal nutrients (Hinsinger et al., 2005; Torri and Lavado, 2009), there is still no well recognized method to evaluate the bioavailability of metals in soil (Sauvé et al., 2000; Wang et al., 2003).

The availability of metals is highly linked with their sources (Bolan et al., 2003). In agroecosystems, livestock manure (LM) has long been applied as a source of organic fertilizer, plant functional nutrients and certain microelements to improve soil quality and benefit plant production (Hsu and Lo, 2001; Ogiyama et al., 2005; Sánchez and González, 2005; Zhou et al., 2005; Lipoth and Schoenau, 2007; Berenguer et al., 2008). In intensive livestock production systems, Cu concentration in LM is usually high because of poor assimilation of Cu additives in livestock feed used for their growth and reproduction (L'Herroux et al., 1997; Nicholson et al., 1999; Hsu and Lo, 2001; Bolan et al., 2003; Mantovi et al., 2003; Cang et al., 2004; Li et al., 2005; Marcato et al., 2009; Achiba et al., 2010). The Cu concentration in LM is up to 1726 mg kg^{-1} of dry matter in China (Cang et al., 2004), and similar results were found by Nicholson in England and Wales (Nicholson et al., 2003). In arable soils, LM is normally applied at the rate of 30 t to 90 t ha^{-1} year^{-1} in fresh weight equivalent to about 0.9–2.8% soil by dry weight considering the water content as 25–30% (Benke et al., 2008). Therefore, the possible diffusion pollution brought by the application of LM attracted more attention because of the accumulation of Cu in soil and the increase of uptake by plants (Qian et al., 2003). However, the Cu distribution pattern in soils, the transformation dynamics and bioavailability to plant after the application of Cu-enriched manure have not been intensively studied.

To estimate Cu accumulation and transformation in soil after the application of LM with different concentrations of Cu, LM-derived Cu was obtained through increasing the quantities or the times of manure in most cases. However, in our study, considering fertilization quantity during the growing period of wheat, the Cu-enriched LM was gained by spiking a series of Cu with gradual concentrations into less contaminated LM. The spiked Cu in LM, despite in ionic form at the beginning, can easily saturate organic matter adsorption sites after a period of time, hence this method was often essential in simulating Cu pollution conditions and predicting the resistance capacity of plants (Bolan et al., 2003; Zhou et al., 2005). Therefore, pot experiments were conducted in a Mollisol with Cu addition either in ionic form or decomposed with LM. We focused on the available forms and different chemical fractions of Cu in the soil, as well as the Cu uptake and transformation in wheat tissues. The aim of our work was to assess the potential effects of LM on Cu distribution and availability, and to elucidate the relationship between Cu forms and wheat uptake in soil-wheat system.

2. Materials and methods

2.1. Soil Samples and livestock manure (LM)

Surface soil samples (0–20 cm in depth) were collected from a representative Mollisol in Hailun City, Heilongjiang Province, Northeast China (126°55′E, 47°27′N). The total Cu concentration was in the range of the national environmental quality standard for agricultural soils (Cu 50 mg kg^{-1} in GB 15618-1995) issued by State Environmental Protection Administration of China. The livestock manure was provided by the College of Environmental and Resource Sciences of Zhejiang University. The properties of the soil and LM were shown in Table 1.

2.2. Experiment design

The soil samples were air-dried, ground, and sieved (<5 mm) prior to the pot experiment. Chemical fertilizers, at the rates equivalent to 150 kg ha^{-1} N (urea), 80 kg ha^{-1} P$_2$O$_5$ (calcium superphosphate), and 70 kg ha^{-1} K$_2$O (potassium chlorine) were mixed with 4.5 kg air-dried soil (bulk soil) thoroughly in plastic pots (20 cm in height and 17 cm in diameter) before sowing. The experiment totally contained 18 treatments. The 12 of the treatments included two levels of LM (1% and 3% to bulk soil by weight) spiked with a series of Cu^{2+} concentration (0, 100, 200, 400, 600 and 800 mg kg^{-1} soil, added as CuSO$_4$) and decomposed for 2 weeks, then the simulated Cu-enriched LM was mixed up with the prepared soil samples. For the other six treatments, the corresponding concentrations of Cu^{2+} solution were added into soil without LM application (referred as control). All the treatments were arranged in a complete random design with four replicates. Twenty-eight seeds of wheat were sowed in each pot and the seedlings were thinned twice to the total of 14 in each pot. The moisture content of 60% field water holding capacity was adjusted by weight during wheat growth. Wheat plants were harvested after 90 d for analysis.

2.3. Sampling

The soil samples were collected destructively and air-dried, ground and sieved to <2 mm for the analysis of available Cu and <0.15 mm for total Cu and different fractions of Cu, respectively.

The collected plant samples (roots, straw and grains) were washed with tap water to remove attached soil, rinsed three times with deionized water and were oven-dried at 70 °C until a constant weight was obtained. The dried plant samples were used for biomass determination, and then they were stored in desiccators for total Cu analysis after being ground and sieved to <0.25 mm.

2.4. Chemical extraction procedures

The total Cu content in soil was hydrolyzed by a mixture of concentrated HCl–HNO$_3$–HF–HClO$_4$ solution.

The total Cu in wheat tissues was digested by concentrated HNO$_3$–HClO$_4$ solution (Zhou et al., 2005).

To evaluate the bioavailability of Cu in soil, DTPA solution (0.005 M DTPA, 0.01 M CaCl$_2$ and 0.1 M triethanolamine (pH 7.3) was added to soil samples (10:1 v/w). After shaking at 25 °C for 2 h, the supernatant from each extraction was collected (Lindsay and Norvell, 1978).

A metal fractionation scheme was carried out following the sequential extraction procedure proposed by the Community Bureau of Reference (BCR) in 1987. The BCR three-step sequential extraction procedures have been used to partition metals into fractions defined as water-soluble, exchangeable and carbonate-bound (acid-extractable fraction, Acid-F), Fe and Mn oxide bound (reducible fraction, RE-F), organic matter and sulphide bound (oxidable fraction, OXi-F), and silicate-bound fraction (residual fraction, RES-F) (Ure et al., 1993). The detailed steps were as follows: (1) Acid-F: 1.0 g soil samples were shaken at 22 °C with 40 mL 0.11 M HOAc for 16 h; (2) RE-F: the residue from the previous extraction was dried and then shaken at 22 °C with 40 mL 0.5 M NH$_2$OH·HCl (pH 2.0) for 16 h; (3) OXi-F: 10 mL 30% H$_2$O$_2$ (pH 2–3) was added to the resulting residue and shaken at 22 °C for 1 h, then dried in a water bath of 85 ± 2 °C; a second 10 mL 30% H$_2$O$_2$ was added and taken to dryness and after cooling, 50 mL 1.0 M NH$_4$OAc (pH 2.0) was added and shaken at 22 °C for 16 h; (4) RES-F: the residue from the third step was digested by HCl–