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Effects of C/N ratio and bulking agent on speciation of Zn and Cu and enzymatic activity during pig manure composting

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ABSTRACT

The aim of this study was to investigate the effects of initial C/N ratio (15, 20, 25) and bulking agent on speciation of Zn and Cu and enzymatic activities (dehydrogenase, urease) during pig manure composting. Rice straw and maize straw were separately chosen as bulking agent. Results showed that maize straw addition greatly enhanced organic matter (OM) degradation. The final NH_4^+-N/NO_3^--N ratio in composting mixture with initial C/N ratio of 25 did not exceed the limit of 0.16 to be considered the index of mature compost. The composting mixture with initial C/N ratio of 25 could reduce the mobility of Cu and Zn, and 90% of Cu belonged to residual fraction in the final compost, while total contents for Zn and Cu increased by 112.8–192.7% and 115.5–132.6%, respectively. In addition, the composting mixture with initial C/N ratio of 25 accelerated the decline of urease activity than those in composting mixtures of initial C/N ratio of 15 and 20, and C/N ratio could affect urease activity by influencing the content of metal ions. Therefore, the final compost with initial C/N ratio of 25 was of low risk and high quality for land application.

1. Introduction

The production of livestock manure is expected to reach approximately 0.23 billion tons nitrogen equivalents per year globally in 2030 due to rapidly developing intensive and industrial livestock production (Davidson, 2012). Large amounts of pig manure have led to serious environmental problems, such as groundwater and surface water contamination by nitrogen and phosphorus, soil accumulation of toxic metals (e.g. Zn, Cu), greenhouse gas emissions and production of pathogens; thus they are not applied to agricultural lands directly (Lu et al., 2014). Besides, corresponding Chinese national standard which were produced for livestock and poultry manure is further restricted. Therefore, it is necessary to find an environmentally friendly alternative to dispose pig manure.

Composting is a competitive alternative for manure management prior to its land application (Huang et al., 2004). Composting followed by land application represents one of the most economical ways for treatment and final disposal of manure, because it simultaneously combines material recycling and biomass disposal (Singh and Kalamdhad, 2013). It allows the conversion of biodegradable organic wastes (such as agricultural leaves, sewage sludge and food waste) into stabilized end products (Amir et al., 2010). The composting process is greatly affected by C/N ratio, moisture content, temperature, pH and aeration (Gao et al., 2010). C/N ratio is one of the key factors influencing both the process and the quality of compost (Zhu, 2007). C/N ratio in the range of 20–30 is considered as the optimum for composting (Sweeten and Auvermann, 2008). Pig manure contains high moisture content and low C/N ratio, and it cannot be successfully composted alone. It needs to be mixed with bulking agents to adjust its properties (Vargas-García et al., 2010). Hence, co-composting of pig manure and bulking agent is an alternative solution (Li et al., 2008). Zhu (2007) studied the effect of low

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initial C/N ratio on the composting of pig manure with rice straw. They reported that a low C/N ratio of 20 showed higher nitrogen loss, shorter thermophilic phase and longer maturity time, while it reduced the amount of bulking agents needed and consequently reduced composting cost, which was in accordance with the results of Huang et al. (2004). Gao et al. (2010) also investigated the effect of C/N ratio (12, 18, 28) on stability and maturity of compost in forced aeration composting. They found the stability and maturity in composting mixture with initial C/N ratio of 28 was superior to those in composting mixtures with initial C/N ratio of 12 and 18.

Pig manure usually contains high concentrations of Zn and Cu, due to feed additives in livestock production (Lu et al., 2014). Heavy metals are non-biodegradable and often become concentrated throughout the composting process, which poses a potential threat to animal and human health through the food chain (Singh and Kalamdhad, 2012). The presence of non-biodegradable and high level of toxic heavy metals in the compost also hinders agricultural land application. Nevertheless, according to previous findings, the potential bioavailability, toxicity and leaching rate of heavy metals largely depend on their specific chemical speciation rather than total content (He et al., 2008; Wang et al., 2013). Several studies have been conducted on the speciation of heavy metals during composting of sewage sludge or pig manure (Cai et al., 2007; Peng et al., 2014; Lu et al., 2014; Lv et al., 2016). It was reported that 70% of Cu belonged to OM fraction, while Zn was mainly concentrated in Fe—Mn oxide and exchangeable fractions in the final sewage sludge composts (Cai et al., 2007). Although many studies have been conducted on C/N ratio and bulking agent impact on the maturity of compost and gas emissions during composting, few have been made on speciation of heavy metals.

Numerous studies have been carried out about the effects of C/N ratio and bulking agent on stability and quality of compost (Dias et al., 2010; Sun et al., 2011; Vargas-García et al., 2010; Zhang and Sun, 2016). The optimal combination of 15% wood chips and 35% composted green waste reduced two-stage composting time to 22 days during green waste composting (Zhang and Sun, 2016). In addition, composting process is driven by microbes and their secreted enzymes (Zeng et al., 2010). Their secreted enzymes play a key role in biological and biochemical transformations of compost matrixes in the process of composting. In particular, activities of dehydrogenase and urease are involved in carbon mineralization and nitrogen cycle, respectively (Vargas-García et al., 2010). Enzymatic activities provide information on the conversion of complex organic compounds into more easily assimilable substances. Hence, enzymes are of interest to evaluate stabilization throughout waste biodegradation (Villar et al., 2015). Therefore, enzymatic activity reflects the dynamics of the composting process involved in organic matter degradation, and it has been used to assess the stability of compost (Liu et al., 2011). However, there is little information available about the effects of C/N ratio and bulking agent on enzymatic activity during pig manure composting.

Therefore, the aim of this study was to evaluate the effects of C/N ratio and bulking agent on enzymatic activity, speciation of Zn and Cu and compost quality. We hypothesized that (1) speciation of Zn and Cu between the control and the treatment with bulking agent or different C/N ratio would differ considerably during composting, (2) there is a close relationship between C/N ratio, urease activity and heavy metal. These results would supplement information on the feasibility of composting technology in management of manure rich in Zn and Cu.

2. Materials and methods

2.1. Composting materials

The pig manure (PM) was collected from a local intensive pig farm (Changsha, China). Rice straw (RS) and maize straw (MS) used as bulking agents to increase the porosity and adjust the C/N ratio, were obtained from a local farmland. The main characteristics of the raw materials are presented in Table 1.

2.2. Composting process

Pig manure and bulking agents were manually mixed to prepare the composting piles following, as shown in Table 2. The moisture content was adjusted to 70% and was maintained by adding tap water. Prior to composting, these materials were shredded to fragments with a length of 3—5 cm. The composting piles were aerated by using natural ventilation and turning periodically. A digital thermometer was put in the central of the composting pile to record temperature.

Each treatment was placed in trapezoidal pile (0.8 m high with 1.5 × 1 m base). The composting piles were manually turned on day 0, 3, 6, 19, 13, 16, 21, 26, 31, 38, 45 and 59. During composting, samples were taken by mixing five locations (30 cm from the top) to get a representative sample, and then they were subdivided into two parts: one part was stored at 4 °C; the second was dried and grounded to 0.25 mm and subsequently sealed in plastic containers.

2.3. Analytical methods

The pH was determined by using a pH meter (Shanghai, China) after mechanically shaking the samples with deionized water (1:10 w/v). Moisture content was measured by drying the sample at 105 °C to constant weight. OM was measured by $K_2Cr_2O_7$ and H_2SO_4 oxidation and its loss was calculated from the initial (X_1) and final (X_2) OM according to Nolan et al. (2011): OM loss (%) $= 100-100 [X_2(100-X_1)]/[X_1(100-X_2)]$. NH_4^+—N and NO_3^-—N were extracted with 2 M KCl solution and measured by Nesster's reagent colorimetry and spectrophotometry, respectively. Total phosphorus and total potassium were digested with H_2SO_4—$HClO_4$ and determined by Anti-Mo-Sb spectrophotometry and flame photometry, respectively. C/N ratio was determined using automatic CHNOS Elemental Analyzer (Vario EL III, Germany). The contents of heavy metals (Zn, Cu, Hg, As, Pb, Cd and Cr) were digested by using HNO_3—HF— $HClO_4$

Table 1
The characteristics of the raw materials.

Parameters	Pig manure	Rice straw	Maize straw
Moisture %	82.9 ± 1.2	8.8 ± 0.3	30.0 ± 0.8
pH	5.58 ± 0.15	8.35 ± 0.06	7.38 ± 0.10
Organic matter %	91.2 ± 3.2	97.1 ± 2.5	99.2 ± 1.6
Total carbon %	27.3 ± 0.7	42.3 ± 1.0	46.7 ± 0.3
C/N ratio	12.6	58.7	97.3
Total potassium %	0.74 ± 0.02	1.95 ± 0.10	1.19 ± 0.05
Total phosphorus %	3.69 ± 0.10	0.39 ± 0.02	0.24 ± 0.05
Zinc mg kg^{-1}	4644.8 ± 25.2	n.d.	n.d.
Copper mg kg^{-1}	1417.4 ± 37.2	n.d.	n.d.

n.d.: not detected.
±: Standard deviation of three replicates.

Table 2
Design of the experiment.

Treatment	Pig manure (kg)	Rice straw (kg)	Maize straw (kg)	C/N ratio
T1	943.0	29.2	/	15.0
T2	943.0	101.7	/	20.0
T3	943.0	195.6	/	25.0
T4	943.0	/	99.6	20.0
T5	943.0	/	/	12.6

(v/v/v 5:5:3) in graphite furnace digestion system and measured by inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer ICP 5300). Fractions of Zn and Cu were determined according to modified BCR three-step sequential exaction procedure (Pueyo et al., 2008). They were divided into four fractions: exchangeable and acid soluble (F1), reducible (F2), oxidizable (F3) and residual fraction (F4). All results were replicated three times.

The dehydrogenase activity was measured by the reduction method of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) as described by a modified method (Gutiérrez et al., 2010). Briefly, 2.0 g sample was mixed with 2.0 mL of (1%) TTC solution, 2.0 mL of (0.1 M) Tris-HCl solution (pH 8.4) and 2.0 mL deionized water. After incubation at 37 °C for 24 h, the reaction was stopped by 5 mL acetone. The filtrate was diluted to 25 mL with acetone and measured for the absorbance at 485 nm. The result was expressed as µg TPF g^{-1} 24 h^{-1}. A control without the addition of TTC was included for each sample.

The urease activity was determined by using spectrophotometry according to Bol et al. (2003). 2.0 g sample was mixed with 1 mL toluene for 15 min at room temperature, and then 20 mL sodium citrate (pH 6.7) and 10 mL of (10%) urea solution were added. The mixture was incubated at 37 °C for 24 h and subsequently filtered. 1 mL filtrate was supplied with 4 mL of (1.35 M) phenolate sodium and 3 mL of (0.9%) active chlorine. After 20 min, it was diluted with deionized water to 50 mL. The absorbance was measured at 578 nm. A control was performed without urea.

2.4. Statistical analysis

Averages and standard deviations of data were calculated by using SPSS 17.0. ANOVA was carried out for each treatment to determine significant differences at level $p < 0.05$.

3. Results and discussion

3.1. Changes of temperature, pH and organic matter

The temperature is strongly correlated with the rate of biological reactions, thus it is often used to reflect the microbial activity and determine the stability of composting (Zhang and Sun, 2016). As shown in Fig. 1a, T2, T3 and T4 reached the thermophilic phase (>50 °C) on day 2, and T1 started on day 9. This phase lasted for 26–31 days, which met the sanitation requirements specified in the Chinese national standards (GB7989-87). It was interesting to note that the temperature for T5 was below 40 °C throughout the process, which did not meet the requirement of pathogen destruction. This was mainly because of the high moisture and low porosity of pig manure. Then, T1, T2, T3, T4 and T5 reached peak temperatures at 70.0 °C, 69.0 °C, 65.0 °C, 68.3 °C and 35.0 °C, respectively. After peaking, the temperature gradually declined and reached the maturing phase at 46 d for T1; 37 d for T2; 31 d for T3; and 44 d for T4. At the beginning of composting with initial C/N ratio of 15, the rise rate of temperature was slower than the composting with C/N ratio of 20 and 25 ($F = 0.077$, $p < 0.001$; $F = 0.097$, $p < 0.001$), which was attributed to insufficient supply of carbon source (Huang et al., 2004). T4 showed a slightly longer thermophilic phase than T2 ($F = 0.172$, $p = 0.045$), indicating that maize straw provided relatively available organic substances for microorganisms.

The changes in pH during composting are shown in Fig. 1b. The pH in T1 rapidly decreased from 7.20 to 5.82 in the first 6 days, and it slightly decreased from 7.88 to 7.36 in the first 13 days for T3. The decrease was attributed to the degradation of organic matter and formation of organic acids by intense anaerobic microbial activity. The pH in T1 and T3 began to increase after the initial decline above, and then the peaks were obtained: 8.37 for T1 on day 13 and 8.21 for T3 on day 16. Differently, pH of T2 and T4 increased right form the initial and reached their peaks later, which were 8.45 on day 13 and 7.96 on day 3, respectively. Besides, pH of T5 slightly increased throughout the process. The increase in pH was caused by degradation of organic acids and release of ammonia through organic nitrogen mineralization. After peaking, pH gradually decreased and stabilized at neutral except T3. The reasons responsible for this decrease were ammonia volatilization, proton-release from microbial nitrification and organic acids production during composting (Wong et al., 2001). As for T3, a relatively high pH in the cooling phase was observed as a result of the reduction of microbial activity.

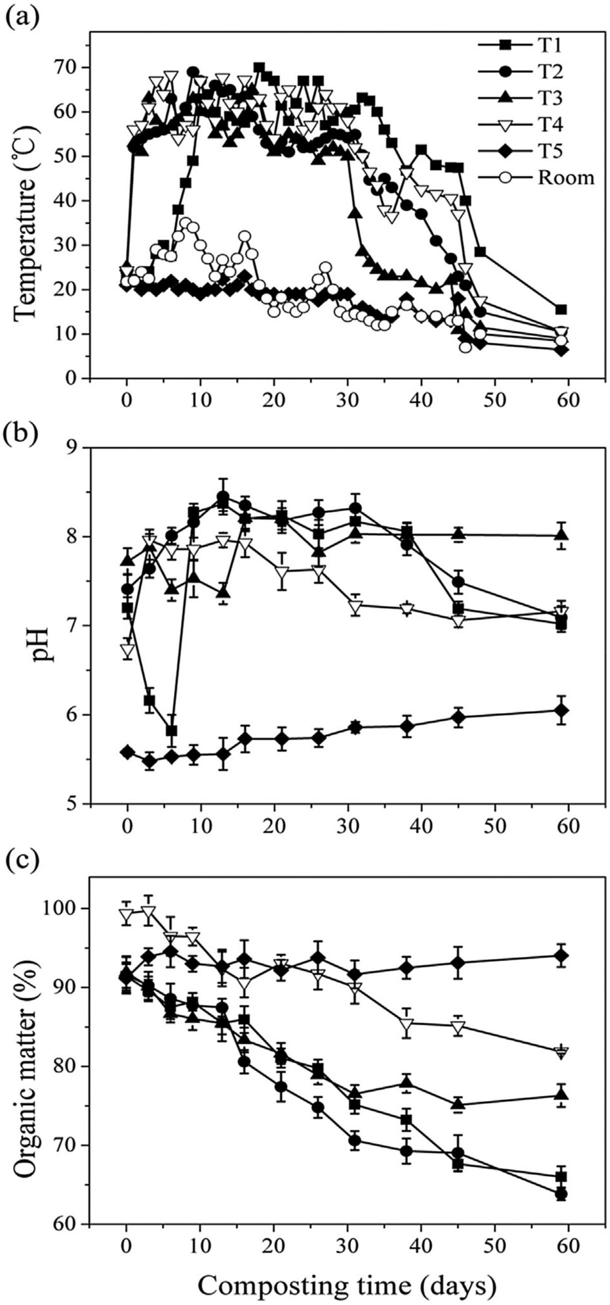

Fig. 1. Change in temperature (a), pH (b) and organic matter (c) during composting.

The OM of all treatments declined gradually except T5. It declined from 91.4% to 66.0% in T1 ($F = 24.686$, $p < 0.001$); from 91.6% to 63.8% in T2 ($F = 40.282$, $p < 0.001$); from 91.9% to 76.3% in T3 ($F = 25.600$, $p < 0.001$); and from 99.4% to 81.9% in T4 ($F = 13.232$, $p = 0.500$) (Fig. 1c). This decrease was caused by the loss of organic matter through microbial degradation. However, the OM in T5 remained unchanged, it might be due to low microbial activity and absence of thermophilic phase (<40 °C) (Fig. 1a). After 59 days of composting, losses of OM were 81.4%, 83.8%, 71.6% and 97.3% for T1, T2, T3 and T4, respectively. OM losses could be related to chemical composition of compost materials, especially the concentrations of fibers. 82.5% of OM was mineralized during composting of coffee husk with poultry manure in 210 days (Dias et al., 2010). This was attributed to the fact that coffee husk contained some of carbohydrates easily hydrolyzed to glucose. In addition, the high lignin materials would inhibit the microbial degradation, which has been confirmed by He et al. (2014). They found that OM was degraded in the following order: aliphatic substances > proteinaceous compounds > polysaccharide and lignin. Addition of MS as bulking agent enhanced the degradation of OM compared to RS ($F = 7.008$, $p = 0.001$), because RS contained large amounts of OM recalcitrant to be degraded, such as cellulose and lignin. As a consequence, RS was extremely resistant to chemical and enzymatic degradation (Huang et al., 2004).

3.2. Changes of NH_4^+–N and NO_3^-–N

Composting of organic wastes rich in nitrogen caused nitrogen loss into the atmosphere, notably through ammonia volatilization; thus it increased air pollution and decreased the agronomic value of end product (Nakhshiniev et al., 2014). As shown in Fig. 2a, the NH_4^+–N contents in all treatments generally increased and then decreased except T5, whose NH_4^+–N content kept increasing throughout the composting process. The NH_4^+–N contents of T1, T2, T3, T4 and T5 increased and reached their peak values on day 16, 13, 16, 3 and 38, respectively. This increase was related to the mineralization of organic nitrogen through ammonification. The NH_4^+–N contents of T1 and T2 were higher than T3 throughout the process ($F = 1.300$, $p < 0.001$; $F = 1.050$, $p < 0.001$), which was mainly due to higher N content in the initial mixture with a lower C/N ratio. After that, the content of NH_4^+–N decreased and stabilized by the end of composting, which was due to NH_3 volatilization under high temperature and pH condition as well as nitrification. After 59 days of composting, the contents of NH_4^+–N were as follows: 1930.8 mg kg^{-1} in T1; 864.8 mg kg^{-1} in T2; 33.2 mg kg^{-1} in T3; 1532.5 mg kg^{-1} in T4; and 12730 mg kg^{-1} in T5. A value of 400 mg kg^{-1} was recommended as the maximum NH_4^+–N content for mature compost (Zucconi et al., 1981). Therefore, all of the final composts except T3 need to be treated to reach maturity.

Changes in NO_3^-–N contents are presented in Fig. 2b. There was no significantly change in the NO_3^-–N content during the initial stage of composting. This was due to the fact that high temperature and excessive NH_3 accumulation had an inhibition on the activity and growth of nitrifying bacteria (Guo et al., 2012). Then, the NO_3^-–N content started to increase rapidly, while there was no obvious change in T5. The increase in NO_3––N content was caused by nitrifying microorganisms. The NO_3^-–N content in T4 was higher than T2 ($F = 0.014$, $p = 0.337$), mainly because of the type of materials used for composting.

The NH_4^+–N/NO_3^-–N ratio has been established to indicate the maturity of compost when it is below the limit value of 0.16 for composting materials such as pig slurry, poultry manure, municipal solid waste and sewage sludge (Bernal et al., 2009). In this paper, NH_4^+–N/NO_3^-–N ratios of T1, T2, T3, T4, and T5 were 1.42, 0.54, 0.02, 0.90 and 23.9, respectively, indicating that T3 has reached maturity after 59 days of composting. However, Rui et al. (2012) suggested that NH_4^+–N/NO_3^-–N ratio might not apply to maturity index for pig manure composts, and needs to be further studied.

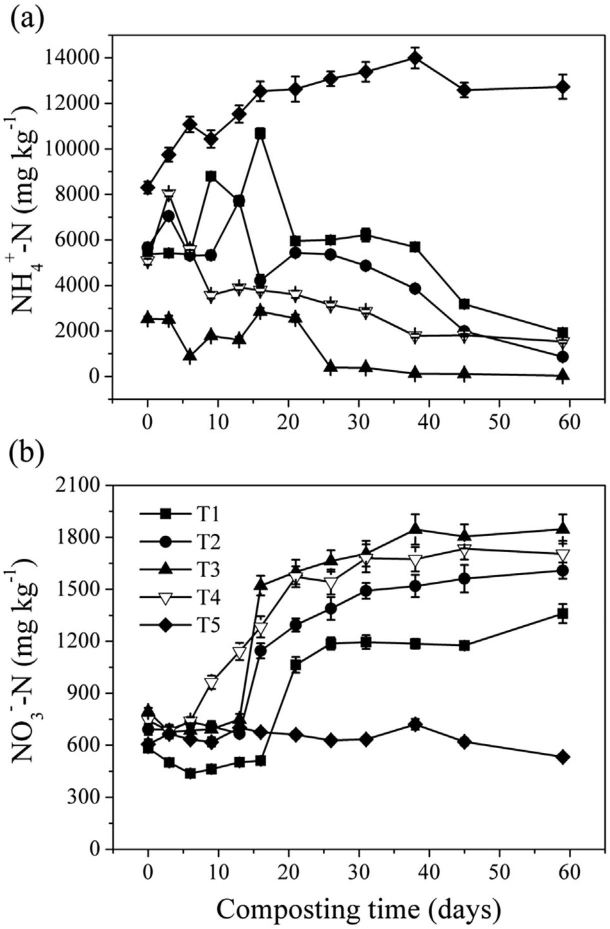

Fig. 2. Change in contents of NH_4^+–N (a) and NO_3^-–N (b) during composting.

3.3. Zinc and copper speciation

Zn and Cu have been reported as the most abundant heavy metals in all composted products, swine manure and sewage sludge (Hseu, 2004). Zn and Cu were derived from feeds additives for shortening the cycle of breeding, and mostly they cannot be absorbed by animals but excreted in animal manures (Guo et al., 2013). The total contents of Zn and Cu remarkably increased in T1, T2, T3 and T4, while T5 decreased during composting (Fig. 3). The total contents in the different treatments increased as follows: 112.8–192.7% for Zn and 115.5–132.6% for Cu. Similar results were confirmed by the studies of Hsu and Lo (2001), who found the total contents of Zn and Cu increased by 2.7–2.8 fold in mature compost compared to the initial values of 576 mg kg^{-1} and 343 mg kg^{-1}, respectively. A higher increment in the toxic metal concentration was due to weight loss in the process of composting following organic matter decomposition, release of CO_2 & water and mineralization processes (Lu et al., 2014). In addition, the total content of Zn in T2 was much higher than T4 ($F = 1.928$, $p = 0.141$), while there was no significantly difference in that of Cu ($F = 0.266$, $p = 0.701$). This result indicated that the types of composting and raw materials had significant effects on metal enrichment. The total metal contents of all treatments were in order of Zn > Cu ($p < 0.001$). The contents of Zn and Cu in final compost were far exceeded by the Spanish legal maximum allowed, which were 1100 mg kg^{-1} and

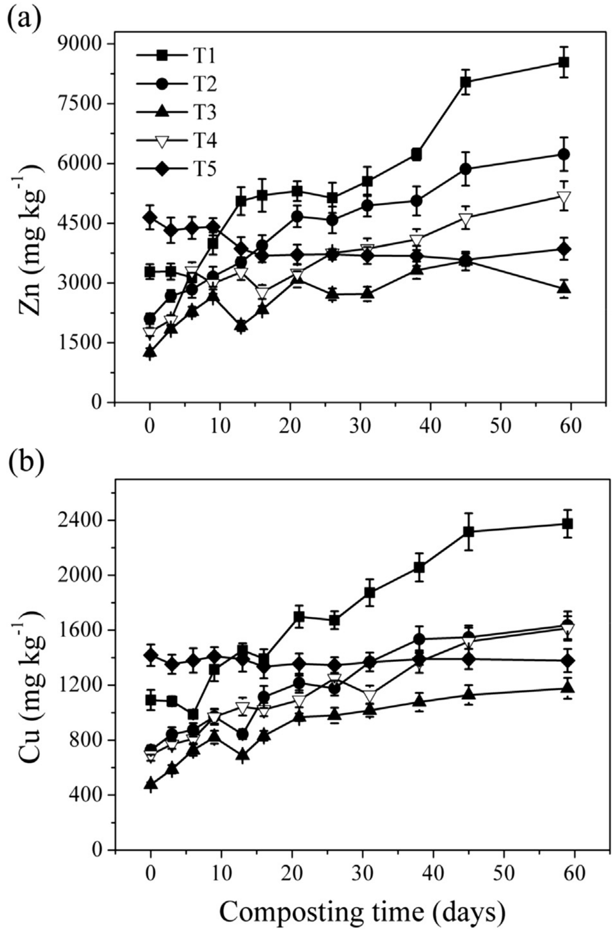

Fig. 3. Change in total concentrations of Zn (a) and Cu (b) during composting.

450 mg kg^{-1} in solid poultry manure, respectively (BOE 2/06/1998). The total content of heavy metal is an indicator of pollution risk but it does not provide comprehensive information about their mobility and bioavailability under different environmental conditions, which largely depends on their specific chemical speciation (Cai et al., 2007). Therefore, the chemical speciation of heavy metal in the composts is attracting extensive attention of researchers.

As shown in Table 3, for Zn, fractions of F1, F2 and F4 were dominant speciation in all treatments. F1 fractions increased from 1914.1 mg kg^{-1} to 2954.2 mg kg^{-1} in T1; from 1179.8 mg kg^{-1} to 2415.1 mg kg^{-1} in T2; from 626.3 mg kg^{-1} to 1044.0 mg kg^{-1} in T3; and from 1557.5 mg kg^{-1} to 1878.9 mg kg^{-1} in T4 during composting, indicating that 1.21–2.05 enrichment of Zn was achieved compared to those in the initial mixture ($p = 0.209$). The decrease in pH might be responsible for the increase in F1 fraction. Toxic metals in F1 and F2 fractions were considered to be mobile and bioavailable, while the F3 and F4 fractions were stable and non-bioavailable (Nomeda et al., 2008). The composting increased the mobile of Zn, which agreed with the result obtained by Amir et al. (2005). Fractions of F2 and F4 were significantly increased, and F3 fraction was increased slightly with the time ($p < 0.05$). On the contrary, F4 fraction was sharply decreased in T5, while fractions of F1, F2 and F3 remained unchanged at the beginning and the ending of composting.

The speciation of Cu was significantly different from that of Zn (Table 3). The residual fractions were dominant speciation in all treatments for Cu. The pH decreased and mineral fraction increased in the compost, which could be responsible for the redistribution of Cu into the residual fraction (Gusiatin and Kulikowska, 2014). The reduction of the F1 fraction in the final compost was 9.3% in T1; 8.2% in T2; 31.4% in T3; and 13.7% in T4 compared to the initial mixture ($p < 0.05$). F4 fraction significantly increased in all treatments except T5 ($F = 0.986$, $p = 0.016$). In T5, fractions of F1 and F4 kept nearly unchanged during composting. Cai et al. (2007) reported that 70% of Cu belonged to organic matter fraction, indicating that Cu had higher affinity for organic matter, which was mainly due to the formation of humic substances. However, F2 and F3 fractions of Cu were not detected in all treatments throughout the composting process.

The mobility factor (MF) which calculated as the ratio of heavy metal in F1 fraction to the total content, could be applied to evaluate the potential mobility of heavy metal (Lv et al., 2016). As shown in Fig. 4, composting reduced the mobility of Cu and Zn. This

Table 3
Change in speciation of Zn and Cu during the composting process.

	Days	Zn (mg kg^{-1})				Cu (mg kg^{-1})			
		F1	F2	F3	F4	F1	F2	F3	F4
T1	0	1914.1 ± 9.9	927.2 ± 5.6	152.1 ± 2.2	1109.5 ± 4.5	157.9 ± 2.1	n.d.	n.d.	933.2 ± 8.6
	21	2678.0 ± 18.5	1258.3 ± 13.3	178.0 ± 3.6	2639.3 ± 16.7	103.3 ± 5.2	n.d.	n.d.	1594.4 ± 3.2
	38	2792.8 ± 52.6	1941.4 ± 12.4	191.7 ± 2.1	3390.9 ± 47.8	116.1 ± 1.5	n.d.	n.d.	1940.4 ± 9.1
	59	2954.2 ± 14.1	2543.4 ± 18.6	195.4 ± 0.0	2858.9 ± 12.4	143.2 ± 1.0	n.d.	n.d.	2231.9 ± 30.1
T2	0	1179.8 ± 16.3	296.6 ± 5.8	118.9 ± 2.2	507.6 ± 3.5	104.4 ± 12.9	n.d.	n.d.	623.8 ± 13.1
	21	1828.8 ± 8.1	1821.8 ± 16.3	129.9 ± 3.6	911.3 ± 31.7	139.0 ± 0.5	n.d.	n.d.	1076.2 ± 24.9
	38	2009.7 ± 21.9	1953.6 ± 5.0	147.2 ± 21.1	949.9 ± 2.5	93.9 ± 0.0	n.d.	n.d.	1440.0 ± 15.3
	59	2415.1 ± 7.9	2378.8 ± 7.4	171.2 ± 0.0	1264.7 ± 43.1	95.8 ± 0.0	n.d.	n.d.	1539.1 ± 3.2
T3	0	626.3 ± 31.9	295.6 ± 1.2	118.5 ± 12.9	219.0 ± 17.9	80.7 ± 3.6	n.d.	n.d.	395.6 ± 12.3
	21	836.8 ± 8.6	716.5 ± 9.2	120.1 ± 11.4	1016.7 ± 14.3	64.6 ± 11.1	n.d.	n.d.	903.0 ± 2.6
	38	980.5 ± 20.8	867.5 ± 15.6	132.2 ± 3.5	1339.4 ± 23.6	57.3 ± 3.3	n.d.	n.d.	1019.0 ± 23.5
	59	1044.5 ± 15.6	1079.9 ± 12.8	179.3 ± 3.5	1350.3 ± 28.1	55.4 ± 24.1	n.d.	n.d.	970.7 ± 21.9
T4	0	1557.5 ± 25.7	391.9 ± 32.9	116.6 ± 13.5	505.1 ± 12.6	137.8 ± 6.5	n.d.	n.d.	555.7 ± 27.1
	21	1493.4 ± 6.9	757.3 ± 12.9	181.3 ± 5.7	875.7 ± 4.3	134.3 ± 12.4	n.d.	n.d.	957.0 ± 1.5
	38	1599.5 ± 18.4	1240.3 ± 17.3	185.4 ± 4.2	1232.3 ± 5.4	121.7 ± 7.4	n.d.	n.d.	1248.8 ± 3.2
	59	1878.9 ± 28.5	1467.3 ± 8.5	223.7 ± 4.7	1755.9 ± 34.1	118.9 ± 15.5	n.d.	n.d.	1494.0 ± 9.3
T5	0	2433.7 ± 12.2	766.8 ± 11.7	146.9 ± 2.2	1297.4 ± 53.2	141.6 ± 18.2	n.d.	n.d.	1275.8 ± 2.9
	21	1861.9 ± 36.1	1095.3 ± 37.4	227.7 ± 2.1	605.0 ± 5.2	130.3 ± 5.4	n.d.	n.d.	1225.7 ± 4.5
	38	1728.8 ± 11.8	1313.6 ± 12.3	168.3 ± 11.4	558.8 ± 17.2	179.3 ± 0.0	n.d.	n.d.	1209.7 ± 6.2
	59	2487.7 ± 23.2	892.9 ± 9.0	144.0 ± 4.2	447.5 ± 2.1	182.4 ± 0.0	n.d.	n.d.	1195.8 ± 3.5

n.d.: not detected; ±: Standard deviation of three replicates.

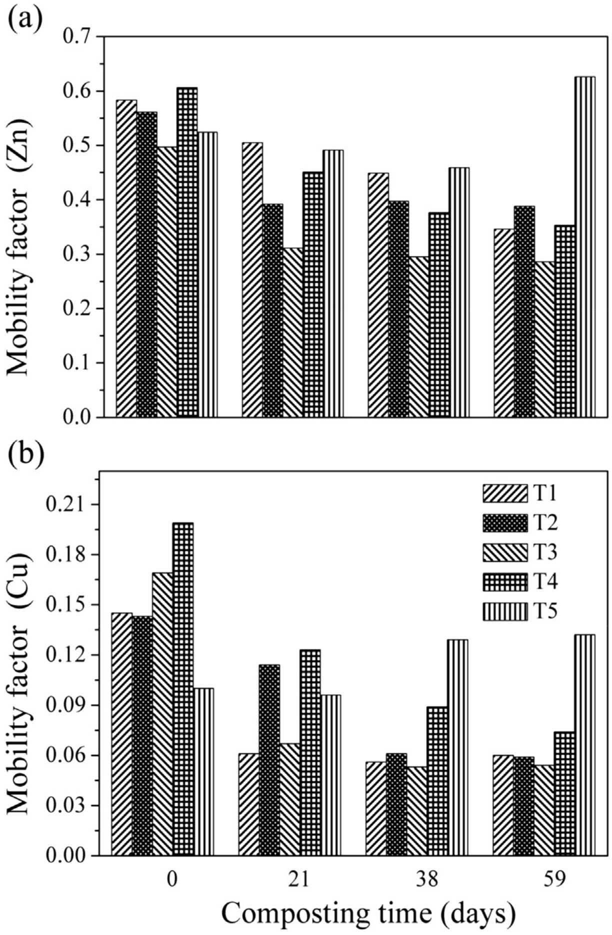

Fig. 4. Change in mobility factor of Zn (a) and Cu (b) during composting.

result was in accordance with previous studies (Lv et al., 2016). The MF of Cu and Zn decreased with the increase of C/N ratio. This result showed that the composting mixture with initial C/N ratio of 25 could alleviate the mobility of Cu and Zn, even though F1 fraction increased with the composting time. Similar results were found by Zhu et al. (2014), they found vermicomposting increased the Cu and Zn concentrations in the earthworms as well as increased the available Cu and Zn concentrations in the vermicomposted residue. At the end of composting, the MF of Zn decreased by 34.6% in T1; 38.8% in T2; 28.6% in T3; and 35.3% in T4 compared to the initial mixture, which suggested that the mobile fractions of Zn were clearly reduced ($p < 0.001$). The mobility of Zn largely depended on the total content of Zn, pH, organic matter and adsorption sites (Amir et al., 2005). The MF of Cu decreased and reached stability on day 21 in T1 and T3, but the stability was achieved on day 38 in T2 and T4 ($F = 4.420$, $p < 0.001$). Despite our initial hypothesis, bulking agents have little effects on the mobility of heavy metal. In T5, the MF of Cu gradually increased. Besides, the MF of Cu and Zn in T4 were higher than T2 ($F = 0.106$, $p = 0.466$). It should be further noted that the MF of Zn was much higher than that of Cu ($p < 0.002$), indicating that the potential mobility of Zn was higher than Cu during composting. Other studies also found the MF of Zn was still higher than Cu in pig manure even though vermicomposting decreased it clearly (Lv et al., 2016).

3.4. Enzymatic activity

Dehydrogenase activity has been defined as a measure of microbial activity in composts, which was directly involved in the respiratory chain and provided information of compost maturity (Vargas-García et al., 2010). Dehydrogenase activity of all treatments decreased drastically from an initial high level during the first week and then levelled off as the process progressed ($F = 3.510$, $p = 0.004$). Interestingly, T3 increased after reaching the lowest value (Fig. 5a). The highest dehydrogenase activity was obtained in T1 (2597.3 μg TPF g^{-1} 24 h^{-1}), T2 (3291.1 μg TPF g^{-1} 24 h^{-1}), T3 (3733.1 μg TPF g^{-1} 24 h^{-1}) and T4 (3205.7 μg TPF g^{-1} 24 h^{-1}) on day 1, T5 (530.6 μg TPF g^{-1} 24 h^{-1}) on day 21. Similar results were observed by other researchers using different composting materials (Vargas-García et al., 2010; Wong and Fang, 2000; Zhu et al., 2012). Readily available carbon sources were exhausted, which slowed down the respiratory process, thereby resulting in a decrease in dehydrogenase activity as well as indicating compost maturity. And the increase in pH also inhibited enzymatic activity during composting (Wong and Fang, 2000).

Urease is involved in the hydrolysis of urea into ammonium and carbon dioxide, which is closely linked to the decomposition of agricultural waste (Vargas-García et al., 2010). The urease activity decreased markedly and fluctuated around the low level for all treatments except T5 (Fig. 5b). Similar results were described for the urease activity by Jurado et al. (2014), who found that urease activities showed a drastic fall during the first five days at the beginning of the process. Besides, the composting mixture with initial C/N ratio of 25 accelerated the decline of urease activity. T1, T2, T3 and T4 reached stability on day 21, 21, 3 and 9, respectively. The urease activity of composting mixtures with different initial C/

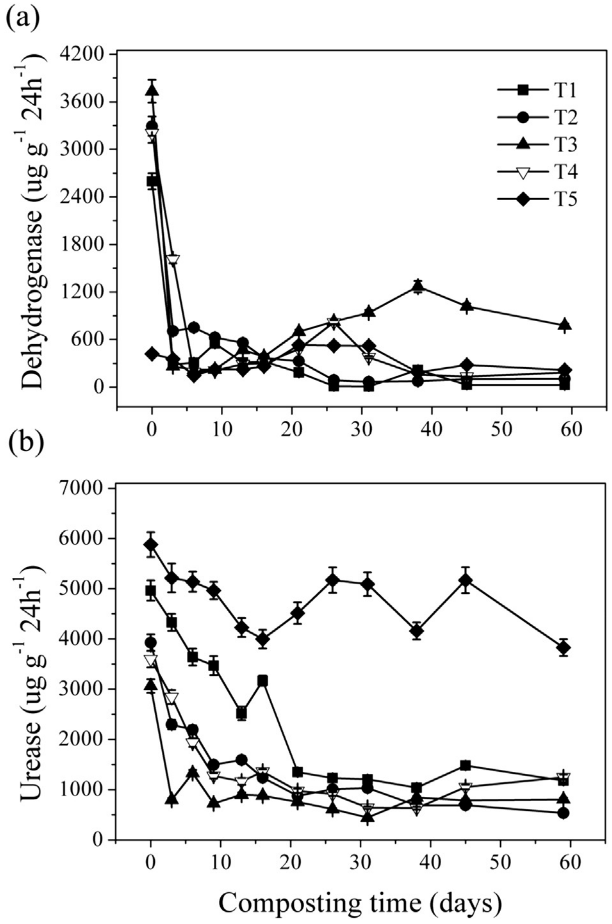

Fig. 5. Change in activities of dehydrogenase (a) and urease (b) during composting.

N ratio were in the order of T1 > T2 > T3, which could be attributed to N content of the initial mixture. The urease activity in T2 had similar trends with T4, indicating that urease activity was not affected by bulking agent. Our results were supported by Zhu et al. (2012), who composted pig manure for producing maggots as feed supplement and organic fertilizer in a two-stage composting system. The urease activity in maggot-treated compost decreased from day 1 and fluctuated at low levels. Similarly, Guo et al. (2012) reported that the enriched Cu (>300 mg kg^{-1}) presented an inhibiting effect on the enzymatic activity. Heavy metal Cu not only showed toxic effects on the enzymatic activity, but also infiltrated into microbial cells, thus interfering with transcription and translation of enzyme. Cu also showed higher inhibition on the urease activities than Zn and Pb when three metals were all present in the soil (Feng et al., 2016). The sludge composting with the addition of lime was abundant in Ca^{2+}, which resulted in an inhibition on urease producing microorganisms, thus it caused sudden decrease in urease activity (Wong and Fang, 2000). Therefore, we deduced that C/N ratio could affect urease activity by influencing the content of metal ions.

3.5. Compost quality

Compost product can be safely applied to soil as organic fertilizer or conditioner depending on products containing high content of organic matter and nutrients, being mature or stable and without toxicity to plant growth (Hu et al., 2015). Quality assessment of compost product was given in Table 4. Compost quality based on physicochemical parameters, nutrient content, heavy metals and toxicity was evaluated by the Chinese national regulations for organic fertilizer (NY525-2012). Except the *Escherichia coli* in T1 and T4 and the mortality of ascarid egg in T2 and T4, other indicators were within the permissible limits. In addition, all compost products showed high quality according to nutrient contents ($N + P_2O_5 + K_2O$). The final compost with initial C/N ratio of 25 also shows a low concentration in heavy metals. The above characteristics make the final compost with initial C/N ratio of 25 suitable for agricultural requirements and suggest that it can be used as an organic fertilizer for plant growth according to Chinese national regulations for organic fertilizer (NY525-2012).

3.6. Environmental implications

The widespread use of unsuitable and unsustainable production techniques in developing countries such as Asia, Africa and Latin America agricultural systems has resulted in extensive deterioration of soil quality, and reductions in soil organic matter content and crop production. Improving and maintaining soil quality and fertility in a sustainable way is an important challenge for modern agriculture (Viaene et al., 2016). Composting followed by land application, represents one of most efficient solution for material recycling with manure disposal simultaneously and is therefore a sustainable way. The incorporation of the composted waste improves soil quality and crop production as well as sustainability, especially the significant increase in availability of N, P, K and soil organic matter content. Composts enriched with minerals also reduced the bioavailability of heavy metals in contaminated soils (Soares et al., 2015). However, the presence of heavy metals in final compost raises serious concern about the adverse environmental impact, as a result of excessive compost application to agricultural lands. Therefore, how to reduce the mobility of heavy metal in compost is a challenge problem.

Table 4
Quality assessment of compost products by the end of composting process.

Properties	NY525-2012	Compost products			
		T1	T2	T3	T4
OM %	≥45.0	65	59.6	75.3	83.1
pH value	5.5–8.5	6.95	6.84	7.98	6.70
Moisture	≤30.0	29.5	29.2	27.9	27.9
Nutrient content % ($N + P_2O_5 + K_2O$)	≥5.0	9.39	10.14	9.21	7.93
Arsenic mg kg^{-1}	≤15.0	2.49	3.26	3.59	1.17
Mercury mg kg^{-1}	≤2.0	1.16	0.42	0.21	0.15
Plumbum mg kg^{-1}	≤50.0	7.39	9.41	6.54	5.04
Cadmium mg kg^{-1}	≤3.0	0.72	0.8	0.64	0.42
Chromium mg kg^{-1}	≤150.0	8.63	9.94	7.43	7.84
Escherichia coli CFU/g	≤100	117	98	93	145
Mortality of ascarid egg	≥95	95.6	90.0	96.0	87.5

On the basis of the results of this study, C/N ratio plays a key role in controlling the mobility of heavy metal in compost. In particular, the final compost with initial C/N ratio of 25 is of high quality and low toxicity for land application. Thus, it will achieve win-win for resource recycling and reduction in environmental burden of manure management.

4. Conclusions

This study investigated the effects of C/N ratio (15, 20, 25) and bulking agent (maize straw, rice straw) on pig manure composting through evaluating their influence on speciation of Zn and Cu and enzymatic activities (dehydrogenase, urease). Maize straw addition as bulking agent greatly enhanced organic matter degradation. The composting mixture with initial C/N ratio of 25 reduced the mobility of Cu and Zn, while their total contents increased during pig manure composting. The residual fractions of Cu were dominant speciation in all treatments. In addition, C/N ratio could affect urease activity by influencing the content of metal ions. Therefore, the final compost with initial C/N ratio of 25 was of low risk and high quality for land application.

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