Effects of earthworm activity on fertility and heavy metal bioavailability in sewage sludge

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Abstract

The potential for using earthworms (*Eisenia fetida*) to improve fertility and reduce copper and cadmium availability in sewage sludge was tested by laboratory incubation experiments. Results comparing sewage sludge with and without earthworm treatment showed that earthworm activity decreased the contents of organic matter, total nitrogen, but increased the contents of available nitrogen and phosphorus and had no significant effect on the contents of total phosphorus, total potassium and available potassium. After incubation of the sewage sludge with earthworms for 60 days, the contents of Cu and Cd in the earthworms increased with the increase of additional Cu up to 250 mg kg⁻¹ and Cd up to 10 mg kg⁻¹. Bioconcentration factors (BCF) were higher than 1 only for Cd when the addition rate was lower than 5 mg kg⁻¹, which indicates that the earthworms can only accumulate Cd when the concentration of Cd is low in sewage sludge. Bioavailability of Cd and Cu was evaluated by applying sewage sludge with and without earthworm treatment to soil and then growing cabbage plants. The results showed that earthworm treatment increased the biomass of cabbage and decreased the bioaccumulation of Cd and Cu in the cabbage plants.

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1. Introduction

The organic matter and high nutrient contents of municipal sewage sludge have promoted its application to agricultural land as an organic fertilizer. However, heavy metals in domestic and industrial wastewater are concentrated in sewage sludge at high levels with the total content ranging from 0.5 to 2% on a dry weight basis (6% in extreme conditions) (Rudd, 1987). Therefore, concerns for the safe application of sewage sludge mainly focus on heavy metal contamination. Copper and cadmium often have high concentrations in sewage sludge and may affect the health of people and animals through the food chain if sludge is applied improperly. Methods for decreasing the contents and bioavailability of these elements in sewage sludge are worthy of research.

Unlike organic compounds, metals cannot be degraded (Lasat, 2000). To decrease the contents and bioavailability of

heavy metals usually requires their removal. Such an approach can be prohibitively expensive. Also, the metal removing process often employs stringent physicochemical agents, which can dramatically inhibit soil fertility with subsequent negative impacts on the ecosystem. Another method is to use chemical immobilization amendments to reduce metal bioavailability. The addition of chemical immobilization amendments may only immobilize lethal levels for restricted periods of time and introduce some other chemicals. Phytoremediation is recognized as the most commercially and environmental friendly available technology (Lasat, 2000), but up to now, only a limited number of plants have been found to have the phytoaccumulation ability and much less can be used for field phytoremediation because of low biomass production.

Earthworms are important ecological contributors to the cycling and release of detritus-bound nutrients as well as ameliorators of soil physical properties. Cooperating with microbes, earthworms can increase the decomposition of organic matter, circulation of carbon, nitrogen and phos-

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Table 1
Selected physico-chemical characteristics of the soil and sewage sludge used

Parameter	Soil	Sewage sludge	Earthworm
Organic matter (%)	1.34	30.91±0.85	
Total N (%)	0.18	2.37±0.11	
Total P (%)	0.12	0.36±0.02	
Total K (%)	1.04	1.12±0.03	
Available N (mg/kg)	85.60	2162.71±6.76	
Available P (mg/kg)	34.40	428.64±7.94	
Available K (mg/kg)	28.10	519.53±11.68	
pH	6.61	5.74±0.25	
Cd (mg/kg, DW)	0.80	1.83	3.81±0.06
Cu (mg/kg, DW)	18.55	163.75	18.66±1.54
Pb (mg/kg, DW)	4.89	26.09	34.65±1.04
Zn (mg/kg, DW)	57.16	488.10	127.51±12.49

The values are the means of three reduplicates ±S.D.

phorus, and enhance the soil fertility (Hameed et al., 1994). In addition, earthworms have the ability to accumulate heavy metals because yellow cells in the yellow tissue of earthworms can accumulate heavy metals (Fisher and Molnar, 1992). Also earthworms have the potential to change the availability of metals in soil by changing the soil characteristics or speciation distribution (Devliegher and Verstraete, 1996; Ma et al., 2002). Studies on the bioaccumulation of metals by earthworm have been extensively investigated mainly for the purpose of using earthworms as bioindicators of soil pollution or metal bioavailability and toxicology (Ireland, 1983; Morgan and Morgan, 1993; Romijn et al., 1994; Spurgeon and Hopkin, 1996). There is a lack of research to examine whether earthworms can be used to reduce the contents and bioavailability of heavy metals in sewage sludge.

The objective of this study was to verify whether earthworms could accumulate heavy metals and change the bioavailability of heavy metals in sewage sludge, and whether earthworm treatment could increase the fertility of sewage sludge.

2. Materials and methods

2.1. Earthworms, sewage sludge and soil samples

Mature (clitellate) specimens of *Eisenia fetida* were used in the experiment, which have a strong ability to endure high

metal concentrations and extreme conditions. The concentrations of heavy metals in the earthworms are shown in Table 1.

The sewage sludge used was the dewatered sewage sludge from Wuhan Wastewater Treatment Plant (containing 70%–80% of water). Sewage sludge was first composted and fermented for 1 month. Copper and Cd were added as chloride salts in aqueous solution and mixed thoroughly. The application rates for Cd and Cu are as follows: C₀: no metals addition as control; C₁: Cd 5 mg/kg, Cu 100 mg/kg; C₂: Cd 10 mg/kg, Cu 250 mg/kg; C₃: Cd 20 mg/kg, Cu 500 mg/kg. The samples were incubated for 2 weeks after addition of the metals and then approximately 5.0 kg of sewage sludge was added to each pot. The basic physico-chemical properties of the sewage sludge are listed in Table 1.

Earthworms were first rinsed with deionised water and blotted with filter paper. 150 earthworms of similar weight were added to each pot with triplicates of each treatment. Earthworms were maintained in the sewage sludge for 60 days. The moisture of sewage sludge was maintained by watering daily. After 60 days, earthworms were taken out, rinsed free of soil particles with deionised water and starved on moistened filter paper for 2 days to eliminate the organic and inorganic content of the alimentary canals. They were then quenched in liquid nitrogen and oven-dried (48 h at 70 °C), crushed and digested with a mixture of HNO₃ and H₂O₂ (Mench et al., 1994).

Soil was collected from the test field in Huazhong Agricultural University. Soil was first ground and sieved. 3 kg of soil was mixed thoroughly with 12 g of air-dried sewage sludge and added to each pot. Each treatment was carried out in triplicate. Chinese cabbages were planted in each pot and grown for 50 days. The plants were then harvested, rinsed with deionised water, oven-dried and then ground.

2.2. Analytical methods

Soil pH was measured in aqueous slurry at 1:2 of soil and water ratio. Organic matter (OM) in soil was measured by K₂Cr₂O₇ digestion (Nelson and Sommers, 1996) and in sewage sludge was estimated from the percentage loss-on-ignition during 12 h in a muffle furnace at 400 °C. Determination of total nitrogen, phosphorus, potassium and available nitrogen, phosphorus and potassium in soil and sewage sludge was determined by the methods proposed by Osen et al., 1954; Chapman and Kelly, 1930.

Fractionation of heavy metals was conducted according to the scheme proposed by Tessier et al. (1979). The fractions were specified as exchangeable, bound to carbonates, bound to iron–

Table 2
Properties and nutrients of sewage sludge with and without earthworm treatment

Measurements	C ₀	C ₀ W	C ₁	C ₁ W	C ₂	C ₂ W	C ₃	C ₃ W
pH	6.09	6.44	5.54	5.78	5.48	5.79	5.43	5.70
OM (%)	29.20	24.14	29.32	24.18	29.48	25.11	28.17	25.17
Total N (%)	2.64	2.49	2.72	2.62	2.42	2.39	2.19	2.09
Available N (mg/kg)	3690.04	4083.26	3234.53	3739.33	3299.76	3537.75	3399.04	3583.40
Total P (%)	0.38	0.36	0.39	0.39	0.53	0.52	0.55	0.54
Available P (mg/kg)	477.53	494.13	473.56	491.91	478.01	488.59	461.96	469.55
Total K (%)	1.01	1.01	1.17	1.07	1.22	1.15	1.07	1.03
Available K (mg/kg)	521.29	461.60	485.59	481.47	491.02	489.64	482.67	468.63

Fig. 1. Contents of Cu and Cd in earthworms after incubation with sewage sludge (mg/kg, DW).

manganese oxides, and bound to organic matter and residual fractions.

Digestion of soil and sewage sludge was by $\text{H}_2\text{SO}_4\text{--HClO}_4$ mixture and for the cabbages digestion by HNO_3 and H_2O_2 was used. Digestion solutions were analyzed for Cu and Cd by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

3. Results and discussion

3.1. Changes of nutrition characteristics of sewage sludge as affected by earthworm treatment

Earthworms had a significant impact on the properties of sewage sludge as shown in Table 2. Comparing the sewage sludge treated with and without earthworms, the pH values were about 0.3 units higher for all application rates of heavy

metals in the presence of earthworm activity. It has been reported (Tisdall et al., 1993) that earthworms tend to neutralize soil pH. Earthworms have three pairs of calciferous glands, situated near the gizzard, which constantly add lime carbonate to all food inputs before digestion. This phenomenon produces castings that are always closer to neutral than the original soil. The contents of organic matter in sewage sludge treated with earthworms were less than that in sewage sludge without earthworm treatment. This might be that abundant microbes together with earthworms accelerated the decomposition of the organic matter in sewage sludge. The contents of total nitrogen in sewage sludge treated by earthworms were less than that untreated by earthworms. However, an increase in available nitrogen was observed after 60 days of incubation and the maximum increase was 89%. This might relate to the decomposition of organic matter and the change in total nitrogen in sewage sludge. The contents of available nitrogen in sewage sludge treated by earthworms increased to a much greater extent than that in the sewage sludge without earthworms. This showed that synergism of earthworms and microbes increased the decomposition of organic matter in sewage sludge, accelerated the change of organic nitrogen to available nitrogen, and increased the fertility of sewage sludge. The contents of total phosphorus in sewage sludge had no significant differences between the treatment with and without earthworms. Higher contents of the total phosphorus concentrations in sewage sludge with heavy metal application higher than 10 mg/kg Cd and 250 mg/kg Cu during incubation could be resulted from the analytical variation affected by heavy metal salt interferences. The contents of available phosphorus in sewage sludge however increased during incubation and the contents of available phosphorus in sewage sludge treated by earthworms were significantly higher than that in sludge without earthworms. Total K contents in sewage sludge showed no change during incubation and there was no difference between the treatments with and without earthworms. Also the difference of the available K contents in sewage sludge between the treatments with and without earthworms was not significant different.

Beneficial roles of earthworms on soil fertility and nutrient cycling have been commonly observed (Willems et al., 1996). However, no studies of the effects of earthworm treatment on the properties and fertility of sewage sludge have been reported. From the above results, we can see that the treatment of sewage sludge by earthworm has significant effects on the nutrition characteristics and especially on nutrient availability.

Table 3

Fractionation of Cd in sewage sludge as affected by earthworm activity after 60 days incubation (% DW)

	Treatment	Exc	Car	Fe–Mn	Oxi	Res
1	– Earthworm	4.04±0.25	10.53±0.24	6.01±0.46	20.58±0.45	59.13±1.25
	+ Earthworm	3.33±0.21	10.27±0.26	5.71±0.41	19.68±1.19	61.10±4.98
2	– Earthworm	7.38±0.12	30.63±0.14	7.34±0.14	31.87±1.03	22.79±0.36
	+ Earthworm	6.27±0.05	30.07±0.62	6.48±0.12	33.84±0.33	23.38±1.47
3	– Earthworm	11.15±0.71	29.94±1.12	5.29±0.38	35.82±0.66	17.82±0.81
	+ Earthworm	10.44±0.28	31.00±0.70	4.21±0.26	36.92±0.79	17.42±0.18
4	– Earthworm	16.70±1.20	36.22±2.43	4.89±0.15	30.97±0.58	11.22±0.11
	+ Earthworm	16.05±0.62	35.57±2.35	4.32±0.25	32.71±0.43	11.34±0.35

Table 4

Fractionation of Cu in sewage sludge as affected by earthworm activity after 60 days incubation (% DW)

	Treatment	Exc	Car	Fe–Mn	Oxi	Res
1	– Earthworm	3.23±0.12	3.40±0.22	71.64±1.58	3.76±0.19	17.97±0.18
	+ Earthworm	2.96±0.15	4.34±0.23	72.03±0.96	4.03±0.32	16.63±0.52
2	– Earthworm	5.77±0.28	8.58±0.27	67.35±0.86	3.15±0.25	15.15±0.35
	+ Earthworm	5.91±0.42	9.48±0.48	64.77±0.69	3.30±0.16	16.54±0.23
3	– Earthworm	5.75±0.20	15.54±1.31	59.23±1.00	2.83±0.20	16.65±0.26
	+ Earthworm	5.52±0.19	17.44±0.78	56.52±1.33	2.85±0.24	17.66±0.18
4	– Earthworm	10.67±0.15	21.66±1.40	55.31±3.12	2.52±0.12	15.75±0.23
	+ Earthworm	10.48±1.04	28.60±1.29	53.79±1.35	2.90±0.14	16.12±1.14

3.2. Absorption of cadmium and copper by earthworms in sewage sludge

After incubation in sewage sludge for 60 days, the contents of Cu and Cd in earthworms increased with increasing Cu application up to 250 mg kg⁻¹ and with Cd up to 10 mg kg⁻¹ (Fig. 1). Decreases in Cu and Cd were observed at the highest application rates of 500 mg kg⁻¹ Cu and 20 mg kg⁻¹ Cd, which could be attributed to the high concentration of Cu being close to the lethal dose, although only a few earthworms actually died. Omitting data for the highest metal application rate, the correlation coefficients (R^2) between the contents of Cu and Cd in earthworms with contents in sewage sludge were 0.996 and 0.903, respectively.

The bioconcentration factor (BCF) was calculated as the ratio of the content of heavy metal in the earthworms to that in the sewage sludge. By using the BCF, we can confirm whether earthworms absorb or accumulate heavy metals. When a $BCF \leq 1$, it shows that earthworms can only absorb but not accumulate heavy metals; when a $BCF > 1$, it shows that earthworms can accumulate metals. The BCFs for Cu were 0.110, 0.113, 0.133 and 0.078 for the application rates of 0, 100, 250 and 500 mg kg⁻¹, respectively. The BCFs for Cd were 1.68, 1.29, 0.79 and 0.32 for the application rates of 0, 5, 15 and 20 mg kg⁻¹, respectively. The BCF gradually decreased with the increase of Cd concentration in sewage sludge. When the contents of Cd in sewage sludge were low, earthworms could accumulate Cd from sewage sludge and when the concentration of Cd was high, earthworms could only adsorb but not accumulate Cd. Mariño and Morgan (1999) indicated that Cd could be strongly accumulated by earthworms. The BCFs of Cd in this work were much lower than that obtained by F. Mariño, which could be attributed to the differences between species of earthworm used in the experiments.

3.3. Effects of earthworm treatment on fractionation of Cu and Cd in sewage sludge

Sequential extraction of heavy metals in soils is a useful technique to determine metal fractions in soils. In this work, we used the sequential extractions proposed by Tessier et al. (1979) to examine whether earthworms could affect the fractional distribution of heavy metals in sewage sludge. Both the sewage sludges with and without earthworm treatment were subjected to the sequential extraction, in which heavy metals were divided into exchangeable, bound to carbonates, bound to iron–manganese oxides, bound to organic matter and residual.

The sequential extraction results for Cu and Cd in the sewage sludge are shown in Table 3, from which we can see that the distributions of Cd and Cu in each fraction are not significantly different between the sewage sludge with and without earthworm treatment. Up to now, there has been no report on the effects of earthworm activity on the fractionation of heavy metals in sewage sludge. In addition, previous research on the effects of earthworm activity on the fractionation of heavy metals in soils is inconsistent, although in most cases, earthworm activity does appear to affect the fractionation of heavy metals in soils. For example, in an investigation of the effects of earthworm on the fractionation of Zn in three different soils, Cheng and Wang (2002) observed that the fractionation of Zn was obviously affected by earthworm activity in acid red soil but not in two other soils (Table 4).

3.4. Effect of earthworm treatment on Chinese cabbage biomass and accumulation of cadmium and copper in Chinese cabbage

Many researches have shown that sewage sludge can increase the growth of plants. In this experiment, the growth of cabbage was improved significantly by the application of sewage sludge. Such results could result from the improvement of soil properties (Sort and Alcaniz, 1996, 1998) and also the increase in available nutrients, such as N and P. The biomass of cabbage grown in the soils amended with sewage sludge treated by earthworms was higher than that without earthworm treatment. Such differences were significant ($P < 0.05$) without metal addition and at the application rate of Cd 5 mg/kg and Cu 100 mg/kg. The

Fig. 2. Biomass of cabbage as affected by sewage sludge with and without earthworm treatment (g/pot, FW).

Fig. 3. Concentration of Cu and Cd in cabbage as affected by sewage sludge with (▲) and without (■) earthworm treatment (mg/kg, DW).

differences were insignificant at higher metal concentration (Fig. 2).

Effects of earthworm activity on the uptake of Cd and Cu in cabbage are shown in Fig. 3. The contents of Cd in cabbage grown in the soil amended with sewage sludge treated by earthworms were significantly lower than that without earthworm treatment ($P < 0.05$). Although it was observed that the contents of Cu in cabbage grown in the soil amended with sewage sludge treated by earthworms decreased comparing with the results without earthworm treatment, the differences were insignificant. It is generally recognized that metal speciation is a very important factor influencing the bioavailability of metals. As mentioned above, earthworm activity did not significantly change metal fractionation in the sludge. Therefore, the change of the soil characteristics and the removal of metals by earthworms could be the main reasons for the reduction in uptake of Cd.

4. Conclusions

The results of this study showed that earthworm activity in sewage sludge could significantly improve soil physical properties and available nutrients, such as N and P. Effects of earthworm activity on the increase of biomass of cabbage and decrease the uptake of Cd by cabbage were observed. The distributions of Cd and Cu among fractions were not significantly different for the sewage sludge with and

without earthworm treatment. We therefore can conclude that earthworm treatment benefits sewage sludge by reducing the bioavailability of Cu and Cd and improving the available nutrients.

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