Immobilization and assessment of heavy metals in chicken manure compost amended with rice straw-derived biochar

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ABSTRACT
In this study, a 30-days laboratory experiment was implemented to investigate the impact of additive biochar on the stabilization of heavy metals in chicken manure compost. Results showed that after the addition of rice straw-derived biochar, heavy metals were more stabilized except Cu, of which the residual fractions distinctly decreased due to the interaction with organic functional groups from biochar. Given the bioavailability of heavy metals, the biochar addition at a 10% proportion decreased the concentration of CaCl2-extractable Cr, Zn, Ni, and Cd. Besides, CaCl2-extractable As did not differ significantly between treatments with and without biochar addition. Furthermore, the CaCl2-extractable Cu was higher than the control, in agreement with the observed changes in speciation. Environment pollution assessment by integrating potential ecological risk assessment explicating the chicken manure compost reached a very high-risk pollution level, and decreased with biochar addition. Therein, Cd was the dominant pollutant with very high potential risk.

1. Introduction

With the increasing demand for poultry products in daily food consumption, poultry breeding in China had been greatly developed [1,2]. Globally, China was the largest producer of meat in recent two decades, the total amount of breeding animals reached 855 million tons in 2016 [3]. Meanwhile, the huge amount of manures and slurries with a total yield of 8.3 billion tons had caused a series of environmental problems, such as greenhouse gas emissions, land-use change, odor and airborne ammonia [4-6]. Therefore, livestock was one of the most significant contributors to present serious environmental problems [7]. In addition, various additives had been wildly adopted during the poultry breeding for the special effects on growth promotion and disease prevention after the earlier study concluded that the addition of Cu could promote the growth of livestock [8]. Therein, most additives contained toxic heavy metals, the majority of which were excreted in feces and urine, thus became important pollution sources once the compost was applied as agricultural fertilizer [9]. According to the previous survey, approximately 20% of agricultural soil exhibited elevated levels of heavy metals due to the application of fertilizer including chicken manure [5]. Therefore, investigation on heavy metals in chicken manure was urgent for proper application and necessary about environmental safety.

With the implementation of ‘Livestock and Poultry Manure Utilization Action Plan (2017–2020)’ policy in 2017 in China, the composting practice had been adopted for livestock and poultry manure utilization [2]. Results of recent studies demonstrated that composting was a viable approach for organic matter stabilization and sanitization [10]. However, during the composting process, toxic heavy metals in chicken manure could not be destroyed or eliminated thus being persistent in the final compost as predominant pollution sources to the subsequent environment [11]. High levels of toxic heavy metals in compost had been extensively reported in China [12]. A recent nationwide survey showed 13.7% and 2.4% compost was characterized with high As and Cd, respectively, exceeding the maximum permissible value [5]. The field study showed that the application of swine manure compost might lead to elevated levels of Cu and Zn in soil, especially after long-term continuous application [13,14]. Besides, Luo et al [15] found that more than half of Cd, Cu and Zn in agricultural soil was attributed to the utilization of livestock manure.

Compared with conventional remediation materials, biochar, which was characterized with high porous micro-structure, active functional groups, high pH, surface area and cation exchange capacity (CEC), had been considered as an effective candidate for re-vegetation and restoration [16], and had been widely
adopted in environmental remediation [17,18]. Biochar being added into soil then increased metal sorption was observed [19], indicating the addition of biochar could reduce metal availability in contaminated soil [20]. Ahmad et al. [21] prepared six different biochar and applied to different soil samples (10% w/w, one was contaminated with Pb and Zn, the other was contaminated with Pb and Cu), it showed that the additive biochar resulted in an increase of soil pH, and the majority of heavy metals were immobilized by biochar through functional group complexation and metal-hydroxide precipitation. Furthermore, Fellet et al. (2011) added various proportions of biochar (0, 1, 5 and 10% w/w) to mine tailings which was contaminated with Cd, Pb and Zn, and found that heavy metal bioavailability decreased with increasing biochar application.

The extensive investigation had been focused on the application of biochar in water and soil remediation [22,23]. However, up to now, there was still limited research on the impact of additive biochar on the stabilization of heavy metals in compost [12]. Only a few studies were put emphasize on the speciation and bioavailability of heavy metals in compost affected by adding biochar, which was of practical significance for composting process optimization and environmental pollution control [24–27]. Therefore, the objectives of the present study were to investigate the impact of additive biochar on the speciation and bioavailability of heavy metals in chicken manure compost, which could provide scientific support and reference for the application of biochar as a modifier in compost.

2. Materials and methods

2.1 Materials

Rice straw-derived biochar was produced at a treatment temperature of 550°C using a slow pyrolysis method for 1 h under limited oxygen supply [28]. Chicken manure samples were collected from commercial composting factory in Anhui Province of China. All the biochar and samples were oven-dried at 105°C for 24 h, then ground using an agate mortar and ground to pass through 2 mm sieves, then thoroughly mixed and stored in labeled plastic bottles before analysis. The ultimate analysis indicated the concentration of carbon, hydrogen, oxygen, nitrogen and sulphur were 83.6, 1.3 13.6, 1.2 and 0.3%, respectively. The biochar had a pH value of 10.5, and a relatively low content of heavy metals (<0.005 mg kg⁻¹) [29].

2.2 Experimental design

According to the Chinese National Agriculture Industry Standard animal manure composting (NY/T 3442–2019), the composting process was performed in PVC reactors (with 30 L effective volume) for 30 days. The composting raw material was mixture of fresh chicken manure with corn straw to adjust the carbon/nitrogen (C/N) ratio to ~25:1. In addition, as shown in Table 1, biochar with five different proportion of 0, 10, 20, 30, and 40% (on dry weight basis) were labeled as CK, C1, C2, C3 and C4, respectively. After the 30-days composting experiment finished, end-product samples were collected and dried at 105°C for 24 h until constant weight and then ground to pass through 2 mm sieves for chemical analysis.

2.3 Sample analysis

2.3.1 Total content of heavy metals

The total content of six selected heavy metals (Zn, Cu, As, Cr, Cd and Ni) in the compost samples (approximately 0.10 g) was determined by inductively coupled plasma atomic emission spectrometer (ICP-AES, PerkinElmer Optima 7300 DV) following digestion with HF-HClO₄-HNO₃ (2:3:2:1) mixed-acid. All plastic-ware and glassware were immersed in a 5% (v/v) nitric acid solution for 24 h and syringed with ultrapure water before determination.

2.3.2 Bioavailability of heavy metals

To predict the bioavailability of heavy metals, neutral salts such as calcium chloride (CaCl₂) was the most suitable extractant with the ability of extract various cations at low concentration with no change of pH during extraction process [29]. In this study, the bioavailability of heavy metals was measured using 0.01 M CaCl₂ solution, as reported by Pueyo et al. [30]. Briefly, compost samples were transferred in 10 mL centrifuge tubes with 10 mL 0.01 M CaCl₂ solution and then shaken at 200 rpm for 2 h at 25 ± 1°C, and then the suspension was separated by centrifugation at 3000 rpm for 30 min and filtered through 0.45 μm membrane. Extracted metal concentration was determined using inductively coupled plasma atomic emission spectrometer.

2.3.3 Chemical speciation of heavy metals

As one of the most widely applied extraction procedures for investigation of metal speciation, the European Community Bureau of Reference (BCR) sequential extraction procedures were adopted in the present study to investigate the chemical speciation of heavy metals [31,32]. Generally, heavy metals speciation can be divided into four different fractions such as
Table 2. Sequential extraction procedure for the speciation of BCR.

| Fraction  | Reagent                           | Shaking |
|-----------|-----------------------------------|---------|
| Acid soluble  | 40 ml 0.11 mol L⁻¹ HAc           | 16 h (3000 r min⁻¹) at 22 ± 5°C |
| Reducible  | 40 ml 0.5 mol L⁻¹ NH₄OH (adjusted pH = 1.5 with HNO₃) | 16 h (3000 r min⁻¹) at 22 ± 5°C |
| Oxidizable | 10 ml 8.8 mol L⁻¹ H₂O₂, (pH = 2–3), followed by 10 ml 8.8 mol L⁻¹ H₂O₂, (pH = 2–3) and 50 ml 1 mol L⁻¹ NH₄Ac, (pH = 2) | 1 h at 85 ± 2 °C, 85 ± 2 °C and 22 ± 5°C |
| Residual   | HF-HCl-HNO₃-HNO₃, digestion     |         |

exchangeable fraction (F1), carbonate fraction (F2), Fe-Mn oxide fraction (F3) and residual fraction (F4), which were regarded as direct eco-toxic and bioavailable (F1 + F2), potentially bioavailable (F3) and non-toxic (F4), respectively [29]. Detailed sequential extraction steps are tabulated in Table 2. The obtained extracted solutions were analyzed by the ICP-AES.

2.4 Risk assessment methods

In this study, based on the chemical speciation of heavy metals, the potential ecological risk index (RI) was applied to gain further insights into the risk assessment of heavy metals in compost [33,34]. RI is an indicator to assess the levels of potential risk of heavy metal pollution, which can be defined as follows:

\[ P_m = \frac{C(F_1 + F_2)}{C_0} \]

\[ ER_m = T_m \times P_m \]

\[ RI = \sum ER_m \]

where, \( P_m \) is the contamination factor of each heavy metal, \( C(F_1 + F_2) \) is the sum of the contents of heavy metals present in fractions F₁ and F₂ in the samples, \( C_0 \) is the background values of heavy metal in Anhui (China), the background concentration of Cr, Ni, Cu, As, Zn and Cd in local soil are 66.5, 29.8, 20.4, 9.0, 62.0, 0.1 mg kg⁻¹, respectively [35,36]. \( ER_m \) is the monomial potential ecological risk coefficient for each heavy metal, \( T_m \) used for reflecting the toxicity of heavy metals are Cr (2), Ni (5), Cu (5), As (10), Zn (1) and Cd (30) [33,37].

2.5 Quality control and evaluation

A certified reference material (GBW07428) as well as reagent blanks, duplicate samples were analyzed simultaneously to check the accuracy and precision of the method. The precision and bias of the analysis results were within ±5%, and the returning recoveries of heavy metal speciation were ranged from 82.5% to 119.48%. The data were presented as mean ± standard deviation (n = 3).

3 Results and discussions

3.1 Content of heavy metals

Total content of heavy metals in the compost samples is given in Table 3. Zinc showed the highest level in chicken manure compost, which was 424.11 mg·kg⁻¹, followed by Cr, Cu, Ni and As, with the mean values of 76.17, 61.66, 28.22 and 27.22 mg·kg⁻¹, respectively, whereas Cd content was the least present in the compost, only 3.09 mg·kg⁻¹. Content of Cr, Cd, Ni and As showed comparable levels to the national statistical data reported by Qin et al. and Wang et al. [38,39]. However, it was noteworthy that heavy metals in present study were higher than the experimental results of Wang and Shan et al. [40,41], which might be attributed to additives used for growth promotion and disease prevention during the breeding process [42,43]. Addition of Cu and Zn to intensive livestock feed had been a common practice to promote optimal nutrition supply [44].

According to the compost standards set by China and other countries, maximum allowable values for heavy metals in compost are summarized in Table 3. On the basis of the Chinese national standard for organic fertilizers [45], Cd and As content in the compost samples were 1.03 and 1.81 times higher than corresponding guideline values, respectively. High As content in compost may pose a significant threat to the environment and human health [46]. Nevertheless, there had been no guideline values set for Cu, Zn, and Ni in China so far. Instead, results were compared with the standard values of heavy metals in mature manure in Germany with upper limit being 400, 20 and 100 mg·kg⁻¹ for Zn, Ni, and Cu [47], where content of Ni exceeding the maximum values by 1.06 times. Furthermore, according to the Australian standards of

| Cr       | Ni     | Cu     | Zn    | As     | Cd     | Source         |
|----------|--------|--------|-------|--------|--------|----------------|
| 76.17    | 28.22  | 61.66  | 424.11| 27.22  | 3.09   | This study     |
| 35.52    | 9.71   | 72.24  | 258.1 | 16.33  | 2.42   | Ding et al., 2017 |
| 0.69 ~ 6603 | 0.68 ~ 72.7 | 3.55 ~ 916 | 11.8 ~ 3692 | 0.37 ~ 71.7 | 0.012 ~ 8.72 | Yang et al., 2017 |
| 0 ~ 1229 | 0 ~ 9600 | 0.7 ~ 501.2 | 0 ~ 53.2 | 0.1 ~ 75.8 | 0.1 ~ 28.0 | Qin et al., 2015 |
| 7.06 ± 3.23 | 5.50 ± 1.60 | 271.2 ± 144.9 | 379.6 ± 181.7 | 5.04 ± 2.65 | 0.73 ± 0.31 | Wang et al., 2015 |
| 33.68 ± 1.03 | 45.25 ± 1.30 | 184.98 ± 2.32 | 364.93 ± 8.38 | 0.93 ± 0.11 | - | Sunur et al., 2016 |
| 16.3     | 7.63   | 314    | 573   | 10.3   | 0.73   | -              |
| 185.89 ± 435.59 | 13.08 ± 5.74 | 96.2 ± 136.1 | 509.18 ± 613.35 | 23.26 ± 96.67 | 0.42 ± 0.65 | Ru et al., 2016 |
200 and 25 mg·kg\(^{-1}\) for Zn and Ni [48], metal content in compost exceeded the limit values by 2.12 and 1.13 times, respectively. Therefore, heavy metal enriched in compost should be taken special consideration for its agricultural applications [49].

### 3.2 Chemical speciation of heavy metals in compost

As the most crucial factor for mobility and bioavailability of heavy metals, chemical speciation would influence their environmental behavior and potential toxicity [50]. Detailed speciation characterization of heavy metals in compost was depicted in Figure 1. It was worth note that Cr, Ni, and Cu were predominantly associated with the residual fraction, which accounted for more than 70% of the total amount, then followed by reducible and oxidizable fractions, indicating that Cr, Ni, and Cu in compost were mostly presented in a more stable form with relatively low risk to the surrounding environment. However, Cd was generally more bioavailable and showed higher mobility in environment [51,52], where reducible fraction was dominant for Cd, with the value of 70.32%, and the acid soluble and oxidizable fraction contributed only 11.58% in present study. Zinc mainly existed in the reducible and residual fraction, which may be attributed to the presence of small molecule humic components in compost [53]. Besides, the predominant chemical partitioning of As was the residual fraction (45.70%), followed by reducible and oxidizable fraction with 22.05 and 22.28%, respectively.

The bioavailability of heavy metal fractions decreased in the order of acid soluble>reducible>oxidizable>residual [54]. Thus, the acid soluble and reducible fractions were more available in environment, whereas the oxidizable and residual fractions were comparably stable [55]. Therefore, given the chemical speciation of heavy metals in compost, the mobility, and bioavailability of the heavy metals could be sequenced as Cd>Zn>As>Cu>Ni>Cr.

### 3.3 Effect of biochar on speciation of heavy metals

Results showed that the speciation of heavy metals in compost was variously affected by the addition of biochar in the 30-days experiment. As displayed in Figure 2, the residual fraction of Cd, As, Cr, Ni, and Zn was increased with the biochar amendment, which indicated that heavy metals were turned to a more stable status after treatment. In addition, the residual fraction of Cd, As, and Zn was mostly increased with 10% biochar as no apparent increasing tendency were found with more biochar. The residual fraction of Ni had risen with the biochar addition within the range from 10% to 30%; then there was no significant change for more biochar addition.

Metal stabilization with biochar had been widely developed for soil and waste water remediation [56]. Chemical speciation of heavy metals was interpreted to be affected by the addition of biochar through direct and indirect interactions: the direct interactions referred to heavy metals were associated with the organic functional groups originated from biochar [57], thus led to the stability of heavy metals [58,59]; while the indirect interactions were related to the release of dissolved organic carbon and increase of pH that would favor metal stability and precipitation [60,61]. The pH was increased from 7.52 to 8.46 in this study, which promoted the formation of stable metal complexes with the dissolved organic matter [62], indicating the predominant effect for heavy metal stabilization, according to the study of Rees et al. [63]. Meanwhile, mineral phases and organic matter from biochar would also provide
3.4 Impact of biochar application on the bioavailability of heavy metals

Bioavailability of heavy metals had been regarded as one of the most crucial issues in agricultural and environmental studies [70], where heavy metals were considered to be bio-absorbed or toxic to organisms [71]. Bioavailability of heavy metals in compost was closely related to the morphology [24,72]. As shown in Figure 3, with the addition of biochar, the proportion of CaCl$_2$-extractable fraction for all metals except Cu showed decreasing tendency. The extractable fraction of Ni, As, Cr, Cd and Zn were observed with a biochar dosage rate of 10%, which were 32.38, 25.57, 56.65, 50.68 and 82.92% lower than those at control sample, respectively. With the increasing amount of biochar, levels of the CaCl$_2$-extractable concentration of Ni, As, Cr and Zn did not show any apparent change, which indicated that more addition of biochar did not make sense. These results were in agreement with the change of speciation of heavy metals aforementioned.

Bioavailability of heavy metals in compost predominantly depended on the specific chemical forms and environmental condition changes. Compost properties included pH, organic matter, sulfate, carbonate, cation exchange capacity, and hydroxide [73,74]. Due to the addition of alkaline biochar, the physical and biological properties of compost were effectively regulated and the pH value of the compost was increased, which enhanced the cation adsorption ability and promoted the precipitation of heavy metals in the forms of oxides, carbonates, hydroxides and phosphates so as to reduce metal bioavailability via sorption and/or precipitation reactions [25,75,76]. Meanwhile, biochar was additional adsorption sites and provide high binding capacity for heavy metals [64].

It was noteworthy that Cu showed an opposite trend, of which the residual fraction was decreased with the biochar amendment. The result was in good agreement with previous studies [65–67]. With the addition of biochar, the dissolved organic carbon increased rapidly, consequently accelerated the combination of Cu and organic matter, especially under high pH value. In a previous study conducted by Hartley et al. [68], higher Cu concentration was found due to the addition of biochar in greenwaste compost. Organic matter may play an important role in Cu speciation in compost, which had also been found in sludge compost [24] and soil [69].
known as well-developed porous structure and abundant surface functional groups including phenolic hydroxyls, carboxyls and carbonyls, which could promote the ion exchange of biochar surface, lower the bioavailability of heavy metals thus alleviate heavy metal pollution in environment [28,77,78]. Besides, biochar had been found to accelerate microbial activity, which also contributed to stimulate metal stabilization in compost [58,79].

As for the level of CaCl₂-extractable Cu, it demonstrated the application of biochar had an adverse effect on Cu immobilization. The extractable level was increased from 0.60 to 1.45 mg·kg⁻¹, which was similar to the result of biochar remedy soil for long time [58,80]. Other studies also observed that compost could increase the proportion of available Cu, which was due to the fact that Cu was temporarily mobilized by humic acids [81]. Results of Ippolito et al. [82] showed that Cu was in complexation with organic ligands under acidic condition, then changed to carbonate, oxide, and hydroxide mineral associations with the increase of pH, which consequently affected the bioavailability of Cu. In summary, the application of biochar can be contributable to change the speciation of heavy metals in compost, thereby lowering the bioavailability of heavy metals.

3.5 Integrated potential ecological risk assessment of heavy metals

To comprehensively evaluate the potential ecological risk of heavy metals in chicken manure compost, potential ecological risk index (RI) was used in this study. Table 5 shows the risk indices (Er) of each heavy metal in compost, ranked in the order of Cd>As>Zn>Cu>Ni>Cr. In comparison with the classification, Cd was the only one element that values for all samples were higher than 40, suggesting the Cd in compost have very high potential environmental risk. With the addition of biochar, the E value was declined, while, no obvious change with the increase of biochar appeared in this research.

With regard to RI values of each sample shown in Figure 4, it was observed that the RI value of composts varied significantly ranging from 480.51 to 740.61, followed the sequence: CK>C1> C2> C3> C4. According to the category of RI [34,83], the compost without
Table 5. Ecological risk assessment of heavy metals in the compost with the addition of biochar.

| Heavy metal | Tr | CK | C1 | C2 | C3 | C4 |
|-------------|----|----|----|----|----|----|
| Cr          | 2  | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Ni          | 5  | 0.25 | 0.18 | 0.18 | 0.09 | 0.12 |
| Cu          | 5  | 1.05 | 1.47 | 0.98 | 1.62 | 1.80 |
| Zn          | 1  | 3.38 | 3.28 | 3.31 | 3.27 | 3.17 |
| As          | 10 | 9.68 | 5.78 | 5.40 | 5.07 | 5.27 |
| Cd          | 30 | 727.23 | 539.85 | 501.89 | 491.87 | 470.20 |
| RI          | 741.61 | 550.57 | 511.76 | 501.94 | 480.57 |

*Er*: 40–40, 80–160, 160–320, and >320 denote low risk, moderate risk, considerable risk, high risk, and very high risk, respectively.

After the biochar addition, the residual fraction of Cd, Cr, Ni, Zn, and As was increased, whereas Cu showed an opposite tendency. Meanwhile, the bioavailability of Zn, Ni, Cr, and Cd was also diminished, while bioavailability of Cu was increased, and with no obvious effect on As. According to the results of risk assessment, the biochar addition reduce the potential ecological risk of heavy metals in compost, however, Cd with very high potential environmental risk should be taken more concern.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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