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Growth and Cu and Zn uptake of two forage grasses affected by application of vermicompost spiked with different metal contents

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To identify environmental risks of vermicompost-borne pollutants after consecutive application for different times, pot experiments were conducted to evaluate the effects of application of polluted vermicompost produced by Eisenia fetida on growth and Cu and Zn uptake of Mexico corn (Euchlaena mexicana) and Sudan grass (Sorghum hybrid Sudangrass) as well as soil characteristics. The results showed that application of vermicompost significantly increased shoot biomass of the two forage grasses, but decreased Mexico corn root biomass. Tissue Cu and Zn contents and soil bio-available fractions of Cu and Zn were significantly increased as the increase of application times, while soil pH and water soluble organic carbon (DOC) decreased. A significantly positive correlation was obtained between soil exchangeable and reducible fractions of Cu and Zn and forage grass tissue metal contents, whereas DOC performed a significantly negative correlation. So, application of polluted vermicompost could contribute to accumulate of soil Cu and Zn exchangeable and reducible fractions, and pose a potential threat to increase tissue Cu and Zn contents of the cultured plants.

Key words: Vermicompost, Cu and Zn, tissue uptake, biomass, fractionations.

INTRODUCTION

More sustainable waste management strategies are indispensable to cope with various environmental problems resulting from immense organic wastes. As is well known, organic wastes regularly contain large amounts of nutrient elements, and recycling of these elements is considered to be practicable for supplying plant with nutrients and improving soil physico-chemical conditions and environmental quality. Negative effects such as salt toxicity to plants, however, occurred by direct land application. Hence, proper treatments are necessary prior to their utilization. Among these treatments adopted worldwide, vermicomposting is emerging as the most appropriate alternative in that this process is rapid, easily controllable, cost effective and energy saving, as well as most efficient recycle of organics and nutrients (Eastman et al., 2001). It has been successfully adopted to convert urban, agricultural and industrial wastes ultimately into vermicompost under laboratory or field condition (Bansal and Kapoor, 2000; Garg et al., 2006; Sangwan et al., 2008; Khwairakpam and Bhargava, 2009; Suthar, 2010). Vermicompost was regarded as an alternate source of organic manure and a substitute for chemical fertilizers in organic farming not only because of the presence of abiotic aspects like readily available plant nutrients, plant growth hormones, and vitamins, but also a number of beneficial microorganisms, namely, nitrogen fixing, phosphorus solubilizing organisms (Gutiérrez-Miceli et al.,
2007; Singh and Gupta, 2010; Warman and Anglopez, 2010). Arancon et al. (2004b, 2006) pointed out that application of vermicompost significantly ameliorated field strawberry growth and yields by elevating the amount of soil NH$_4$-N, NO$_3$-N, orthophosphates and dehydrogenase activity as well as microbial biomass-N. Nonetheless, vermicompost contaminated by heavy metals are ubiquitous, especially those made out of polluted pig manure derived from intensive pig farms. Cang et al. (2004) investigated the status of heavy metal pollution concerning poultry and livestock manure from intensive farms in Jiangsu province, China, and ascertained that Cu and Zn concentrations reached up to 1726.3 and 1505.6 mg/kg, respectively. Although, earthworm (Eisenia fetida) could inhabit such pig manure and accumulate heavy metals (Zhang et al., 2009; Li et al., 2010), they would egest casts enriched with high metals content over the course of vermicomposting when concentrations of metals outstripped the utmost that they could endure, thereby, rendering metals concentrations in vermicompost abnormally excessive. Additionally, the fractionation of heavy metals would be changed subsequent to digestion by earthworm, for instance, Li et al. (2009) noted that the content of Cu bound to organic matter increased from 60 to 75% after pig manure transmitted through the gut of earthworm (E. fetida). However, most researches have been focused on the choice of raw materials, bio-chemical properties alteration of substrate during vermicomposting, and the beneficial aspects of resultant vermicompost, little attention is paid to the effects of vermicompost-borne heavy metals on the environment, especially on soil-plant systems.

In recent years, along with the adjustment of flock structure and increased herbivore livestock number raised in crop areas, planting quality and effective forages have been urgent in China. Also, fertilizers are widely recommended for sustainable agricultural pro-duction in China (Yang and Li, 2000). Vermicompost has been successfully adopted as a viable alternative container media component for the horticulture industry, the production of vegetables or agriculture to restore soil fertility, but fewer literatures were available for forage grasses. Sudangrass and Mexico corn are graminaceous forage grasses in China. So, it is essential and practical to assess the growth and Cu and Zn contents of forage grasses after polluted vermicompost application for sustainable and healthy agroecosystems.

Application of polluted vermicompost may prompt adverse effects, just as Zhou et al. (2005) observed that agricultural utilization of livestock and poultry manures from intensive farms stepped up soil extractable Cu and Zn content, and ultimately increased Cu and Zn concen-trations in radish and pakchoi tissues. Zinc concentration (28.7 mg/kg) in above-ground part of radish even exceeded the Chinese Food Hygiene Standard of 20 mg/kg on a fresh weight basis after pig manure containing heavy metals was applied for four times. Lin et al. (2006) also documented that application of earthworm casts could boost soil exchangeable Cu concentration and promote Cu translocation from roots to shoots and Cu accumulation in shoots of ryegrass (Lolium multiflorum).

Therefore, the aims of this study are to: (1) assess growth and Cu and Zn uptake of two forage grasses affected by spiked vermicompost application for different times; (2) investigate the speciation of metals in the soil after application; (3) further examine the relationship between basic properties of tested soil and Cu and Zn content in forage grass tissues.

**MATERIALS AND METHODS**

**Soil and vermicompost**

A surface (0 to 20 cm) garden soil was sampled at Jianggan district, Hangzhou, Zhejiang, China. Vermicompost (VM) was produced by earthworm (E. fetida) treating pig manure, and was collected from four different greenhouses in an intensive farm located in Yuhang country, Zhejiang. The vermicompost was thoroughly mixed, stored in sealed plastic boxes and then kept in a refrigerator at 4°C. The soil and vermicompost were air-dried, ground and sieved to < 2.0 mm nylon fiber mesh before use. Selected physical and chemical properties of the tested samples were shown in Table 1. The pH of vermicompost was lower than that of the soil, while Cu and Zn contents were much higher.

As is known to all, organic matter as well as nutrients would be mineralized and consumed, whereas most of the heavy metals accumulated in soils after utilizing manures in farmlands. In order to simulate soil metal accumulation by consecutive application for different times, vermicompost was spiked with CuSO$_4$ and ZnSO$_4$ solutions for obtaining different accumulate of Cu and Zn in soil after consecutive application for different times, and the increase of Cu and Zn were 20.0 and 26.5 mg/kg for one time, respectively, according to 2% vermicompost being applied in soil for one time (w/w). Then, the spiked vermicomposts were equilibrated for 2 weeks by the cycle of dryness and wetting (the moisture content of 40%). Five treatments, namely, VM$_0$, VM$_1$, VM$_2$, VM$_3$, and VM$_4$, were adopted, where VM$_0$ (Cu 30.7, Zn 70.6 mg/kg) represented no application of vermicompost in soil (the control), VM$_1$ (Cu 57.7, Zn97.1 mg/kg) represented once application, VM$_2$ (Cu 84.7, Zn 123.6 mg/kg) represented two times application, VM$_3$ (Cu 138.7, Zn 165.1 mg/kg) represented three times application, VM$_4$ (Cu 202.7, Zn 209.8 mg/kg) represented four times application, and VM$_5$ (Cu 266.7, Zn 253.5 mg/kg) represented five times application.

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**Table 1. Selected physical and chemical properties of the tested soil and vermicompost.**

<table>
<thead>
<tr>
<th>Tested sample</th>
<th>pH (H$_2$O)</th>
<th>Organic matter (%)</th>
<th>Total N (%)</th>
<th>Total P (%)</th>
<th>Total K (%)</th>
<th>Total Cu (mg/kg)</th>
<th>Total Zn (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garden soil</td>
<td>8.0</td>
<td>0.17</td>
<td>0.04</td>
<td>0.08</td>
<td>0.32</td>
<td>30.7</td>
<td>70.6</td>
</tr>
<tr>
<td>Vermicompost</td>
<td>6.4</td>
<td>20.8</td>
<td>1.06</td>
<td>2.62</td>
<td>0.86</td>
<td>1352.2</td>
<td>1326.4</td>
</tr>
</tbody>
</table>

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*Note: The pH and organic matter content were measured using a pH meter and an organic meter, respectively. The total N, P, K, Cu, and Zn contents were determined using a Total Nitrogen meter, Total Phosphorus meter, Total Potassium meter, Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), respectively.*
178.6 mg kg$^{-1}$) represented three times application, and VM$_4$ (Cu 246.7, Zn 282.6 mg kg$^{-1}$) represented four times application, respectively.

**Experimental procedures**

Soil/vermicompost mixtures were prepared and un-amended soil was served as the control. After being mixed thoroughly, the mixtures were placed in plastic pots with 18 cm diameter and 22 cm height. Each pot was filled with 0.5 kg, and then de-ionized water was added to keep the moisture content of 70% soil water holding capacity for 2 weeks.

Seeds of Mexico corn (*Euchlaena mexicana* Schrad) and Sudan grass (*Sorghum hybrid* Sudangrass) were purchased from Xinnongfeng Agricultural Research Institute of Agriculture Technology located in Beijing, China. The seeds were sterilized in 1% potassium permanganate solution for 5 min, followed by proper washing and then soaked overnight. The soaked seeds were cultured in Petri dishes until seed germination in an incubator at 25 ± 1°C. Thirty consistent seedlings were planted in each pot. Three replicates were performed for each treatment. Pots were placed in a greenhouse from July to August, 2011 in a completely randomized design, watered regularly with de-ionized water to keep the moisture content of 70% soil water holding capacity. After being thinned twice, eight seedlings of Mexico corn and fifteen seedlings of Sudan grass finally grew in each pot, respectively. Both plants were harvested after 30 days (days after the second thinning), and the soil was sampled at the same time.

**Chemical analysis**

**Analysis of Cu and Zn contents in forage grass tissues**

According to the procedures described by Wang et al. (2003), the forage grasses were harvested for the above-ground part and the root, respectively. The tissues were washed thoroughly, and then were dried in an oven at 70°C to a constant weight for dry biomass determination. After that, the dried samples were digested with concentrated HNO$_3$ and HClO$_4$, and Cu and Zn concentrations of the digestion solution were determined by Flame Atomic Absorbance Spectrometer (FAAS).

**Analysis of Cu and Zn fractions in soil**

A modified three-step sequential extraction procedures were adopted to determine the fractionation of Cu and Zn in the soil, into exchangeable fraction, reducible fraction, oxidizable fraction, and residual fraction, where the exchangeable, reducible fraction, and oxidizable fraction were considered as the bio-available fractions in this study, and specific details of the extraction procedures were adopted according to Rauret et al. (1989, 1998). All extracts were stored in a refrigerator at about 4°C prior to analysis. Cu and Zn in extracts were determined by FAAS.

The pH of soil was measured in a 1:2.5 (w/v) fresh sample/de-ionized water mixture using a PHS-3C pH meter fitted with a glass electrode. To determine water soluble organic carbon, 20 ml of de-ionized water was added to 4 g fresh sample and was shaken for 5 h at room temperature. The mixture was centrifuged at 12000 rpm for 4 min, and then the supernatant filtered through a number 0.45 µm filter member to obtain the water extracts. Water soluble organic carbon in the extract was determined using the standard dichromate oxidation method of Bao (2005).

**Statistical analysis**

The results were presented as an average of three replicates. Statistical analysis was performed by analysis of variance (ANOVA) using the software Statistical Package for Social Sciences (SPSS) 17.0 for Windows and considering the treatment as the independent variable. The means were separated by the Tukey’s test, considering a significant level of P<0.05 throughout the study.

**RESULTS AND DISCUSSION**

**Biomass of Sudan grass and Mexico corn affected by vermicompost application**

Biomass is an effective indicator for the plant growth (Luciano et al., 2002). Table 2 shows the biomass of Mexico corn and Sudan grass affected by vermicompost application for different times. Many studies have suggested that the potting mixture partly substituted with vermicompost could improve plant growth (Arancon et al., 2004a; Singh et al., 2008). Similar results were also observed in the present study. Higher aerial biomass was recorded in VM$_4$ treatment, which were 2- and 2.4-times of those in the control for Sudan grass and Mexico corn, respectively. With increase of application times, the two forage grasses shoot biomass also increased for Mexico corn; VM$_4$ treatment is significantly higher than other

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sudan grass (mg plant$^{-1}$)</th>
<th>Mexico corn (mg plant$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Above-ground</td>
<td>Root</td>
</tr>
<tr>
<td>VM0</td>
<td>31.8 ± 1.1$^a$</td>
<td>4.7±0.2$^a$</td>
</tr>
<tr>
<td>VM1</td>
<td>43.6 ± 1.2$^{ab}$</td>
<td>5.1±0.3$^a$</td>
</tr>
<tr>
<td>VM2</td>
<td>45.4 ± 3.7$^{ab}$</td>
<td>5.6±0.2$^a$</td>
</tr>
<tr>
<td>VM3</td>
<td>61.6 ± 1.5$^{ab}$</td>
<td>5.3±1.2$^a$</td>
</tr>
<tr>
<td>VM4</td>
<td>64.6 ± 5.3$^c$</td>
<td>8.5±0.2$^a$</td>
</tr>
</tbody>
</table>

*Means within the column with different letters represent a significant difference at P<0.05 according to Tukey’s Multiple Comparisons, the same as follows.

Table 2. Biomass of Sudan grass and Mexico corn affected by vermicompost application.
Table 3. Copper and Zn contents of Sudan grass and Mexico corn tissues affected by vermicompost application.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cu content</th>
<th>Zn content</th>
<th>Cu content</th>
<th>Zn content</th>
</tr>
</thead>
<tbody>
<tr>
<td>VM0</td>
<td>17.5±1.4a</td>
<td>95.6±11.6a</td>
<td>101.1±0.2a</td>
<td>571.5±5.7a</td>
</tr>
<tr>
<td>VM1</td>
<td>21.9±0.5d</td>
<td>469.8±10.5d</td>
<td>110.6±6.8d</td>
<td>567.9±2.8a</td>
</tr>
<tr>
<td>VM2</td>
<td>22.6±4.5d</td>
<td>608.8±5.0c</td>
<td>133.4±4.8d</td>
<td>577.1±2.8a</td>
</tr>
<tr>
<td>VM3</td>
<td>27.1±3.2c</td>
<td>1170.4±10.9e</td>
<td>175.0±1.1c</td>
<td>629.0±43.4a</td>
</tr>
<tr>
<td>VM4</td>
<td>27.4±0.5c</td>
<td>1046.3±32.5e</td>
<td>180.7±0.9c</td>
<td>763.6±35.1d</td>
</tr>
</tbody>
</table>

Copper and Zn contents in Sudan grass and Mexico corn tissues affected by vermicompost application

Table 3 presented Cu and Zn content in Sudan grass and Mexico corn tissues. Copper and Zn content in the above-ground part of Sudan grass increased with increase in vermicompost application times, and it ranged from 21.9 to 27.4 µg/g/plant and from 110.6 to 180.7 µg/g/plant, respectively, implying a significant difference versus the control. The discrepancies amongst treatments were also significant, especially between the low application times (VM₁, VM₂) and the higher ones (VM₃, VM₄), which might reside in higher bioavailable fractions of Cu and Zn in soils due to higher application times (Figure 1). In the roots of Sudan grass, Cu and Zn content also increased with increase in application times and performed significant differences among the treatments. The highest Cu content was observed in VM₄ treatment (up to 1170 µg/g/plant), whereas Zn content (up to 21.9 µg/g/plant) was higher in VM₃ treatment. Analogous trends for Cu and Zn content in Mexico corn tissues were also identified. In principle, Cu had a higher affinity with soil organic matter (Díaz-Barrientos et al., 2003) and Zn was more easily accumulated in vegetable tissues than Cu (Zhou et al., 2005). In the present study, however, there was higher Cu content in the forage grass tissues under almost the same amount of Cu and Zn being applied to the soil. It may be attributed to the different plant species and manures. The aforementioned results indicated that application of polluted vermicompost could increase Cu and Zn content in the two forage grass tissues, and might eventually pose a potential transferring threat to the food chain, especially in the wake of higher application times.

Soil basic properties affected by vermicompost application

Effects on fractionation of soil Cu and Zn

Figure 1 showed the effects of vermicompost application on the fractionation of soil Cu and Zn. Compared with the control, application of vermicompost redistributed soil Cu and Zn, and significantly increased reducible fraction of Cu and exchangeable fraction of Zn, respectively, whereas the oxidizable fraction was preponderant among the studied fractions in the control. In our previous investigation pertaining to pig manure used as the feed of earthworm (E. fetida), assay of Cu...
fractionation showed that reducible fractions was the overwhelming existence (data not shown), and Li et al. (2009) also indicated that Cu was more bio-available after digestion by earthworm, which might account for the fact that reducible fraction was the main fraction in soil upon vermicompost application. However, the main existence of Zn fraction was oxidizable fraction in pig manure, and Zn was less bio-available after assimilation by earthworm (Li et al., 2009). In contrast, soil Zn exchangeable fraction was significantly lifted in this study, which may be ascribed to the soil environmental factors.

As application times increase, exchangeable, reducible, and oxidizable fractions of Cu and Zn in soils all significantly increased, and the highest values were all observed in VM4 treatment. Results obtained in this study were partly consistent with Zhou et al. (2005), who revealed that the application of polluted pig manure, chicken manure, and organic manure raised soil extractable Cu and Zn concentrations. But in contrast to our findings, there was no significant difference among the treatments with increasing of soil Cu and Zn content, which may be associated with different soils and manures.

**Effects on soil pH and water soluble organic carbon (DOC)**

Soil pH was considered as an important factor affecting the fractionation and mobility of heavy metals. Figure 2 unfolded the changes of soil pH after vermicompost application. Compared with the control, application of vermicompost significantly reduced soil pH, which were in good agreement with findings of Gutiérrez-Miceli et al. (2007) and Atiyeh et al. (2001), who set forth that addition of sheep manure vermicompost or pig manure vermicompost to soil lowered soil pH. This was due to the fact that pH value of vermicompost was lower than that of the soil (Table 1). No significant change was observed with increase in vermicompost application times. In contrast to our results, Atiyeh et al. (2001) reported that pH dwindled progressively with increase of application rates of vermicompost with pH 5.7. The stability of pH among VM treatments in this study could be accredited to
the same amount of vermicompost applied to soil, while the soils used in the study of Atiyeth et al. (2001) were combined with different proportion of vermicompost.

Water soluble organic carbon (DOC) is recognized as the direct carbon source consumed by microorganisms, and also affects the mobility, transformation, and adsorption of soil organic and inorganic matter (Davidson et al., 2003; Hartley et al., 2010). Alan et al. (2008) reported that application of compost fertilizer increased soil DOC, in which DOC was 75, 78, and 101% greater in 29 months after application of 0, 80, and 160 Mg compost/ha. However, application of vermicompost significantly reduced soil DOC in the present study as shown in Figure 3. The lowest content of DOC was observed in VM₃ treatment. The obtained phenomenon might be related with the stimulation of soil microorganism activities. Vermicompost contained a wide variety of microorganisms and its application would result in increasing quantities and diversities of soil microorganisms (Marinari et al., 2000) as well as soil Cu and Zn concentrations, which might exert stress to the soil microorganisms (Brooks, 1984). As a result, more soluble organic carbon was consumed by microorganisms so as to maintain the growth and resist the deleterious surroundings.

Additionally, correlation analysis indicated a significantly negative correlation between soil DOC and metal content in the forage grass tissues (Table 4). It implied that decreasing soil DOC is accompanied by the increase of tissue metal content after application of vermicompost. However, there was no significant difference for soil DOC among treatments when Cu and Zn concentrations in forage grass tissues significantly elevated as the increase of application times, illustrating that soil DOC might not be the main factor affecting the translocation of Cu and Zn to forage grass tissues with the increase of application times.

Relationship between tissue Cu and Zn contents and soil basic properties

The correlation of tissue metal content with soil basic properties appeared in Table 4. Cu and Zn content in the forage grass tissues significantly positively correlated with soil bio-available Cu and Zn fractions, and negatively correlated with soil DOC, respectively. There was no significant correlation between soil pH and tissue metal content except for shoot Cu and root Zn content of Mexico corn. It implied that increasing of tissue Cu and Zn content in the two forage grasses was caused by the increase of soil Cu and Zn bio-available fractions and decrease of soil DOC after vermicompost application. This corresponded with the published studies, which had demonstrated that soil extractable or bio-available heavy metals took on a positive correlation with plant tissues metal content after application of polluted manures.
**Table 4.** Correlation between the forage grass tissue metal contents and soil metal fractions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sudan grass</th>
<th></th>
<th></th>
<th></th>
<th>Mexico corn</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cu shoot</td>
<td>Cu root</td>
<td>Zn shoot</td>
<td>Cu root</td>
<td>Zn shoot</td>
<td>Zn root</td>
<td>Zn root</td>
<td>Zn root</td>
</tr>
<tr>
<td>CuEx</td>
<td>0.910**</td>
<td>0.916**</td>
<td>0.909**</td>
<td>0.729*</td>
<td>0.830**</td>
<td>0.977**</td>
<td>0.942**</td>
<td>0.954**</td>
</tr>
<tr>
<td>CuRe</td>
<td>0.888**</td>
<td>0.863**</td>
<td>0.859**</td>
<td>0.945**</td>
<td>0.881**</td>
<td>0.991**</td>
<td>0.896**</td>
<td>0.831**</td>
</tr>
<tr>
<td>CuOx</td>
<td>0.759*</td>
<td>0.726*</td>
<td>0.949**</td>
<td>0.806**</td>
<td>0.861**</td>
<td>0.852**</td>
<td>0.933**</td>
<td>0.853**</td>
</tr>
<tr>
<td>pH</td>
<td>-0.611*</td>
<td>-0.576*</td>
<td>-0.384*</td>
<td>-0.085</td>
<td>-0.784**</td>
<td>-0.570</td>
<td>-0.597</td>
<td>-0.664*</td>
</tr>
</tbody>
</table>

**and* Correlation coefficients were statistically significant at P<0.01 and P<0.05. CuEx, CuRe and CuOx represented the exchange fraction, the reducible fraction and the oxidizable fraction of Cu, respectively.

(Walker et al., 2003; Zhou et al., 2005; Padmavathamamma and Li, 2010). However, it must be pointed out that soil bio-available fractions, including exchangeable, reducible and oxidizable fraction, all increased as application time increased, but no significant difference was obtained in soil DOC among treatments, which unraveled that increment of soil bio-available metal fractions might be the main factor behind the increase of Cu and Zn content in forage grass tissues with increase in application times.

**Conclusion**

This study suggested that application improved forage grass growth, but simultaneously increased tissue Cu and Zn content in virtue of increment of soil bio-available metal fractions. It should be emphasized that pot experiments were different from field trials, and heavy metal mobility to further profiles and their leaching to groundwater after application should also be evaluated, just as Hao et al. (2008) pointed out that application of polluted poultry and livestock manures bolstered Cu and Zn concentrations in the leachate and posed a threat to the groundwater quality.

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