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Phytotoxicity and speciation of copper, zinc and lead during the aerobic composting of sewage sludge

Miao-miao He, Guang-ming Tian*, Xin-qiang Liang

Department of Environmental Engineering, College of Environment and Resources Science, Zhejiang University, Hangzhou 310029, China

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ABSTRACT

The content and speciation of heavy metals in composted sewage sludge is the main cause of negative impacts on environment and health of animal and human. An aerobic composting procedure was conducted to investigate the influences of some key parameters on phytotoxicity and speciation of Cu, Zn and Pb during sewage sludge composting. The pH value reached the optimal range for development of microorganisms, and content of organic matter (OM) and dissolved organic carbon (DOC) decreased with the composting age. The total amounts of Cu, Zn and Pb were much lower in the final compost. The results from sequential extraction procedure of heavy metals showed that composting process changed the distribution of five fractions of Cu, Zn and Pb, and reduced the total contents and sum percentages of four mobile fractions (exchangeable (EXCH), carbonate (CAR), reducible iron and manganese (FeMnOX), and organic matter bound (OMB)), indicating that the metal mobility and phytotoxicity decreased after aerobic composting. The seed germination and root growth of Pakchoi (*Brassica Chinensis* L.) were enhanced with composting age and reached the highest value at the end of compost. The decrease of OM and DOC was significantly correlated to changes of metal distribution and germination index (GI) of Pakchoi. Only for Cu in the compost, the GI could be predictable from the sum mobile metal fractions (EXCH + CAR + FeMnOX + OMB) ($R = -0.814^{*}$). For Zn and Pb, R value was significantly increased by use of other components, such as pH, OM and DOC, which suggested that the transformation of heavy metal speciation and phytotoxicity of sewage sludge during an aerobic composting was rather strongly dependent on multiple components than a single element.

1. Introduction

Land application of composted sewage sludge represents one of the most cost-effective methods for treatment and final disposal of sewage sludge, because the valuable components (N, P, K, organic matter (OM) and other necessary nutrients for plant growth) in stable sludge can be recycled and the properties of soil can be improved [1–3]. Unfortunately, the presence of non-biodegradable and toxic heavy metals limits agricultural application of composted sludge, which tends to accumulate along the food chains and bring potential risks to animal and human [4].

Total heavy metal concentration is an important indicator of pollution. It has been reported that total content of metals in sewage sludge was about 0.5–4% (on a dry weight basis) [5]. However, heavy metals associated with different fractions had different impacts on the environment [6] and their phytotoxicity would connect to some forms rather than the total concentration of metals [7]. The sequential chemical extraction procedures could provide an understanding of chemical fractions of heavy metals and was useful for predicting metal mobility, bioavailability and leaching rates [8].

Many researchers have focused on the heavy metal speciation and phytotoxicity of composted sewage sludge or raw sludge for agricultural use [9–13]. They found that heavy metals in the soils amended by composted sludge presented the higher stability and lower bioavailability [14,15]. Walter et al. [12] suggested that composting procedure changed the mobility of heavy metals and reduced the phytotoxicity of sludge.

The composting process accelerates decomposition of organic matter, especially in the stages with high temperature. Consequently, the significant variation of properties in compost materials occurred within a relatively short period, such as moisture, pH, ammonia, dissolved organic carbon and humus [16–20]. These changes could influence the distribution of heavy metal speciation and phytotoxic behavior of the compost materials. Some authors have studied the evolution of metal contents and fractions in the composting system [19–21]. However, the phytotoxicity of heavy metals was ignored, and the contribution of heavy metals in mobile

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* Corresponding author. Tel.: +86 571 86971975; fax: +86 571 86971898.
E-mail addresses: hemiaomiao0301@163.com (M.-m. He), gmtian@zju.edu.cn (G.-m. Tian), liang410@zju.edu.cn (X.-q. Liang).

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speciation associated with other physicochemical parameters on the phytotoxic behavior is also needed to evaluate.

In the current study, an aerobic composting experiment was conducted on sewage sludge to evaluate the changes of heavy metals speciation and phytotoxic behavior in terms of Pakchoi seed germination. Meanwhile, the effects of some key parameters on metal phytotoxicity and speciation during composting were also investigated.

2. Materials and methods

2.1. Composting procedure

Dewatered anaerobically digested sewage sludge, collected from Sibao Wastewater Treatment Plant in Hangzhou, China, was mixed with sawdust as a bulking agent at 4:1 (w/w fresh weight) to obtain a water content of 60–70% and C/N ratio of 25. The composting reactor was cuboid and with inner dimension of 1.0 m × 0.8 m × 0.8 m (length × width × height). The mixture was composted for 42 days. Composting process was controlled by a forced-aeration static pile system and the air was supplied to the composting mass at 50 L min^{-1} and 12 times per day (15 min with intermission of 105 min per time, totally 3 h per day). At the depth of 0.3 m, temperature was monitored daily. Three phases were observed during composting: the mesophilic phase (32–50 °C, 0–4 days), the thermophilic phase (50–65 °C, 5–21 days, the highest value appeared on day 7) and the cooling phase (fall to room temperature, 22–42 days). About 200 g sample was collected from 5 positions in the composting reactor on days 0, 4, 7, 14, 21, 28 and 42, and mixed for chemical and biological testing by triplicate.

2.2. Chemical analysis

The moisture content of fresh compost sample was determined by oven-drying at 70 ± 5 °C [22]. The aqueous extracts of compost were obtained by shaking at 200 rpm with distilled water at a solid:water ratio of 1:10 (w/v) for 16 h at 20 °C. After the suspension was centrifuged at 10,000 rpm for 20 min and filtered through 0.45 μm membrane filter papers, the pH, EC and dissolved organic carbon (DOC) were determined by pH meter, conductivity meter and a total organic carbon analyzer (Apollo 9000, USA), respectively. Concentration of organic matter was determined by the Walkley and Black wet dichromate oxidation method [23]. For total N, the method of Kjeldahl digestion–distillation was used [24]. Total P was analyzed by the molybdenum blue color method after H_2SO_4–$HClO_4$ digestion [25]. Total Cu, Zn and Pb concentrations were analyzed by HF–HNO_3–$HClO_4$ digesting procedures [26] and measured by AAS (Thermo Solar MKII-6). The selected physicochemical properties of sewage sludge were listed in Table 1.

Table 1
Selected physicochemical properties of sewage sludge

Properties	Sewage sludge (SS)
Moisture (%)	82.4 ± 3.5[a]
pH	6.1 ± 0.07
Electrical conductivity (EC, ds m^{-1})	1.3 ± 0.1
Organic matter (%)	37.6 ± 1.4
Dissolved organic carbon (DOC, g kg^{-1})	10.0 ± 1.7
Total nitrogen (TN, g kg^{-1})	27.7 ± 0.9
Total phosphorus (TP, g kg^{-1})	15.4 ± 0.03
Cu (mg kg^{-1})	346.8 ± 10.6
Zn (mg kg^{-1})	3241.6 ± 52.9
Pb (mg kg^{-1})	194.6 ± 9.5

[a] The values are means ± standard deviations ($n = 3$).

2.3. Sequential extraction

Speciation of Cu, Zn and Pb in this study was conducted by using the procedure of Tessier et al. [27], which was widely used in the studies on heavy metals in sludge [12,21,28,29]. Five fractions of heavy metal were defined: (1) exchangeable (EXCH): 1 g air-dried sample was extracted with 1.0 M $MgCl_2$ at pH 7 with agitation at 220 rpm for 1 h at 25 °C; (2) carbonate (CAR): residue from (1) was extracted with 1.0 M NaOAc at pH 5 with agitation at 220 rpm for 5 h at 25 °C; (3) reducible iron and manganese (FeMnOX): residue from (2) was extracted with 0.04M NH_2OHHCl in 25% HOAc (v/v) for 6 h at 96 °C in water bath with occasional agitation; (4) organic matter bound (OMB): residue from (3) was extracted with 0.02 M HNO_3 and 30% H_2O_2 of pH 2 for 5 h at 85 °C, and then 3.2 M NH_4OAc in 20% HNO_3 (v/v) was added and agitated for 0.5 h at 25 °C; (5) residual (RES): residue from (4) was digested by HF–HNO_3–$HClO_4$ procedures [26]. After centrifugation and filtration, the supernatant from each extraction was analyzed by AAS (Thermo Solar MKII-6).

According to Tessier, the fraction of EXCH is readily influenced by changes of ionic composition in the liquid; CAR is susceptible to pH variations; FeMnOX is unstable in reductive conditions; OMB decomposes and changes under the oxidizing conditions; and RES permanently fixed in crystal lattice and not enter the food chain. In the normal conditions, fractions of EXCH and CAR mainly represent the heavy metal mobility in the short-term. During the composting, however, all of the fractions except RES make contributions to the metal mobility and bioavailability due to the rapid evolutions of compost properties.

2.4. Pseudo values of heavy metals

Since volatilization of gases and leaching of liquids following the decomposition and mineralization procedures of organic matter during composting, the Cu, Zn and Pb were concentrated in the compost mass [30]. To correct this excessive part of heavy metal contents during composting, all concentration values were normalized by the moisture content. The corrected pseudo values of heavy metals were evaluated using following formula as described similarly by Amir et al. [19]:

$$C_p = \frac{C_a \times (1 - m_0)}{1 - m_t}\,(\mathrm{mg\,kg^{-1}}) \qquad (1)$$

where C_a is the actually measured value of heavy metals (mg kg^{-1} dry compost); m_0 is moisture content in the compost sample of 0 day; m_t is the moisture content at each sampling time (0, 4, 7, 14, 21, 28 and 42 days).

2.5. Germination test for the pakchoi seeds

The germination assay was tested using Pakchoi (*Brassica Chinensis* L.) seed, which has a quick growth rate (harvest in about 2 months from sowing) and was popular to be used as a phytotoxic indicator to evaluate environmental risk of soil contamination by metals [31].

Compost extracts (three replicates for each sample) were prepared by shaking fresh samples with distilled water at a solid:water = 1:10 (w/v) for 1 h. The suspensions were then centrifuged, filtered and kept at 4 °C before testing. For germination tests, 5 mL of each extract were dispensed into a sterilized Petri-dish, which was lined with a filter paper. 50 seeds of Pakchoi were placed in one dish and incubated at 25 °C in the dark for 3 days. Distilled water was used as the control. Treatments were evaluated by counting the number of germinated seeds and measuring the length of the root. The percentage of relative seed germination

Table 2
pH values, organic matter contents (OM), and dissolved organic carbon (DOC) concentrations during sewage sludge composting

Time (day)	pH	OM (%)	DOC ($g\,kg^{-1}$ DW)
0	7.1 ± 0.1[a]b[b]	65.2 ± 2.2a	14.0 ± 1.3a
4	7.0 ± 0.1b	62.2 ± 0.0ab	13.6 ± 0.5a
7	7.9 ± 0.2a	60.1 ± 2.7b	9.9 ± 0.7b
14	7.3 ± 0.1b	56.5 ± 1.4bc	10.1 ± 0.4b
21	7.0 ± 0.1b	55.6 ± 3.9c	8.6 ± 0.4c
28	6.9 ± 0.6b	52.7 ± 2.0c	6.0 ± 0.6d
42	6.8 ± 0.3b	47.0 ± 0.9d	3.5 ± 0.2e

[a] The values are means ± standard deviations ($n = 3$).
[b] Means with the same letter in a column are not different at $P < 0.05$ (LSD test).

(RSG), relative root growth (RRG) and germination index (GI) were calculated according to the following formula [32,33]:

$$\mathrm{RSG}(\%) = \frac{\text{number of seeds germinated in sample extract}}{\text{number of seeds germinated in control}} \times 100 \quad (2)$$

$$\mathrm{RRG}(\%) = \frac{\text{root length in sample extract}}{\text{root length in control}} \times 100 \quad (3)$$

$$\mathrm{GI} = \frac{\mathrm{RSG} \times \mathrm{RRG}}{100} \quad (4)$$

2.6. Statistical analysis

All results were presented as the average of three replicates. Correlation (Pearson correlation) and linear regression analysis were performed by SPSS Version 12.0 for windows.

3. Results

3.1. Changes of key parameters during composting

The pH of compost decreased slightly during the mesophilic phase, but increased to the maximum value (pH 7.9) on the day 7 (Table 2). Then, pH fell gradually to 6.8 in the final compost, which was in the range of optimum environment for microorganism. The decline of pH might be due to decomposition of organic matter, the release of ammonia or the nitrifying process [34].

The organic matter was biodegraded and transformed to the steadier and hydrated fractions in the process of composting [35]. The OM content decreased gradually from 65.2% to 47.0% during sludge composting (Table 2). Similarly, compost DOC fell from 14.0 to 3.5 $g\,kg^{-1}$, although a slight increase occurred during the thermophilic stage. This conformed to the results of Inbar et al. [36] and Zheng et al. [20] for composting of cattle manure and sewage sludge, respectively.

3.2. Heavy metal speciation during composting

The total amounts of Cu, Zn and Pb decreased remarkably during the initial 21 days of composting (Fig. 1b, d, f). This was probably due to the higher leaching rate of soluble metals during the mesophilic and thermophilic stages. After thermophilic phase, changes of total metals tended to be steady. According to the limitation of pollutants for agricultural use of China [37], the concentrations of Cu and Pb were within "clean" concentrations (in the soil of pH <6.5: Cu 250 $mg\,kg^{-1}$; Pb 300 $mg\,kg^{-1}$), which indicated the lower risk of mature compost for land application.

The exchangeable and carbonate Cu only accounted for the small parts of total Cu. Their concentrations increased, although a decrease tendency appeared during thermophilic phase (Fig. 1a). The maximum increasing percents of EXCH-Cu and CAR-Cu were 1.26% and 1.10%, respectively, which occurred in the cooling and thermophilic stages, respectively (Fig. 2a). Both of FeMnOX-Cu and OMB-Cu dropped sharply during initial 21 days, and then leveled off in the remainder composting period (Fig. 1a and b). The relative percentage of Fe–Mn oxide and organic matter bound Cu also declined, especially during thermophilic stage (Fig. 2a). These results indicated that composting process transformed FeMnOX and OMB fractions of Cu into the more extractible fractions of EXCH-Cu and CAR-Cu. The residual Cu was almost stable (95.0 ± 4.0 $mg\,kg^{-1}$) in the whole process (Fig. 1b), and its relative percent increased from 37.46% in the initial compost to 48.68% in the final compost. OMB-Cu and RES-Cu accounted for the most predominant speciation throughout sludge composting.

The contents of EXCH-Zn and CAR-Zn increased in the mesophilic phase and then tended to remain constant (Fig. 1c). The relative percentages of EXCH-Zn and CAR-Zn, respectively, increased 6.82% and 9.06% during mesophilic and thermophilic phases (Fig. 2b). Conversely, FeMnOX-Zn declined rapidly from 668.9 $mg\,kg^{-1}$ (28.59% of initial total Zn) to 302.3 $mg\,kg^{-1}$ (16.15% of final total Zn) during mesophilic and thermophilic stages, and kept steadily at about 300 $mg\,kg^{-1}$ in the cooling stage (Figs. 1c and 2b). This decreasing trend led dominant fraction of Zn shifted from FeMnOX-Zn in the initial compost to CAR-Zn in the final compost. Comparatively, the concentrations of OMB-Zn declined more tardily during composting, and its relative percentage fell from 25.58% to 18.21% (Figs. 1d and 2b). The concentration of RES-Zn fluctuated slightly and reached the lowest level (357.5 $mg\,kg^{-1}$) in the final compost (Fig. 1d). The relative percent of residual Zn increased 3.38% during mesophilic and thermophilic phases but decreased 1.07% in the cooling stage.

There was a significant decrease in exchangeable Pb during the initial 7 days (Fig. 1e). The content of EXCH-Pb increased during the later thermophilic stage, but dropped again in the cooling period. The variation percentage of EXCH-Pb was larger than other fractions, which declined from 12.43% in the initial compost to 6.91% in the final compost (Fig. 2c). CAR-Pb increased slightly and reached the maximum content (10.24 $mg\,kg^{-1}$) at the end of composting. The concentrations and relative percent of FeMnOX-Pb decreased during thermophilic stage and then leveled off. The change of OMB-Pb was not substantial during sludge composting, and only an increase of 0.93% in the mesophilic phase could be distinctly found in the relative percentage of OMB-Pb (Figs. 1f and 2c). Residual Pb, which accounted for the majority of total Pb, decreased slightly but its percentage climbed from 67.28% to 70.92% during the composting process.

3.3. Phytotoxicity assay

The germination test was usually used to evaluate the compost maturity and phytotoxicity of biowaste materials [38]. This index had been proved to be a more sensitive parameter to illuminate both low toxicity affecting root growth and high toxicity affecting germination [39].

The seed germination and root growth of Pakchoi was highly inhibited by the compost in the mesophilic stage, indicating that immature sewage sludge would cause phytotoxic effects to the plant (Fig. 3). During the thermophilic period, relative germination rate (RSG) and root elongation (RRG) climbed rapidly from 5.66% and 12.21% to 67.58% and 73.05%, respectively. The germination index increased gradually in the cooling phase and reached the maximum value in the final compost extracts (Fig. 3). These results reflected that the phytotoxicity of compost bulk reduced and the beneficial effects on Pakchoi were more and more remarkable following the composting process.

4. Discussion

4.1. Influences on transformation of metal speciation during sludge composting

The composting process changed the distribution of five fractions of Cu, Zn and Pb, and reduced the total concentrations and sum percentages of four mobile fractions (EXCH, CAR, FeMnOX, and OMB), suggesting that the metal mobility and phytotoxicity decreased after aerobic composting. For Cu and Zn, the proportions of FeMnOX and OMB fractions decreased, while the percents of EXCH, CAR and RES fractions increased, especially during the mesophilic and thermophilic periods. The evolution tendency of Pb speciation was different from Cu and Zn. The relative percentages of EXCH-Pb and FeMnOX-Pb decreased, while the proportions of CAR-Pb, OM-Pb and RES-Pb increased. Pb is known to be not readily detected in the mobile forms and is preferentially bound to the residual fraction [40]. In contrast, Cu and Zn prefer to bind with exchangeable and carbonate fractions following the formation of humic substances during composting process.

The pH values had no significant effects on transformation of metal speciation in our study (Table 3). Some authors reported that pH was a factor which could influence the evolution of metal distribution during composting [20,21]. Other authors, however, also did not find the relationships between pH and transformation of heavy metals in the process of compost [17,19]. The diverse results might depend on the different raw materials or different durations of compost. For example, Liu et al. [21] only conducted 288 h of sludge compost at a sampling interval of 24 h, and the compost bulk was always in the acid condition; while Hsu and Lo [17] and Amir et al. [19] did the composting for more than 100 days, and compost samples were neutral or alkaline. In our study, the pH values of compost were shown in the neutral or alkaline conditions (Table 2) and compost duration was much longer than Zheng et al. [20] and Liu et al. [21].

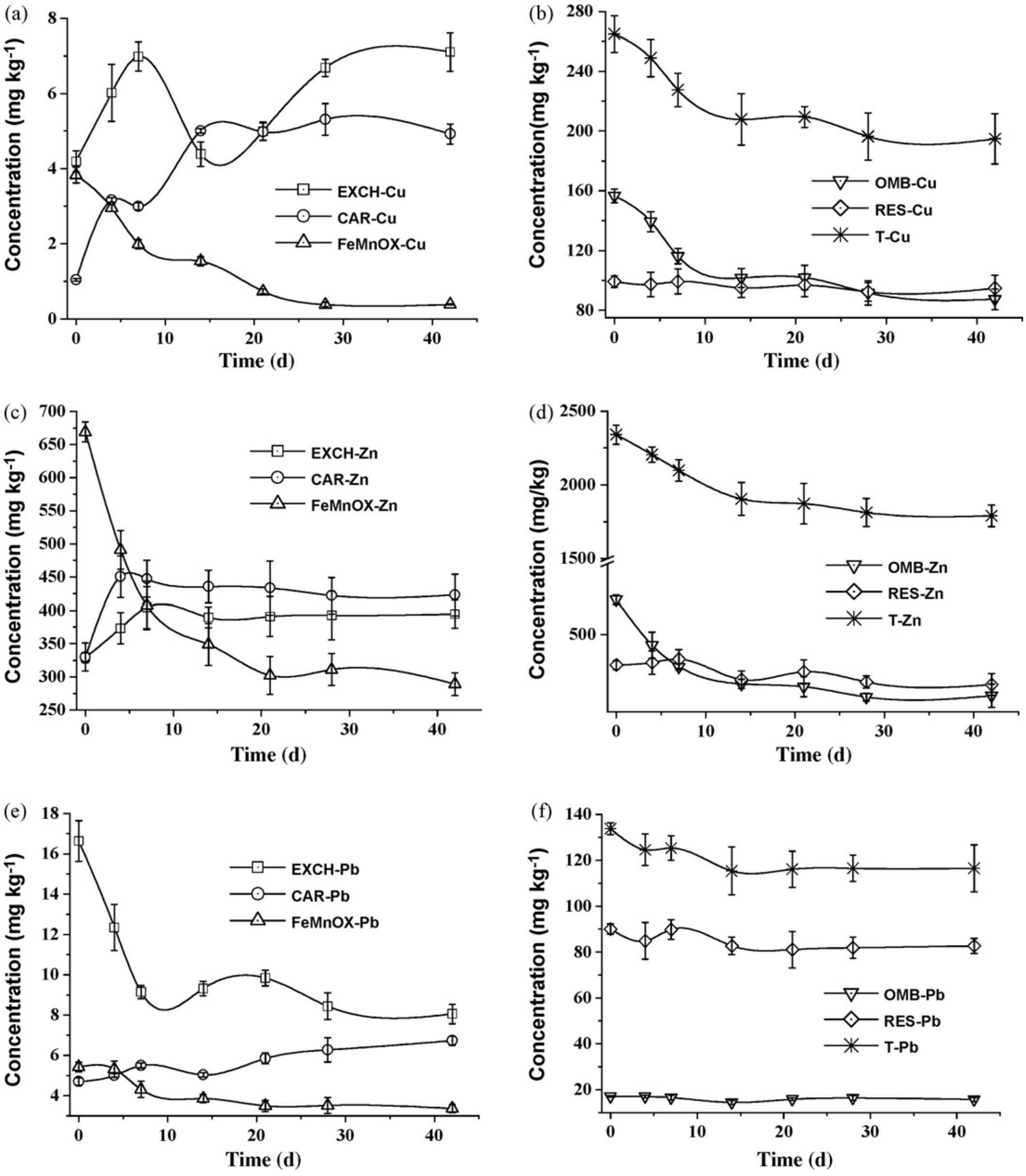

Fig. 1. Changes of Cu, Zn, and Pb speciation (EXCH-M, CAR-M, FeMnOX-M, OMB-M and RES-M) and total concentrations (T-M) during sewage sludge composting.

The evolutions of CAR-Cu, FeMnOX-Cu, OMB-Cu and sum of four mobile fractions presented significant correlations to the decrease of OM and DOC (Table 3). Residual Cu was positively related to OM, but had no remarkable relationship with DOC. No correlations were found between EXCH-Cu and OM or DOC. For Zn, the decrease of OM and DOC did not significantly correlate to increase of EXCH-Zn and CAR-Zn (Table 3). However, FeMnOX-Zn, OMB-Zn, RES-Zn and sum of four mobile fractions showed remarkable relationships with OM and DOC. Unlike Cu and Zn, EXCH-Pb and CAR-Pb have more affinity for OM and DOC (Table 3). However, the decrease of OMB-Pb, RES-Pb and sum of four mobile fractions did not relate to the decline of OM and DOC. Our results were inconsistent with those of Amir et al. [19] and Liu et al. [21], who found the significant correlations between easily extractable fractions (exchangeable fraction, or fractions with extractant of water-soluble or KNO_3) of Cu and Zn and organic matter contents. For exchangeable or water-soluble Pb, they did not find the correlations with organic matter. The exchangeable or water-soluble fractions of metals could easily leach out with H_2O during composting; on the other hand, these forms could be transformed from other fractions following the decomposition of organic matters. It is difficult to determine how much the leaching and transformation, respectively, account for the overall variations, so that there are diverse results on the most easily extractible speciation. However, in our study, changes of EXCH-Cu during later thermophilic and cooling stages of sludge composting were significantly correlated to decrease of OM ($R = -0.902^{**}$) and DOC ($R = -0.975^{**}$), and EXCH-Zn in the thermophilic phase was related to OM ($R = 0.775^{*}$) and DOC ($R = -0.817^{*}$). For other fractions, the results were similar to those of Amir et al. [19] and Liu et al. [21].

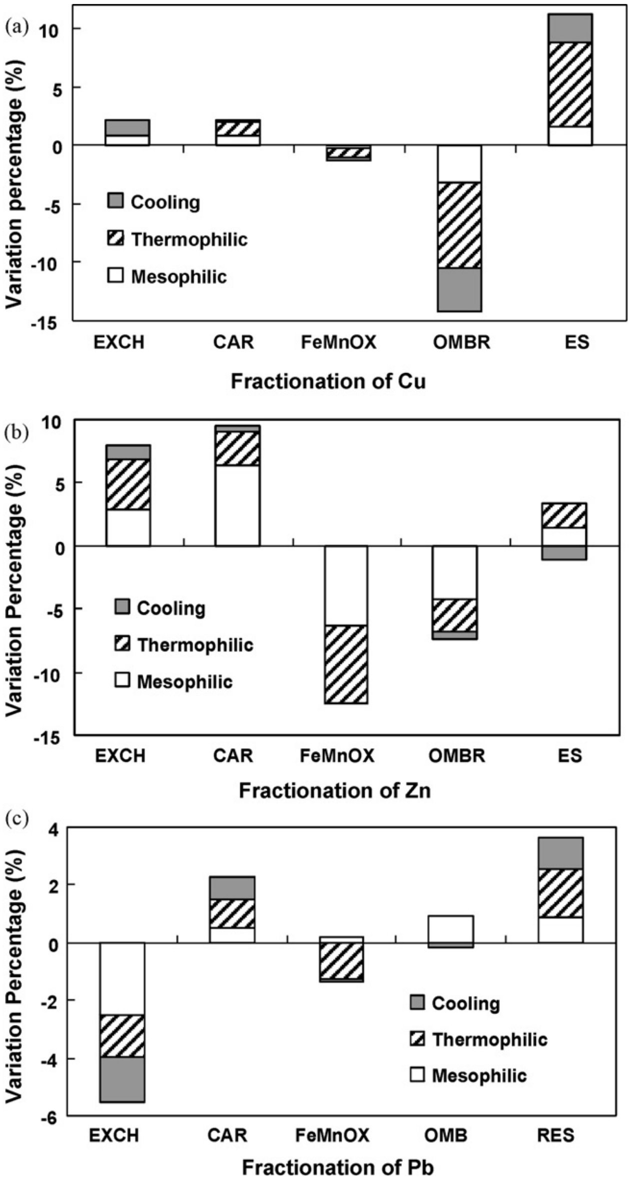

Fig. 2. Variations of relative percentage in Cu, Zn and Pb speciation (EXCH, CAR, FeMnOX, OMB and RES) during mesophilic, thermophilic and cooling phases of sewage sludge composting.

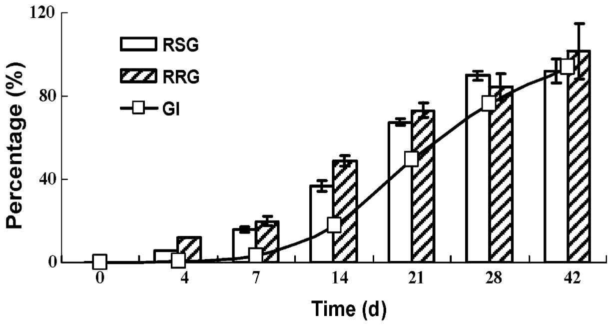

Fig. 3. Changes of the percentage of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) during sewage sludge composting.

4.2. *Influences on metal phytotoxicity during sludge composting*

The seed germination and root growth of Pakchoi significantly increased with composting age and reached the highest value at the end of compost (RSG = 92.3%, RRG = 101.7% and GI = 93.9%), indicating the lowest phytotoxicity existed in the final compost. As reported by Zucconi et al. [39], the compost was phytotoxic-free when GI values were higher than 80%. However, results about GI should be carefully used on the agricultural application. If the composts with a GI of about 95% are applied to agricultural land, it could

Table 3
Linear correlation coefficients (*R*) of heavy metals speciation with pH, organic matter contents (OM), and dissolved organic carbon concentrations (DOC) during sewage sludge composting

	pH	OM	DOC
Cu			
EXCH	0.040	−0.517	−0.606
CAR	−0.353	-0.836^{*}	-0.769^{*}
FeMnOX	0.322	0.925^{**}	0.929^{**}
OMB	0.221	0.926^{**}	0.919^{**}
RES	0.589	0.763^{*}	0.703
EXCH + CAR + FeMnOX + OMB	0.224	0.923^{**}	0.916^{**}
Zn			
EXCH	0.211	−0.650	−0.671
CAR	0.194	−0.373	−0.323
FeMnOX	0.189	0.865^{*}	0.841^{*}
OMB	0.621	0.854^{*}	0.778^{*}
RES	0.183	0.869^{*}	0.848^{*}
EXCH + CAR + FeMnOX + OMB	0.304	0.818^{*}	0.798^{*}
Pb			
EXCH	−0.013	0.816^{*}	0.822^{*}
CAR	−0.420	-0.923^{**}	-0.965^{**}
FeMnOX	0.233	0.898^{**}	0.907^{**}
OMB	0.013	0.517	0.407
RES	0.652	0.722	0.598
EXCH + CAR + FeMnOX + OMB	−0.035	0.717	0.686

(*and **): Statistically significant at the probability level 0.05 and 0.01, respectively (2-tailed).

Table 4

Linear correlation coefficients (*R*) of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) with pH, organic matter contents (OM), dissolved organic carbon concentrations (DOC), and sum of mobile fractions (EXCH + CAR + FeMnOX + OMB) during sewage sludge composting

	pH	OM	DOC	EXCH + CAR + FeMnOX + OMB		
				Cu	Zn	Pb
RSG	−0.569	-0.942^{**}	-0.941^{**}	-0.896^{**}	-0.815^{*}	−0.688
RRG	−0.551	-0.971^{**}	-0.946^{**}	-0.924^{**}	-0.741^{*}	−0.706
GI	−0.643	-0.940^{**}	-0.938^{**}	-0.814^{*}	−0.629	−0.513

(*and **): Statistically significant at the probability level 0.05 and 0.01 level, respectively (2-tailed).

still lead the negative effects on seed germination and plant growth. Therefore, it's better to prolong the compost maturation duration to further decline the negative effects.

To determine if and how the key parameters affect the compost phytotoxicity, the linear regression analysis of Pakchoi germination assay with pH, OM, DOC, and sum of mobile metal fractions were analyzed (Tables 4 and 5).

The pH value did not significantly related to the Pakchoi germination and root growth (Table 4), which varied from the data of Kim et al. [41]. This might be due to the usage of different compost curing progresses. However, similar to Kim et al. [41], the changes of Pakchoi germination and root growth were highly correlated to progressive degradation of OM and DOC (Table 4). The organic matter and dissolved organic carbon during composting were important factors in compost maturity [42,43] and controlling phytotoxicity of compost [20,44]. The ethylene oxide, short-chain aliphatic acids and various phenolic compounds, which are produced during the decomposition of organic matter and presented in dissolved organic matter, were identified as the phytotoxic agents and might suppress seed germination [45,46]. Once these materials disappeared, plant growth was markedly enhanced.

The Pakchoi seed germination (RSG) and root growth rate (RRG) had the significantly negative correlations with sum mobile fractions (EXCH + CAR + FeMnOX + OMB) of Cu and Zn, but no relationship was found with Pb (Table 4). The germination index, which was used to evaluate the phytotoxicity of compost, could only be estimated by overall mobile fractions of Cu ($R = -0.814^{*}$, Tables 4 and 5). For Zn and Pb, the *R*-values were significantly increased by utilizing other components, such as pH, OM and DOC (Table 5). Although the neutral or alkaline conditions (pH >7.0) in our research did not significantly influence mobility and bioavailability of heavy metals, the pH still could enhance the phytotoxicity of metals as an accessorial factor. Decomposition of organic matter and dissolved organic carbon could decrease organic hazardous agents of compost; meanwhile, it reduced mobility of heavy metals and consequently decreased the phytotoxic behavior. Additionally, DOC can form stable and soluble complexes with heavy metals, so that the risks of heavy metals in the compost on plant might be weakened with the decomposition or leaching of DOC [16].

Table 5

Data on the linear regression analysis for germination index (GI) in relation to the sum of mobile fractions (EXCH + CAR + FeMnOX + OMB) of Cu, Zn and Pb, and other key parameters (pH, organic matter content (OM), and dissolved organic carbon (DOC))

Linear regression analysis	*R*
GI = −1.268Cu(EXCH + CAR + FeMnOX + OMB) + 192.856	0.814^{*}
GI = −1.099Cu(EXCH + CAR + FeMnOX + OMB) − 49.56pH + 524.619	0.914^{*}
GI = 0.57Cu(EXCH + CAR + FeMnOX + OMB) − 0.812OM + 426.481	0.950^{**}
GI = 0.112Cu(EXCH + CAR + FeMnOX + OMB) − 36.109pH − 9.03DOC + 362.358	0.997^{***}
GI = −0.170Zn(EXCH + CAR + FeMnOX + OMB) + 308.529	0.629
GI = −0.143Zn(EXCH + CAR + FeMnOX + OMB) − 44.037pH + 578.83	0.925^{*}
GI = 0.11Zn(EXCH + CAR + FeMnOX + OMB) − 36.849pH − 8.807DOC + 362.311	0.997^{***}
GI = −5.674Pb(EXCH + CAR + FeMnOX + OMB) + 241.237	0.570
GI = 3.052Pb(EXCH + CAR + FeMnOX + OMB) − 11.969DOC + 35.626	0.959^{**}
GI = 2.785Pb(EXCH + CAR + FeMnOX + OMB) − 13.196pH − 0.698OM + 425.055	0.978^{*}
GI = 0.80Pb(EXCH + CAR + FeMnOX + OMB) − 34.132pH −9.04DOC + 333.237	0.998^{***}

(*, ** and ***): Statistically significant at the probability level 0.05, 0.01, and 0.001 levels, respectively (2-tailed).

These results confirmed that the evaluation of metal phytotoxicity during sewage sludge composting was dependent on the multiple components, besides the mobile metal fractions, rather than the single element. In arriving at a meaningful model for assessment of influences on heavy metal phytotoxicity and speciation during composting, the knowledge about heavy metals associated with other physicochemical properties is important.

In summary, decomposition of organic matter during composting was the most important accessorial factor to influence phytotoxicity and speciation of heavy metals. From an agricultural point of view, therefore, decomposition degree of organic matter during composting should be paid particular attentions. Additionally, the land application of stable sewage sludge with high OM or DOC content needs long-term field studies to determine their potential toxicity and minimize the negative environmental effects.

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References

[1] T. Hemández, J.L. Moreno, F. Costa, Influence of sewage sludge application on crop yields and heavy metal availability, Soil Sci. Plant Nutr. 37 (1991) 201–210.

[2] S.R. Smith, Agricultural Recycling of Sewage Sludge and the Environment, C.A.B. International, Wallingford, 1996, pp. 207–236.

[3] R. Zufiaurre, A. Olivar, P. Chamorro, C. Nerín, A. Callizo, Speciation of metals in sewage sludge for agricultural uses, Analyst 123 (1998) 255–259.

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

[5] L. Wong, J.G. Henry, Bacterial leaching of heavy metals from anaerobically digested sludge, in: D.L. Wise (Ed.), Biotreatment System, vol. 2, CRC Press, Boca Raton, FL, 1988, pp. 125–169.

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

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

[8] W. Grazebisz, W.Z. Kocialkowski, B. Chudzinski, Copper geochemistry and availability in cultivated soils contaminated by a copper smelter, J. Geochem. Explor. 58 (1997) 301–307.

[9] B.J. Alloway, A.P. Jackson, The behavior of heavy metals in sewage sludge-amended soils, Sci. Total Environ. 100 (1991) 151–176.

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

[11] V. Antoniadis, B.J. Alloway, The role of dissolved organic matter in the mobility of Cd, Ni and Zn in the sewage sludge-amended soils, Environ. Pollut. 117 (2002) 515–521.

[12] I. Walter, F. Martínez, V. Cala, Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses, Environ. Pollut. 139 (2006) 507–514.

[13] Q.Y. Cai, C.H. Mo, Q.T. Wu, Q.Y. Zeng, A. Katsoyiannis, Concentration and speciation of heavy metals in six different sewage sludge-composts, J. Hazard Mater. 147 (2007) 1063–1072.

[14] N. Korboulewsky, S. Dupouyet, G. Bonin, Environmental risks of applying sewage sludge compost to vineyards: carbon, heavy metals, nitrogen, and phosphorus accumulation, J. Environ. Qual. 31 (2002) 1522–1527.

[15] P.R. Warman, W.C. Termeer, Evaluation of sewage sludge, septic waste and sludge compost applications to corn and forage: yields and N, P and K content of crops and soils, Bioresour. Technol. 96 (2005) 955–961.

[16] J.H. Hsu, S.L. Lo, Chemical spectroscopic analysis of organic matter transformations during composting of pig mature, Environ. Pollut. 104 (1999) 189–196.
[17] J.H. Hsu, S.L. Lo, Effect of composting on characterization and leaching if copper, manganese, and zinc from swine manure, Environ. Pollut. 114 (2001) 119–127.
[18] F. Amlinger, B. Götz, P. Dreher, J. Geszti, C. Weissteiner, Nitrogen in biowaste and yard waste compost: dynamics of mobilization and availability—a review, Eur. J. Soil Biol. 39 (2003) 107–116.
[19] S. Amir, M. Hafidi, G. Merlina, J.C. Revel, Sequential extraction of heavy metals during composting of sewage sludge, Chemosphere 59 (2005) 801–810.
[20] G.D. Zheng, D. Gao, T.B. Chen, W. Luo, Stabilization of nickel and chromium in sewage sludge during aerobic composting, J. Hazard Mater. 142 (2006) 216–221.
[21] Y.S. Liu, L.L. Ma, Y.Q. Li, L.T. Zheng, Evolution of heavy metal speciation during the aerobic composting process of sewage sludge, Chemosphere 67 (2007) 1025–1032.
[22] U.S. Department of Agriculture and U.S. Composting Council, Test Methods for the Examination of Composting and Compost, Edaphos International, Houston, TX, 2001.
[23] D.V. Nelson, L.E. Sommers, Methods of Soil Analysis, Part 3: Total Carbon, Organic Carbon and Organic Matter, SSSA, Madison, 1982, pp. 539–579.
[24] J.M. Bremner, Methods of Soil Analysis, Part 3: Chemical Methods, SSSA, Madison, 1996, pp. 1103–1108.
[25] S. Kuo, Methods of Soil Analysis, Part 3: Chemical Methods, SSSA, Madison, 1996, pp. 908–910.
[26] M.R. Carter, Soil Sampling and Methods of Analysis, Lewis Publishers, London, 1993.
[27] A. Tessier, P.G.X. Campbell, M. Bisson, Sequential extraction procedure for the speciation of particulate trace metals, Anal. Chem. 51 (1979) 844–851.
[28] Y.M. Luo, P. Christie, Bioavailability of copper and zinc in soils treated with alkaline stabilized sewage sludge, J. Environ. Qual. 27 (1998) 335–342.
[29] J.W.C. Wong, K. Li, M. Fang, D.C. Su, Toxicity evaluation of sewage sludges in Hong Kong, Environ. Int. 27 (2001) 373–380.
[30] S. Canaruttto, G. Petruzzelli, L. Lubrano, G.V. Guidi, How composting affects heavy metal content, BioCycle 32 (1991) 48–50.
[31] M.P. Hirsch, Availability of sludge-borne silver to agricultural crops, Environ. Toxicol. Chem. 17 (1998) 610–616.
[32] N.J. Hoekstra, T. Bosker, E.A. Lantinga, Effects of cattle dung farms with different feeding strategies on germination and initial root growth of cress (*Lepidium sativum* L.), Agric. Ecosyst. Environ. 93 (2002) 189–196.
[33] A. Fuentes, M. Lloréns, J. Sáez, M.I. Aguilar, J.F. Ortuño, V.F. Meseguer, Phytotoxicity and heavy metals speciation of stabilised sewage sludges, J. Hazard Mater. 108 (2004) 161–169.
[34] S.H. Atchley, J.B. Clark, Variability of temperature, pH, and moisture in aerobic composting process, Appl. Environ. Microb. 38 (1979) 1040–1044.
[35] O. Reinikainen, M. Herranen, Different methods for measuring compost stability and maturity, in: B. Balis, K. Lasaridi, R.A.K. Szmidt, E. Stentiford, J. Lopez-Real (Eds.), Acta Horticulturae: Proceedings of the International Symposium on Composting of Organic Matter, vol. 549, ISHS, Belgium, 2001, pp. 99–102.
[36] Y. Inbar, Y. Hadar, Y. Chen, Recycling of cattle manure: the composting process and characterization of maturity, J. Environ. Qual. 22 (1993) 857–863.
[37] Chinese EPB, Limitation of pollutants in solid wastes for agricultural use of China (GB4284-84) [S], Chinese State Standard, 1984.
[38] N. Roe, P. Stoffella, D. Graetz, Compost from various municipal solid wastes feed stocks affect vegetable crops. I. Emergence and seedling growth, J. Am. Soc. Hortic. Sci. 122 (1997) 427–432.
[39] F. Zucconi, A. Pera, M. Forte, M. De Bertoldi, Evaluating toxicity of immature compost, BioCycle 22 (1981) 54–57.
[40] G. Petruzzelli, L. Ottaviani, E. Lubrano, Veschetti, Characterization of heavy metal mobile species in sewage sludge for agricultural utilization, Agrochimica 38 (1994) 277–284.
[41] K.Y. Kim, H.W. Kim, S.K. Han, E.J. Hwang, C.Y. Lee, H.S. Shin, Effect of granular porous media on the composting of swine manure, Waste Manage 28 (2008) 2336–2343.
[42] C. Garcia, T. Hernandez, F. Costa, Changes in carbon fractions during composting and maturation of organic wastes, Environ. Pollut. 15 (1991) 433–439.
[43] S. Zmora-Nahum, O. Markovitcha, J. Tarchitzky, Y. Chen, Dissolved organic carbon (DOC) as a parameter of compost maturity, Soil Biol. Biochem. 37 (2005) 2109–2116.
[44] K.A. Bolton, L.J. Evans, Elemental composition and speciation of some landfill leachates with particular reference to cadmium, Water Air Soil Pollut. 60 (1991) 43–53.
[45] M.H. Wong, Y.H. Cheung, C.L. Cheung, The effects of ammonia and ethylene oxide in animal manure and sewage sludge on the seed germination and root elongation of *Brassica parachinensis*, Environ. Pollut. 30 (1983) 109–123.
[46] F. Zucconi, A. Monaco, M. Forte, M. De Bertoldi, Phytotoxins during the stabilization of organic matter, in: J.K.R. Grasser (Ed.), Composting of Agricultural and Other Wastes, Elsevier Science, Amsterdam, 1985, pp. 73–86.