Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 19(2007) 1482–1490

JOURNAL OF
ENVIRONMENTAL
SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Effects of two sludge application on fractionation and phytotoxicity of zinc and copper in soil

HE Miao-miao[1], TIAN Guang-ming[1,*], LIANG Xing-qiang[1], YU Yi-tong[1],
WU Jian-yang[1], ZHOU Gen-di[2]

1. Department of Environmental Engineering, Zhejiang University, Hangzhou 310029, China. E-mail: hemiaomiao0343@126.com
2. Department of Life Science, Hangzhou Teachers College, Hangzhou 310006, China

Received 23 January 2007; revised 7 June 2007; accepted 26 August 2007

Abstract

The potential harm of heavy metals is a primary concern in application of sludge to the agricultural land. A pot experiment was conducted to evaluate the effect of two sludges on fractionation of Zn and Cu in soil and their phytotoxicity to pakchoi. The loamy soil was mixed with 0%, 20%, 40%, 60% and 80% (by weight) of digested sewage sludge (SS) and composted sludge (SC). The additions of the both sludges caused a significant raise in all fractions, resulting in that exchangeable (EXCH) and organic matter (OM) became predominance for Zn and organic bound Cu occupied the largest portion. There was more available amount for Zn and Cu in SS treatments than SC treatments. During the pot experiment, the concentration of Zn in EXCH, carbonate (CAR) and OM and Cu in EXCH and OM fractions decreased in all treatments, so their bioavailability reduced. Germination rate and plant biomass decreased when the addition rate was high and the best yield appeared in 20% mixtures at the harvest of pakchoi. The two sludges increased tissue contents of Zn and Cu especially in the SS treatments. Zn in pakchoi was not only in relationship to ΔEXCH and ΔCAR forms but also in ΔOM forms in the sludge-soil mixtures. Tissue content of Cu in pakchoi grown on SC-soils could not be predicted by ΔEXCH. These correlation rates between Zn and Cu accumulation in pakchoi and variation of different fractions increased with time, which might indicate that sludges represented stronger impacts on the plant in long-term land application.

Key words: sludge; heavy metal; fraction; pakchoi; phytotoxicity

---

Introduction

With more wastewater treatment plants appearing, the accumulation of sewage sludge becomes a growing loading to the environment. Reusing stabilized sludge to agricultural land may bring considerable agronomic, economic and environmental benefits (Logan and Harrison, 1995; Sims and Pierzynski, 2000; Ramos, 2006), because it enables the recycling of valuable components such as N, P, K, organic matter and other necessary nutrients for plant growth and improves the soil structure (Smith, 1996; García-Orenes *et al*., 2005). However, the presence of potentially toxic elements (PTEs), such as heavy metals, organic compounds or any low molecular weight fatty acids (Wollan *et al*., 1978; Roe *et al*., 1997; Wong *et al*., 2001), limits the agricultural application of sewage sludge due to its inhibition to the seed germination and food chain contamination (Chang *et al*., 1992).

In China, zinc (Zn) and copper (Cu) are of major concerns (Wu *et al*., 1998; Dai *et al*., 2006), since the developing industry made excessive levels exist in the sewage sludge and their maximum concentrations in Agricultural Use Limitation (in China) are difficult to meet. The guidelines of sludge land application through the world refer to the total metal concentration of trace elements. However, depending on sludge properties, application of various sludges with similar total amount of Zn, Cu, and other elements may result in different impacts on the soil and plants (Tam and Wong, 1996; Walter *et al*., 2005). Composting offers an attractive method for not only treating sludge to stabilize organic materials and eliminate pathogens, but also influence the trace element concentration, availability and mobility (Bernal *et al*., 1998; Amir *et al*., 2005). Theis *et al*. (1998) found that the metal concentration of leachate from composted sludge was lower than dewatered sludge. Walter *et al*. (2005) showed that sludge compost had the higher total concentration of heavy metals but its bioavailability was lower compared with anaerobic sewage sludge. Therefore, speciation and fractionation of trace elements in sludges and sludge-amended soils are needed to predict elemental mobility in the soil and phytoavailability to plants (Flyhammar, 1998). Moreover, biological tests can be accurate to predict the phytotoxicity of sludge and provide information about

---

Project supported by the Provincial Technique Program of Zhejiang Province (No. 2004c23024) and the Key Science and Technology Program of Hangzhou City (No. 2005113A07). *Corresponding author.
E-mail: gmtian@zju.edu.cn.

toxicity of heavy metal, as well as for obtaining their optimum application rate.

The previous researches on biosolid-amended soils demonstrated that the changes of fraction and extractability of Zn and Cu depended on the soil type and length of incubation time (Obrador *et al*., 1997; Qiao *et al*., 2003; Hseu, 2006). However, they only focused on the bioavailability of heavy metals during incubation of amended soils. Hence, the further research is needed to focus on the soil-plant system to assess the movement and bioavailability of Zn and Cu from sewage sludge (SS) and composted sludge (SC) and to estimate application levels of sludge that might be safe for agricultural crops. The objectives of this study were: (1) to evaluate and compare the effect of SS and SC on fractionation evolution of Zn and Cu in the sludge-treated soil and correlate it to plant uptake; (2) to assess the impacts of different SS and SC addition rates on plant germination and growth; and (3) to explore the opportune application rate of sludge in the soil.

1 Materials and methods

1.1 Sewage sludge, sludge compost and soil

An anaerobic digested sewage sludge and a composted sludge were used in this study. The anaerobic digested SS was collected from Sibao Wastewater Treatment Plant in Hangzhou, China, which treated sludge from both domestic and industrial sources. The SC used in this experiment was obtained from a mixture of above digested sewage sludge and sawdust at an initial ratio of 5:1 (w/w, fresh weight). The compost was performed in a concrete container with forced aeration system at 0.1 $m^3/(min \cdot m^3)$ for 63 d and the temperature increased quickly at the beginning of the process. The top layer (0–20 cm) of loamy soil was collected from Shuangqiao Farm in Jiaxing Fountry, Zhejiang Province, China and geographically situated at 120°40′E and 30°50′N. The sand, silt, and clay contents were 27%, 38% and 35% (w/w), respectively. In Table 1, the main chemical properties of sludges and soil are presented. The compost used met the maturity criteria, namely, the ratio of C/N was lower than 15. According to the limitation of pollutants for agricultural use of China (Chinese EPB, 1984), the contents of Cu were within “clean” concentration (500 mg/kg), while Zn in the two sludges exceeded the standard (1000 mg/kg) greatly. Before use, SS, SC and the loamy soil were air dried at room temperature, ground to pass the 2 mm sieve and then mixed. For both of sludges, five treatments were made respectively, which consisted 0% (100% soil), 20%, 40%, 60% and 80% of SS or SC to soil by weight. The soil-sludge mixtures were prepared with air-dried soil and sludges, and were thoroughly mixed.

Table 1 Chemical properties of the two sludges and soil used in the experiment

Parameter	Sewage sludge (SS)	Composted sludge (SC)	Soil
pH	6.10±0.07[a]	6.77±0.03	6.85±0.05
EC (ds/m)	1.29±0.15	2.73±0.11	0.11±0.06
CEC[b] (cmol/kg)	50.73±3.69	45.36±5.87	12.12±0.17
OM (%)	37.62±1.36	16.98±0.95	1.86±0.77
Total-N (g/kg)	27.70±0.92	11.72±0.72	1.74±0.07
Total-P (g/kg)	15.41±0.03	6.63±0.12	1.35±0.02
Total-K (g/kg)	11.45±1.54	9.71±0.74	15.23±1.22
Total-Ca (mg/kg)	73.92±6.83	60.77±4.32	7.23±0.51
Total-Mg (mg/kg)	10.64±1.02	8.79±0.93	8.65±0.74
Zn (mg/kg)	3241.63±52.91	2970.96±34.38	83.81±2.75
Cu (mg/kg)	264.75±2.59	230.96±8.31	11.82±0.67
Pb (mg/kg)	65.14±4.87	60.49±5.93	10.39±0.80
Cr (mg/kg)	140.28±13.92	162.75±13.37	35.16±2.55
Cd (mg/kg)	3.56±0.12	3.08±0.38	1.15±0.09
Ni (mg/kg)	15.66±0.79	20.31±1.94	2.38±0.16

[a] The values are means±standard deviations (n=3); [b] cation exchange capacity (pH 7.0).

1.2 Experiment design

Pakchoi (*Brassica Chinensis* L.), which has a quick growth rate (harvest in about 2 months from sowing) and is popular to be used as the phytotoxic indicator to evaluate environmental risk of soil contamination by metals (Hirsch, 1998), was chosen for the experimental work.

1.2.1 Germination test

The germination assay was carried out to estimate their acute phytotoxicity before using soil-sludge mixtures for growth. For each treatment, 70 g mixture was put in Petri dishes (diameter 10 cm and depth 1.5 cm) and watered to saturated water content. Then 50 seeds of Pakchoi were placed in the surface soil and incubated 120 h at 25°C. Each treatment was triplicate. The percentage of seed germination was calculated as follows:

$$R_g\ (\%\ ) = \frac{N_g}{N_s} \times 100 \qquad (1)$$

where, R_g is the germination rate (%), N_g is the number of germinated seeds, N_s is the number of sowed seeds.

1.2.2 Pot experiments

The pot experiment of Pakchoi was carried out in the greenhouse (15-h light and 9-h dark, 15–25°C) at Zhejiang University and was conducted with plants in plastic containers with 14 cm diameter and 12 cm high. Each pot contained 600 g mixtures, and then was saturated at water content of 70% with water for 5 d before sowing. No additional fertilizer was used during experiment. The Pakchoi started from seeds. Ten seeds were sown in every container, in 6 replicates. One week after emergence (about on the day 10), number of Pakchoi was reduced to 2 per pot. At the day 20 after sowing, plants of each treatment in 3 pots of 6 replicates were removed. The other 3 pots of Pakchoi in each treatment were harvested on day 60 after sowing, and all plants were removed with roots. The fresh weight of plant samples was measured immediately after harvest, and then dried at 60°C for 72 h for the dry weight of biomass and concentrations of Zn and Cu analysis. After pot experiment, sequential extraction of Zn and Cu of the soil-sludge mixtures were analyzed.

1.3 Analytical procedure

The pH and electrical conductivity (EC) were determined in sludge or soil extracts (mixture/distilled water ratio is 1:10 (w/v)) using a pH and conductivity meter, respectively. Concentration of organic matter (OM) was determined by the Walkley and Black wet dichromate oxidation method (Nelson and Sommers, 1982). Cation exchange capacity (CEC) was determined with the ammonium acetate method (pH 7.0) (Rhoades, 1982). Total N was determined by the Kjeldahl digestion-distillation method (Bremner, 1996). Total P was determined by H_2SO_4-$HClO_4$ digestion and analyzed by the molybdenum blue color method (Kuo, 1996). Total K, Ca and Mg and total concentration of heavy metals were analyzed by digesting it with HF-HNO_3-$HClO_4$ procedures (Carter, 1993) and measured by AAS (Thermo Solar MKII-6).

The sequential extraction in this study followed the method by Tessier *et al.* (1979), which defined metal fractions as exchangeable and soluble (EXCH), carbonate (CAR), Fe-Mn oxides (FeMnOX), organic matter and sulfides (OM), and residual (RES).

For heavy metal analysis in plant, samples were first washed with distilled water and oven-dried (72 h at 60°C), then ground and sieved through a 2-mm screen. The concentrations of Zn and Cu were measured by AAS after HNO_3-$HClO_4$ digestion (Environmental Protection Agency of China, 1999).

Data were analyzed using one-way analysis of variance (ANOVA). Significant differences among the treatment means were calculated by the least significant difference (LSD) test, at $P < 0.05$. Germination rate was compared between treatments against the control using χ^2 test. All the statistical analyses were performed by SPSS Version 10.0 for window.

2 Results and discussion

2.1 Variation of Zn fractionation during pot experiment

The fractionation changes of zinc are shown in Fig.1. Before the pot experiment, the order of distribution of Zn in fractions of loamy soil was OM > FeMnOX > RES > EXCH > CAR. The sludges increased concentrations of Zn in all forms. In the soil-SS mixtures, increasing additions resulted in the amount of EXCH-Zn increased significantly especially in the 60% and 80% treatments, which were more than 20 times higher than natural soil (Fig.1). The 60% and 80% sludge additions led concentration of CAR-Zn 35 and 45 times higher than soil respectively, which is the biggest augment of Zn in single fraction. The FeMnOX-Zn only increased 1–3 times comparing to natural soil. The amount of OM-Zn accounted for nearly 30%–40% of the total concentration and increased with SS additions. Overall, the order of concentrations of Zn in SS treatments was OM > EXCH ⩾ CAR > RES > FeMnOX. Application of composted sludge to soil caused almost the same trend. Zn in OM form also accounted for the biggest part in SC-soil mixtures, which was probably due to the high content of organic matters in sludge and compost (Walter and Cuevas, 1999). However, EXCH-Zn in different SC additions was 30%–40% lower while the RES fraction was 30%–50% higher than those in the SS treatments. The dissimilarities between two treatment series probably resulted from different properties of sewage sludge and composted sludge. After composting, the mobile forms (i.e. EXCH fraction) were lower and inactive forms (i.e. Residual form) were higher than them in the sludge (Simeoni *et al*., 1984; Amir *et al*., 2005). Korolewicz *et al*. (2001) had reported that compost process decreased the EXCH-Zn considerably and increased the concentration of CAR-Zn, which reflected our results indirectly. In addition, perhaps the insoluble salts formed from release of P and mineralization of organic matters during compost process could limit metal bioavailability (Ross, 1994). The order of Zinc of different distribution in SC treatments was as following: OM > CAR > EXCH ⩾ RES > FeMnOX.

During the pot experiment, the Zn fractions in the natural soil were basically invariable (Fig.1). In amended treatments, Zn in EXCH, CAR, and OM fractions decreased, significantly in the first 20 d. More than 50% of EXCH-Zn decreased in soil-SS mixtures, while 10%–30% reduction occurred in SC treatments (Figs.1a1 and 1a2). However, between 20 to 60 d, decreasing rate of EXCH-Zn became much lower and its concentration occupied the smallest part of total Zn in soil-sludge mixtures. Less slightly reduction was observed in CAR and OM fractions (Figs.1b1, 1b2, 1d1 and 1d2). These observations possibly caused by the lower initial pH value after addition of sludge to the soil, which resulted in the faster decomposition rate of organic matter and would help to reduce the metal ions from exchangeable site or labile organic compound in the beginning period of pot experiment. Another potential reason in this soil-plant system may be that these fractions were more readily to be adsorbed by the plant, which indicated that plant uptake might play a significant role in redistribution of heavy metals. Noticeably, the only augment was observed in the FeMnOX-Zn fraction (Figs.1c1 and 1c2), which represented the largest part of total Zn at the end of pot experiment. Due to the increasing pH value and decreasing organic matter content in the soils, Zn would have relatively higher affinity for sorption on the surfaces of Fe and Mn oxides (Shuman, 1975). Additionally, according to Carmen and Murray (2001), the co-precipitation of Zn with ferrihydrite might also increase with the incubation time at the pH lower than 7.0. Zn in residue form changed insignificantly during the pot experiment (Figs.1e1 and 1e2) but its proportion was increased. Our results showed that the mobility of Zn decreased especially in the SC mixtures during the pot experiment, as Shuman (1999) had reported that the pH, adsorption and complexation probably redistributed Zn from EXCH to less bioavailable forms in organic matter-rich amended soils. At the end of pakchoi growth, the fractionation order in soil-sludge treatments became FeMnOX > OM ⩾ CAR > RES > EXCH. That might be suggested that the important outlet of fixation of Zn during plant growth was through precipitation of oxides when it released from sludges. These results were different from Hseu (2006) who found that RES-Zn was the major

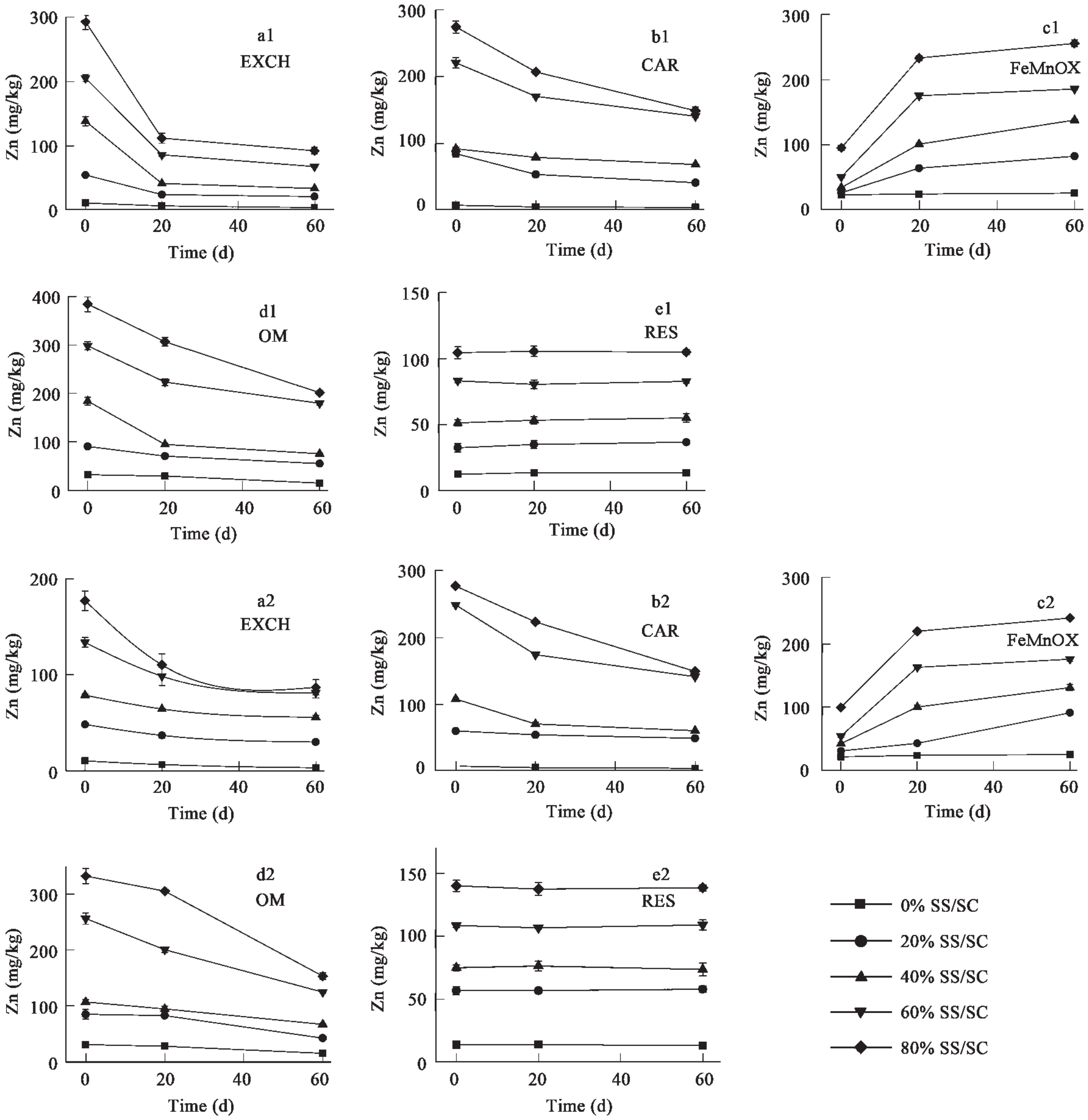

Fig. 1 Variation of Zn speciation in soils amended with sewage sludge (SS) (a1, b1, c1, d1, e1) and sludge compost (SC) (a2, b2, c2, d2, e2) during pot experiment. Values are means of 3 replicates, and bars represent standard errors.

fraction after one year incubation for sludge amended soil without plant growth. Although it has been explored that distribution of Zn depended on the sludge and soil properties in the sludge-treated soil, plant growth could influence the transformation and cycling of elements in the soil and the distribution of heavy metals might represent differently between the soil in the presence and absence of plant uptake.

2.2 Variation of Cu fractionation during pot experiment

From Fig.2, it was observed that before mixture, copper distribution in loamy soil was in the following order: OM $\gg$ RES > CAR > FeMnOX > EXCH. Sewage sludge increased Cu in EXCH fraction significantly, especially in 80% SS additions it reached 13 times higher than the natural soil (Fig.2a1). Cu in CAR and FeMnOX fractions increased relatively slightly (Figs.2b1 and 2c1). Addition of SS resulted in the higher concentration of OM-Cu, which occupied 60%–75% of total concentration. In the 80% SS treatment, OM-Cu reached 10 times more than the original soil (Fig.2d1). Overall, copper distribution in the SS-soil mixtures was OM $\gg$ RES > EXCH > CAR > FeMnOX. The similar variety was also observed in the SC treatments. Both sludges increased OM-Cu largely, which could be attributed to the high content of organic carbon in sewage sludge and composted sludge and proved that Cu was predominantly associated with the organic compounds (Walter and Cuevas, 1999; Wong *et al*., 2001). These results were in agreement with Sim and Kline (1991) who reported that the application of sludge increased Cu in all fractions and biggest increase was detected in organic fraction. Some other reports also suggested these results (Sloan *et al*., 1997; Luo and Christie, 1998). However, SC increased CAR-Cu triplicate and decreased RES-Cu twice comparing with respective SS-soil mixtures

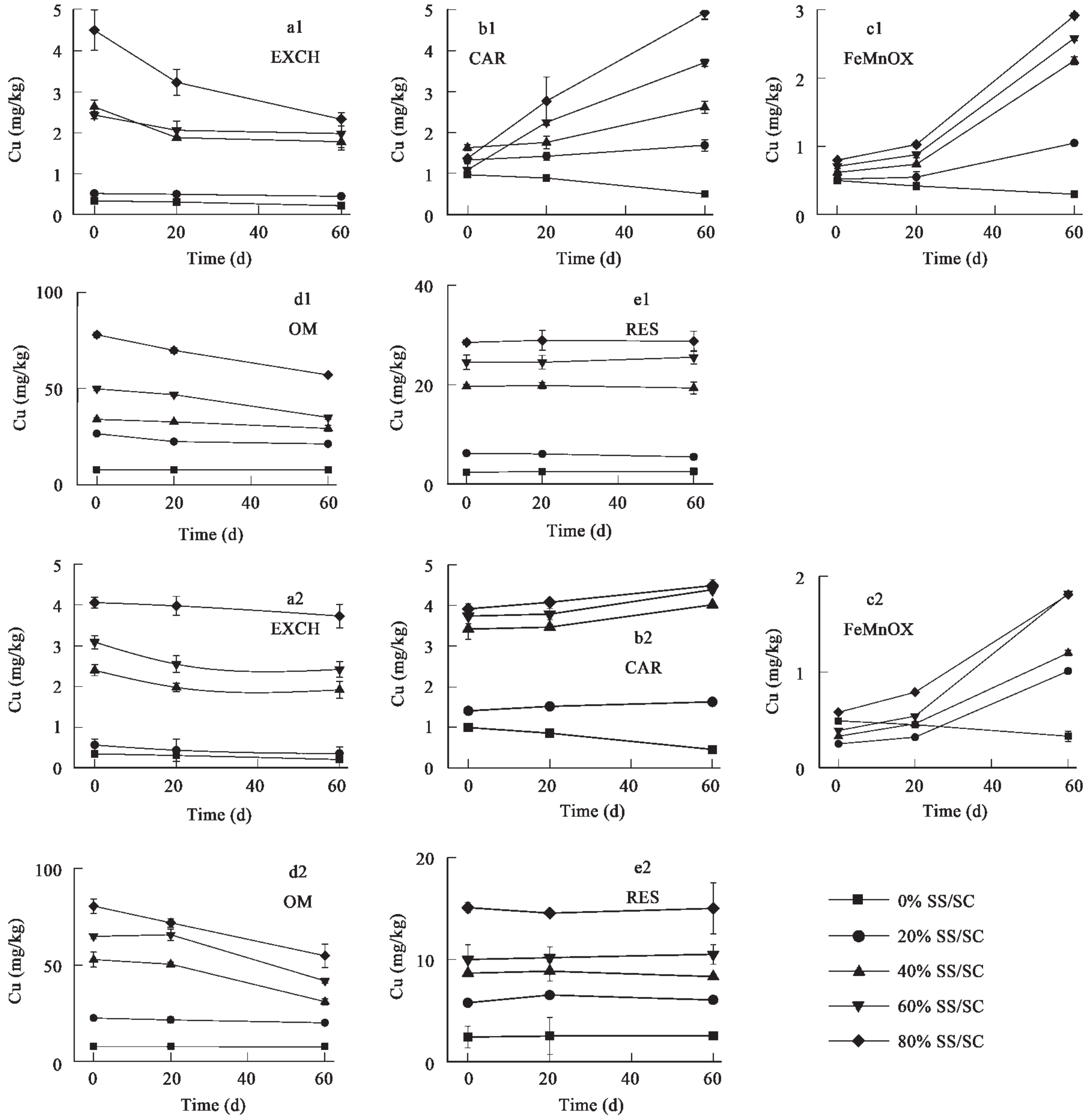

Fig. 2 Variation of Cu speciation in soils amended with sewage sludge (SS) (a1, b1, c1, d1, e1) and sludge compost (SC) (a1, b1, c1, d1, e1) during pot experiment. Values are means of 3 replicates, and bars represent standard errors.

(Fig.2b2). That might result from composting process which could increase mobility fraction of Cu and decrease the concentration of residue form (Richard *et al*., 1997; Amir *et al*., 2005). Therefore, the distribution of Cu in different fractions in SC treatments was OM ≫ RES > CAR > EXCH > FeMnOX.

The distribution variety of Cu fractions during pot experiment is different from Zn. Cu in EXCH and OM fractions decreased in all treatments. Concentrations of EXCH-Cu had a faster decreasing rate in SS-soils, especially in the first 20 d and this decreasing process led EXCH-Cu turn into the smallest part after pot experiment (Fig.2a1). This result was similar to the evolution of EXCH-Zn. The hypothesis was the existence of a “plateau” in EXCH fraction uptake during the later period of plant growth, but plant uptake of specific fractions was still rarely studied. The decrease in the OM-Cu also had the similar variety trend to OM-Zn (Figs.2d1 and 2d2). However, the decreasing rate of Cu in EXCH and OM fractions was much lower than Zn, which was different from the results of Lu *et al.* (2005) who only studied the incubation of soils. The results were ascribed to two reasons: one is the concentration of Cu introduced by sludges application was much lower than Zn; another is the available Cu to Pakchoi was small due to its higher affinity with soil organic matter than Zn (Diaz-Barrientos *et al*., 2003).

On the other hand, slight but significant increase was observed in the amount of Cu abound with CAR fraction (Figs.2b1 and 2b2), which was different from the reduction of CAR-Zn. This different variation might result from the abundant carbonate ions brought from sludges have precedence of combination with Cu over Zn at the pH lower than 7.0. The increasing rate of FeMnOX-Cu was nearly the same as the decreasing rate of OM-Cu, which could be considered that transformation of Cu from OM bound fractions to the FeMnOX form occurred in the relatively oxidized conditions (Mclaren and Crawfold, 1973). In our study, the higher concentration of organic matter and clay might supply more Cu-binding sites. With the decomposition of organic matter during the pot experiment, the Fe-Mn oxides would retain Cu ions from the soil solution with Cu^{2+} released from OM bound fractions. In addition, the FeMnOX-Cu was also probably transformed from loosely bound fractions such as EXCH form, which had been reported by Lu *et al*. (2005) and Yu *et al*. (2004) during incubation of soil. The proportion of Cu in various fractions in the SS treatments became: OM > RES > CAR > FeMnOX > EXCH, while for the SC mixtures the distribution was unaltered.

2.3 Germination rate and growth of pakchoi

The germination test was usually used to determine the compost maturity and phytotoxicity of biowaste materials (Roe *et al*., 1997). In our study, solid materials were used directly instead of extracted solution to test the acute toxicity of mixtures, which could affect not only chemical characteristics but also the physical properties. As Fig.3 shown, no significant differences in pakchoi germination rate resulted from the increasing additions (for SS treatment series: χ^2 = 3.71, d*f* = 4, *P* = 0.447; for SC treatment series: χ^2 = 3.65, d*f* = 4, *P* = 0.455), and germination rate of pakchoi between SC and SS treatments was no significant differences (*F* = 0.984, *P* = 0.794). For sewage sludge, the lowest germination rate was in the 80% treatment and the peak value appeared in 40% SS-soil. For composted sludge, the lowest germination appeared in the 80% treatment but the highest was 60% SC-soil. Although the concentrations of heavy metals in mixtures were much higher than those in natural soil, the application SS and SC have the weak acute toxicity on pakchoi germination at the low addition rates. This may be result from the beneficial effects, such as increasing nutrients and improving of properties of soil with the sludge addition, were the major effects when application rate was lower.

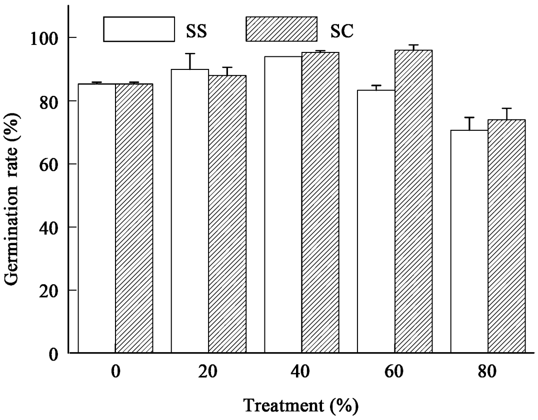

Fig. 3 Germination rate of pakchoi per treatment. SS, soil amended with sewage sludge; SC, soil amended with composted sludge.

The results of plant growth were quite different from that in germination test. As Table 2 shown, sludge amendments in the soil led to reduction in dry and fresh weights of pakchoi at the high application rates. In the first 20 d, the growth of pakchoi was best in the 40% mixtures for both of SS and SC treatments and composted sludge enhanced 50%–120% fresh biomass comparing to sewage sludge (Table 2). However, at the harvest (60 d), the peak value transferred to 20% mixtures and difference between SS and SC treatments augmented to 70%–150% by fresh weight (Table 2). Singh and Sinha (2004) also observed that growth parameters showed reduction at the high concentration of amendment. In contrast, some researchers (Pinamonti *et al*., 1997; Atiyeh *et al*., 2001; Perez-Murcia *et al*., 2006) found the biomass of plants increased with

Table 2 Fresh weight, dry weight and concentration of Zn and Cu of pakchoi per treatment during pot experiment

Treatment (%)	Fresh weight (g)	Dry weight (g)	Tissue (mg/kg)	
			Zn	Cu
Day 20				
0	3.60±0.23 aab	0.31±0.09 ac	29.31±3.17 ab	3.95±0.35 abc
20 SS	4.37±0.43 acg	0.37±0.01 ade	54.8±6.83 ab	7.86±0.56 ab
40 SS	4.98±0.22 aceg	0.42±0.06 adf	73.14±5.26 ab	8.98±1.25 bc
60 SS	4.05±0.63 a	0.34±0.02 adf	92.93±6.46 bd	10.23±1.03 bde
80 SS	2.16±0.32 a	0.18±0.01 ad	101.21±6.60 a	10.35±2.82 ac
0	4.80±0.19 ab	0.40±0.07 adf	25.43±6.01 ac	3.49±0.02 abd
20 SC	5.36±0.23 acd	0.46±0.19 bd	44.72±3.89 ac	7.59±1.44 ac
40 SC	7.55±0.46 a	0.63±0.05 ac	76.22±10.45 ac	8.06±0.52 ac
60 SC	6.32±0.13 a	0.54±0.22 bc	90.23±5.35 be	8.44±1.84 bdf
80 SC	4.91±0.25 acef	0.46±0.13 bd	99.32±12.98 c	8.56±0.96 bdg
Day 60				
0	23.30±1.24 a	1.28±0.23 abe	43.00±7.28 ab	6.84±1.77 ab
20 SS	25.86±0.29 ab	1.42±0.12 ace	88.30±9.34 ab	18.24±2.36 abc
40 SS	19.85±0.66 a	1.09±0.22 ad	122.72±8.00 a	20.24±1.58 abd
60 SS	15.33±0.78 a	0.84±0.23 ae	170.28±12.76 a	28.21±6.40 abc
80 SS	9.86±0.33 a	0.54±0.06 ab	207.49±17.74 a	40.96±2.79 a
0	30.6±1.24 ab	1.68±0.01 ab	43.55±3.59 ac	6.60±0.68 ac
20 SC	42.37±1.19 a	2.19±0.06 a	70.76±3.64 a	15.75±0.76 abe
40 SC	32.46±0.76 a	1.86±0.08 abf	80.26±6.12 ac	17.52±3.22 abf
60 SC	30.66±0.29 ac	1.79±0.29 a	147.28±12.09 a	25.28±1.46 abd
80 SC	25.39±1.33 ac	1.33±0.36 adf	187.00±5.38 a	38.61±2.36 a

SS: soil amended with sewage sludge; SC: soil amended with composted sludge. The values are means±standard deviations (*n*=3), means with the same letter in a column within one day for two additions are not different at *P* < 0.05 (LSD test).

the increasing biowaste presence in mixtures. These results from present study indicated that SS and SC caused both beneficial and harmful effects on pakchoi depending on the application rates. The low application could increase nutritious elements and improve soil physicochemical properties. However, when the application rate was higher, toxicity of heavy metals and other toxic matters released over time and had more potential harm to growth of plants. On the day 20 and 60, plant biomass was significantly different between two sludges (20 d, $F = 7.79$, $P = 0.024$; 60 d, $F = 11.391$, $P = 0.010$). Sewage sludge showed a more inhibitory effect on growth of pakchoi, which could be attributed to heavy metal availability or EC since they were higher in SS than in SC. Furthermore, it might be attributable to other phytotoxic compounds present in the sludge. Ammonia (Ells *et al*., 1991), ethylene oxide (Wong *et al*., 1983), short-chain aliphatic acids and various organic compounds produced during the anaerobic decomposition of organic matter have been identified as phytotoxic agents to plants (Mathur *et al*., 1993).

2.4 Zn and Cu concentrations in pakchoi

During the growth of pakchoi, the plant readily adsorbed more Zn and Cu from the soil under higher sludges application rates due to the higher availability, especially in the SS treatments (Table 2). In the first 20 d, Zn and Cu contents of pakchoi were slightly higher in soil-SS mixtures. While on the day 60, tissue Zn and Cu concentration was 1–2 times higher than those in the first 20 d and concentration gap of two sludge-soils series became more significant. Soils with high concentration of heavy metals had stronger negative effect on vegetation growth, which presented more significantly in long-term plantation. At the harvest, contents of Zn in pakchoi in 20% and 40% additions did not exceed the toxic levels, and tissue Cu in 20% SC, 40% SC and 20% SS treatments was also lower than toxic limit (Toxic limit of Zn was 150 mg/kg; Cu was 20 mg/kg (Chapman, 1966)). Some researches reported that biowaste application did not increase Zn and Cu concentrations of plant (Warman and Havard, 1998; Mendoza *et al*., 2006; Kidd *et al*., 2007). Furthermore, plant species showed different responds to heavy metals (McBride, 2003). Other authors, however, have reported increased Zn and Cu content in plant tissue following sludge application (Zheljazkov and Phil, 2004; Singh and Agrawal, 2007).

The accumulation of metals in plants was connected with not only total amounts of metals in the soils but also metal fractions. In present study, the linear regression analysis was used to investigate the concentration of Zn and Cu in pakchoi and the variation of different fractions of the elements in soil-sludge mixtures (Table 3).

The regression analysis of data suggested that Zn uptake by pakchoi was positively linearly related to decrease of EXCH-Zn, CAR-Zn and total Zn in sludge-soil mixtures and those positive correlations was more significant over time (Table 3). Hseu (2006) had the similar results to us, who found the positive correlations between sum

Table 3 Linear regression analysis and Pearson correlations for varieties of Zn and Cu speciation and pakchoi uptake at different application rates

Fraction change	Zn		Cu	
	Linear regression expression	Pearson correlation	Linear regression expression	Pearson correlation
	SS for 20 d			
ΔEXCH	$y = 0.7903x + 72.016$	0.962**	$y = 3.3412x + 6.6435$	0.675*
ΔCAR	$y = 1.893x + 78.245$	0.864**	$y = -2.9674x + 6.6716$	–0.776**
ΔFeMnOX	$y = -1.1533x + 12.45$	–0.988**	$y = -20.705x + 6.3277$	–0.962**
ΔOM	$y = 1.3058x + 71.467$	0.870**	$y = 0.5598x + 6.4210$	0.663*
ΔRES	$y = 13.208x + 150.97$	0.462	$y = -2.8537x + 7.9087$	–0.221
ΔTotal	$y = 0.7587x + 66.373$	0.947**	$y = 0.5757x + 6.5262$	0.615*
	SC for 20 d			
ΔEXCH	$y = 2.062x + 79.89$	0.838**	$y = 5.0381x + 6.0088$	0.531
ΔCAR	$y = 1.8493x + 69.943$	0.918**	$y = -16.985x + 6.4467$	–0.909**
ΔFeMnOX	$y = -1.1258x + 66.525$	–0.968**	$y = -20.728x + 5.0723$	–0.925**
ΔOM	$y = 2.1026x + 92.235$	0.746*	$y = 0.2256x + 6.7219$	0.400
ΔRES	$y = 21.49x + 122.47$	0.553	$y = 0.552x + 7.3064$	0.120
ΔTotal	$y = 1.7714x + 96.081$	0.657*	$y = 0.2207x + 6.7278$	0.401
	SS for 60 d			
ΔEXCH	$y = 2.0517x + 73.709$	0.981**	$y = 12.465x + 13.774$	0.849**
ΔCAR	$y = 3.2287x + 94.264$	0.953**	$y = -7.4867x + 12.342$	–0.974**
ΔFeMnOX	$y = -2.4615x + 46.62$	–0.947**	$y = -11.42x + 9.263$	–0.892**
ΔOM	$y = 2.3718x + 53.1$	0.969**	$y = 1.4474x + 9.611$	0.974**
ΔRES	$y = 36.775x + 340.6$	0.463	$y = -6.5283x + 22.428$	–0.334
ΔTotal	$y = 1.2514x + 83.547$	0.993**	$y = 1.8126x + 9.5606$	0.968**
	SC for 60 d			
ΔEXCH	$y = 3.5275x + 85.267$	0.982**	$y = 23.85x + 12.023$	0.424
ΔCAR	$y = 2.1008x + 95.024$	0.983**	$y = -17.771x + 15.421$	–0.740**
ΔFeMnOX	$y = -2.0592x + 45.189$	–0.930**	$y = -17.213x + 6.1894$	–0.824**
ΔOM	$y = 1.4157x + 115.83$	0.913**	$y = 0.7896x + 9.2222$	0.815**
ΔRES	$y = 36.094x + 206.63$	0.355	$y = 3.0901x + 21.067$	0.083
ΔTotal	$y = 0.8921x + 142.62$	0.899**	$y = 0.8156x + 9.5636$	0.797**

SS: soil amended with sewage sludge; SC: soil amended with composted sludge. *, **: correlation is significant at the 0.05, 0.01 level (2-tailed), respectively. n=15 (3 replications in 5 levels of compost application).

of EXCH-Zn and CAR-Zn and plant uptake. However, Zheljazkov and Phil (2004) did not detect the relationship between uptake of Basil and Chard and concentration of Zn in any fractions. Noticeable, ΔOM-Zn was also related to Zn concentration of pakchoi. The results were in agreement with Babjoke and McGrath (1991), who showed that Zn in EXCH, CAR and OM was closely related to the bioavailability of Zn to maize plants. This finding suggested that pakchoi-available Zn was not only in EXCH and CAR forms but also in OM forms in the sludge-soil mixtures. It is probable that the organic matter in the sludges existed in the labile fractions which are easy to transform, decompose and be adsorbed by plants.

Tissue content of Cu in pakchoi grown on SS-soils was related to decrease of total Cu and changes of all fractions except ΔRES. In SC amended soils, however, uptake of Cu could be only predicted by the variety of CAR-Cu and FeMnOX-Cu during the first 20 d. For the whole pot experiment period, significant correlations between changes of total-Cu and OM-Cu and pakchoi uptake of Cu were also observed. It probably ascribed that the outlet of EXCH-Cu and OM-Cu was more in transformation to other fractions than plant uptake when the plants were young seedlings. It suggests Cu in composted sludge may have the lower risk to the pakchoi growth. Luo and Christie (1998) had the different results with us, who reported that Cu uptake of spring barley was closely associated with EXCH-Cu and CAR-Cu in sludge-amended soils. While Zheljazkov and Phil (2004) found that Cu content of dill and peppermint was related to all fractions in soil amended with high-Cu compost. These results suggested that different sludge types and plant species showed the different responses to accumulation of heavy metals. Furthermore, these correlation rates between Zn and Cu accumulation in pakchoi and variation of different fractions increased with time, which might indicate that sludges represented stronger impacts on the plant over time.

3 Conclusions

Addition of sewage sludge or composted sludge to the loamy soil increased total concentrations of zinc and copper in the soils. Sequential extraction showed that the large proportion of extractable Zn was found in the EXCH, CAR and OM fractions and predominance of Cu was found in OM fraction. There was more available amount of Zn and Cu in SS treatments than SC treatments. During the pot experiment, EXCH-Zn, CAR-Zn and EXCH-Cu decreased. Zn in FeMnOX fraction turned into the largest portion and might control the solubility of Zn in sludge-soils. Both sludges had the weak acute toxicity on pakchoi germination except in the 80% addition treatments. During the growth of pakchoi, sewage sludge represented more potential harm. In the first 20 d, the biomass of pakchoi was highest in the 40% mixtures for both of SS and SC treatments and the peak value transferred to 20% mixtures on the day 60. Addition of SS and SC increased the levels of Zn and Cu in pakchoi with the increasing application rate, especially in SS treatments.

Our study indicated that the evolution of heavy metals speciation in the soil-plant system was different from incubation of soil system. The plants, which are the acceptor of heavy metals, might influence the redistribution of these elements. On the other hand, the heavy metals in sludge-amended soils affect growth of plants. If we try to relate the sludge application rates used in this study to agricultural use, 40% sewage sludge or composted sludge treatments would be rather extreme and the 20% composted sludge may be feasible for growth of pakchoi. However, metal transfer into the plants was not the only factor for evaluation of overall effects of sludge application on environment. Furthermore, as the bioavailability and maximum loading rate of heavy metals varied drastically between species, the exact application rate must be confirmed by field trial and the long-term effect should be monitored so that the negative environmental impacts of sludge application could be minimized.

References

Amir S, Hafidi M, Merlina G *et al*., 2005. Sequential extraction of heavy metals during composting of sewage sludge[J]. Chemosphere, 59: 801–810.

Atiyeh R M, Edwards C A, Subler S *et al*., 2001. Pig manure vermicompost as a component of a horticultural bedding plant medium: Effects on physicochemical properties and plant growth[J]. Biores Technol, 78: 11–20.

Babjoke V A, McGrath S P, 1991. Studies of the distribution and bioavailability of soil zinc fractions[J]. J Sci Food Agric, 57: 325–334.

Bernal M P, Navarro A F, Sánchez-monedero M A *et al*., 1998. Influence of sewage sludge compost stability and maturity on carbon and nitrogen mineralization in soil[J]. Soil Bio and Bioche, 30(3): 305–313.

Bremner J M, 1996. Methods of soil analysis. Part 3: Chemical methods[M]. Madison: SSSA. 1103–1108.

Carmen E M, Murray B M, 2001. Cd, Cu, Pb, and Zn copreipitates in Fe oxide formed at different pH: aging effects on metal solubility and extractability by citrate[J]. Environ Toxicol Chem, 20: 122–126.

Carter M R, 1993. Soil sampling and methods of analysis[M]. London: Lewis Publishers.

Chang A C, Granato T C, Page A L, 1992. A methodology for establishing phytotoxicity criteria for Cr, Cu, Ni, and Zn in agricultural land application of municipal sewage sludges[J]. J Environ Qual, 21: 521–536.

Chapman H P, 1966. Diagnostic criteria for plants and soils[M]. Berkley: University of California Press.

Chinese EPB, 1984. Limitation of pollutants in solid wastes for agricultural use of China (GB4284-84)[S]. Chinese State Standard.

Dai J Y, Chen L, Zhao J F *et al*., 2006. Characteristics of sewage sludge and distribution of heavy metal in plants with amendment of sewage sludge[J]. J Environ Sci, 6: 1094–1100.

Diaz-Barrientos E, Madrid L, Maqueda C *et al*., 2003. Copper and zinc retention by an organically amended soil[J]. Chemosphere, 50: 911–917.

Ells J E, McSay A E, Workman S M, 1991. Toxic effects of manure, alfalfa and ammonia on emergence and growth of cucumber seedlings[J]. HortScience, 26: 380–383.

Environmental Protection Agency of China, 1999. Monitoring and analytical methods of environment[M]. Beijing: Chinese Environmental Science Press.

Flyhammar P, 1998. Use of sequential extraction of heavy metals on anaerobically municipal solid waste[J]. Sci Total Environ, 212: 203–215.

García-Orenes F, Guerrero C, Mataix-Solera J *et al*., 2005. Factors controlling the aggregate stability and bulk density in two different degraded soils amended with biosolids[J]. Soil and Tillage

Research, 82: 65–76.

Hirsch M P, 1998. Availability of sludge-borne silver to agricultural crops[J]. Environ Toxicity and Chem, 17(4): 610–616.

Hseu Z Y, 2006. Extractability and bioavailability of zinc over time in three tropical soils incubated with biosolids[J]. Chemosphere, 63: 762–771.

Kidd P S, Domłnguez-Rodrłguez M J, Dłez J *et al.*, 2007. Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge[J]. Chemosphere, 66: 1458–1467.

Korolewicz T, Turek M, Ciba J *et al.*, 2001. Speciation and removal of zinc from composted municipal solid wastes[J]. Environ Sci Technol, 35: 810–814.

Kuo S, 1996. Methods of soil analysis. Part 3: Chemical methods[M]. Madison: SSSA. 908–910.

Logan T J, Harrison B J, 1995. Physical characteristic of alkaline stabilized sewage sludge (N-Viro soil) and the effects of soil physical properties[J]. J Environ Qual, 24: 153–164.

Lu A X, Zhang S Z, Shan XQ, 2005. Time effect on the fractionation of heavy metals in soils[J]. Geoderma, 3/4: 225–234.

Luo Y M, Christie P, 1998. Bioavailability of copper and zinc in soils treated with alkaline stabilized sewage sludges[J]. J Environ Qual, 27: 335–342.

Mathur S P, Owen G, Dinel H *et al.*, 1993. Determination of compost biomaturity. I. Literature review[J]. Biol Agric Horticult, 10: 65–85.

McBride M B, 2003. Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks?[J]. Adv Environ Res, 8: 5–19.

Mclaren R G, Crawfold D V, 1973. Studies on soil copper: The fractionation of copper in soils[J]. J Soil Sci, 24: 172–181.

Mendoza J, Garrido T, Castillo G *et al.*, 2006. Metal availability and uptake by sorghum plants grown in soils amended with sludge from different treatments[J]. Chemosphere, 65: 2304–2312.

Nelson D V, Sommers L E, 1982. Methods of soil analysis. Part 3: Total carbon, organic carbon and organic matter[M]. Madison: SSSA. 539–579.

Obrador A, Rico M I, Mingot J I *et al.*, 1997. Metal mobility and potential bioavailability in organic matter-rich soil-sludge mixtures: effect of soil type and contact time[J]. Sci Total Environ, 206: 117–126.

Perez-Murcia M D, Moral R, Moreno-Caselles J *et al.*, 2006. Use of composted sewage sludge in growth media for broccoli[J]. Biore Techno, 97(1): 123–130.

Pinamonti F, Stringari G, Gasperi F *et al.*, 1997. Heavy metal levels in apple orchards after the application of two composts[J]. Commun in Soil Sci and Plant Anal, 28(15/16): 1403–1419.

Qiao X L, Luo Y M, Christie P *et al.*, 2003. Chemical speciation and extractability of Zn, Cu and Cd in two contrasting biosolids-amended clay soils[J]. Chemosphere, 50: 823–829.

Ramos M C, 2006. Metals in vineyard soils of the Peneds area (NE Spain) after compost application[J]. J Environ Manag, 78: 209–215.

Rhoades J D, 1982. Methods of soil analysis, Part 2: Cation exchange capacity[M]. Madison: SSSA. 149–157.

Richards B K, Peverly J H, Steenhuis T S *et al.*, 1997. Effect of processing mode on trace elements in dewatered sludge products[J]. J Environ Qual, 26: 782–788.

Roe N, Stoffella P, Graetz D, 1997. Compost from various municipal solid waste feed stocks affect vegetable crops. I. Emergence and seedling growth[J]. J Am Soc Hortcult Sci, 122(3): 427–432.

Ross S M, 1994. Retention, transformation and mobility of toxic metals in soils[M]. Chichester, U.K.: John Wiley & Sons Ltd. 63–152.

Shuman L M, 1975. The effect of soil properties on zinc adsorption by soils[J]. Soil Sci Soc of Ame Pro, 39: 454–458.

Shuman L M, 1999. Organic waste amendments effect on zinc fractions of two soils[J]. J Environ Qual, 28(5): 1442–1447.

Singh S, Sinha S, 2004. Scanning electron microscopic studies and growth response of the plant of *Helianthus annus* L. grown on tannery sludge amended soil[J]. Environ Int, 30: 389–395.

Singh R P, Agrawal M, 2007. Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants[J]. Chemosphere, 67: 2229–2240.

Simeoni L A, Barbarick K A, Sabey B R, 1984. Effect of small-scale composting of sewage sludge on heavy metal availability to plants[J]. J Environ Qual, 13: 264–268.

Sims J T, Kline J S, 1991. Chemical fractionation and plant uptake of heavy metals in soils amended with co-composted sewage sludge[J]. J Environ Qual, 20(2): 387–395.

Sims J T, Pierzynski G M, 2000. Assessing the impact of agricultural, municipal and industrial by-products on soil quality[M]. Madison: SSSA. 237–261.

Sloan J J, Dowdy R H, Dolan M S *et al.*, 1997. Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils[J]. J Environ Qual, 26: 966–974.

Smith S R, 1996. Agricultural recycling of sewage sludge and the environment[M]. Wallingford: CAB International. 207–236.

Tam N F Y, Wong Y S, 1996. Retention and distribution of heavy metals in mangrove soils receiving wastewater[J]. Environ Pollu, 94(3): 283–291.

Tessier A, Campbell P G X, Bisson M, 1979. Sequential extraction procedure for the speciation of partionlate trace metals[J]. Anal Chem, 51: 844–851.

Theis T L, Brown R L, Gibbs J *et al.*, 1998. Land application of biosolids: comparison among stabilization methods[C]. In: Water Resources and Urban Environment-98. Chicago, Illinois, June 1998, Reston, VA. Proceeding of the National Conference on Environmental (Wilson T. E., ed.).

Walter I, Cuevas G, 1999. Chemical fractionation of heavy metals in soil amended with repeated sewage sludge application[J]. Sci Total Environ, 226: 113–119.

Walter I, Martinez F, Cala V, 2005. Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses[J]. Environ Pollu, 3: 507–514.

Warman P R, Havard K A, 1998. Yield, vitamin and mineral contents of organically and conventionally grown potatoes and sweet corn[J]. Agri Ecosy Environ, 68(3): 207–216.

Wollan E, Davis R D, Jenner S, 1978. Effects of sewage sludge on seed germination[J]. Environ Pollu, 17: 195–205.

Wong M H, Cheung Y H, Cheung C L, 1983. The effects of ammonia and ethylene oxide in animal manure and sewage sludge on the seed germination and root elongation of *Brassica parachinensis*[J]. Environ Pollu, 30: 109–123.

Wong J W C, Li K, Fang M *et al.*, 2001. Toxicity evaluation of sewage sludges in Hong Kong[J]. Environ Int, 27: 373–380.

Wu Q T, Nyirandege P, Mo C H *et al.*, 1998. Removal of heavy metals from sewage sludge by low costing chemical method and recycling in agriculture[J]. J Environ Sci, 10: 122–128.

Yu S, He Z L, Huang C Y *et al.*, 2004. Copper fractionation and extractability in two contaminated variable charge soils[J]. Geoderma, 123: 163–175.

Zheljazkov V D, Phil R W, 2004. Phytoavailability and fractionation of copper, manganese, and zinc in soil following application of two composts to four crops[J]. Environ Pollu, 131: 187–195.