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# Saudi Journal of Biological Sciences

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# The effects of applying sewage sludge into Jiangxi red soil on the growth of vegetables and the migration and enrichment of Cu and Zn



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Received 14 October 2015; revised 27 October 2015; accepted 29 October 2015 Available online 2 December 2015

### **KEYWORDS**

Sewage sludge; Heavy metal; Red soil; Migration; Enrichment

Abstract Jiangxi red soil was used as the tested soil and water spinach (*Ipomoea aquatic*) and Chinese chive (Allium tuberosum) were used as the tested vegetables in this study to investigate the effects of different amounts of sewage-sludge application on the growth of vegetables and the migration and enrichment patterns of Cu and Zn in vegetables using the potted method. The results indicated that the application of sewage sludge could improve the properties of red soil and promote vegetable growth. The dry weight of water spinach and Chinese chive reached the maximal levels when treated with the amount of sewage sludge at 4% and 10%, which was  $4.38 \pm 0.82$  g and  $1.56 \pm 0.31$  g, respectively. The dry weights after the application of sewage sludge were all larger than control treatment (CK) without sludge application. With increases in the applied amount of sewage sludge, the concentrations of Cu and Zu in red soil continued to increase, and the peak value was not reached. After the two vegetables were planted, the concentrations of Cu and Zn in red soil decreased by different degrees. The degrees of decrease of Zn were generally higher than those of Cu. The enrichment coefficient of water spinach on Cu showed a trend of increase followed by a decrease and reached the peak value of  $1.04 \pm 0.38$  when the applied amount was 4%. The enrichment coefficient of Chinese chive on Cu overall showed a decreasing trend and did not reach the peak value under the treatment levels used in this experiment. The enrichment pattern of Chinese chive on Zn was not obvious, and the differences among all treatment levels were not significant (p < 0.05). However, the enrichment coefficient after the application of sewage sludge was significantly lower than that without the application of sludge.

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Peer review under responsibility of King Saud University.

#### 1. Introduction



Residual activated sludge is sewage effluent from the municipal sewage treatment plant. The current disposal of sewage sludge

http://dx.doi.org/10.1016/j.sjbs.2015.10.028

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in China mainly relies on landfills. However, with the production of a large amount of sewage sludge and the shortage of land resources, landfills are severely restricted. Land application of sewage sludge has become one of the promising disposal methods for municipal sewage sludge due to the potential to recycle organic material among soil, crops, and sewage sludge, which is conducive to the sustainable development of cities and agriculture (Mo et al., 2000). Sewage sludge contains a large amount of organic material. Application of organic material to the soil can significantly increase the ratio of stable soil aggregates (Ashraf et al., 2013a), decrease soil bulk density, and increase the cation-exchange capacity of soil. However, heavy metals in sewage sludge become the main factor that limits its agricultural application. Therefore, the migration and transformation patterns of heavy metals in sludge-soil-crops are key issues that need to be solved for scientific and rational utilization of sewage sludge in crop systems (Zhang and Fu, 2007; Li et al., 2009).

When heavy metals in sewage sludge enter the soil, different environmental effects will result. Cu and Zn are the most common heavy metals in municipal sewage sludge (Chen et al., 2003). After sewage sludge is applied to the soil, the Cu and Zn in the sewage sludge will accumulate in the soil and different parts of crops (Yan et al., 2014; Li et al., 2014). Jiangxi is the province in China that has the most extensive distribution areas of concentrated red soil. The area with red soil accounts for 64% of the total land area in the province (Wang, 1987). Jiangxi red soil has the features of strong acidity, susceptibility to stickiness and compaction, low organic content, and poor fertilizer and water retention properties (Cai and Huang, 1998). The application of sewage sludge can significantly improve the pH in the soil and increase the soil fertility. The effect is better than that of chemical fertilizers, with an equal amount of nutrients, and is equivalent to that of compound fertilizers on the market (Mo et al., 1997). Therefore, the application of sewage sludge to red soil has very good potential.

At present, there are few reports on the effects of the application of heavy metals in sewage sludge into Jiangxi red soil on soil and crops. This study aimed to use pot experiments and several treatment levels to investigate the effects of different applied amounts of sewage sludge on the growth of two types of tested vegetables and the migration and enrichment pattern of heavy metals between soil and vegetables in order to provide a reliable theoretical basis for the allocation of sewage sludge into red soil.

## 2. Material and methods

## 2.1. Tested material

Experiment soil was collected from the bare surface of acidic red soil in the campus of the Jiangxi Agricultural University (0–20 cm). The sewage sludge used in the experiment was collected from the sludge in the dehydration plant of the Qingshan Lake sewage treatment plant in Nanchang City. The moisture content of the sewage sludge was approximately 78%. The properties are shown in Table 1. The collected soil and sludge samples were spread onto a plastic film, dried, and crushed. Foreign material was sieved through a 2 mm

 Table 1
 Heavy metal concentrations in the tested sludge (unit: mg/kg, except pH).

0, 0, 1 1	/		
Item	pH	Cu	Zn
Tested sludge GB4294-84	8.05 ± 0.26	$235.6 \pm 3.12$ 250	$\begin{array}{c} 425.6 \pm 4.68 \\ 500 \end{array}$

nylon sieve and removed. The tested vegetables were water spinach (*Ipomoea aquatic*) and Chinese chive (*Allium tuberosum*). Seeds for these vegetables were purchased from a local farmer's market.

#### 2.2. Experimental methods

The sewage sludge was thoroughly mixed with soil at mass ratios (after each material was air dried) of 0% (CK), 2% (T1), 4% (T2), 6% (T3), 8% (T4), 10% (T5), 12% (T6), 14% (T7), and 16% (T8), and the mixtures were placed in plastic flower pots with diameters of 20 cm. The basic material weight of each pot was approximately 1.5 kg, and each ratio was replicated three times. Two plants of water spinach at approximately 10 cm of seedling height were planted in the above basic material. Equal amounts of Chinese chive roots were planted in the above basic material. Watering was performed each day to maintain the soil water retention rate at approximately 60%. The period of growth was 60 days. During the harvest, the parts of water spinach and Chinese chive above the ground were cut along the ground surface and washed in deionized water, and deactivation of enzymes was performed at 105 °C for 30 min. The samples were baked at 60 °C until the weight became constant. The soil at the root system was shaken off and was considered the rhizophere soil (Batool et al., 2015).

### 2.3. Measurement methods

Heavy metals in soil and sewage sludge were digested and extracted using perchloric acid-nitric acid. Heavy metals in water spinach and Chinese chive were extracted using the concentrated nitric acid digestion method. The above extraction solutions were analyzed using the inductively coupled plasma atomic emission spectroscopy (ICP-AES) method. Two parallel samples were set up for each sample. Analysis of each parallel sample was repeated twice. The experimental results were the arithmetic mean  $\pm$  the standard deviation of 4 data points.

#### 2.4. Data processing

Statistical analyses and plotting of all the obtained data were performed using SPSS19.0 and origin8.0.

## 3. Results and discussion

# 3.1. The effects of different treatments on the biomass of vegetables

Biomass is the most direct response of the normal growth of crops to external heavy metals. The changes in dry weight of water spinach and Chinese chive under different treatment levels of sewage sludge application are shown in Table 2.

With the increase of the amount of sewage sludge applied, the dry weights of water spinach and Chinese chive both showed an initial increase followed by a decrease (Table 2). In addition, under different application levels of sewage sludge, the dry weights were all higher than that in the control CK. For water spinach, the dry weight ( $4.38 \pm 0.82$  g) in the T2 (4%) treatment level was significantly higher than the weights in the other treatment levels (p < 0.05). The order of dry weights in all the treatment levels from high to low was T2 > T3, T4 > T1, T5 > T6, T7, T8 > CK. For Chinese chive, the dry weight ( $1.56 \pm 0.31$  g) in the T4 (10%) treatment level was significantly higher than that in the other treatment levels (p < 0.05). The order of dry weights in all treatment levels from high to low was T5 > T4 > T3 > T6, T7 > T2, T8 > T1 > CK.

These results indicated that the application of appropriate amounts of sewage sludge to red soil could improve the red soil properties, promote crop growth, and increase crop biomass. However, the application of an overly high amount of sludge would inhibit the growth of crops, as concluded in related studies (Li et al., 1998; Yu et al., 2011). Because sewage sludge contained rich nutrient elements, it could significantly increase the nutrient content of soil when applied into the soil (Wang et al., 2002). However, when the concentration of heavy metals in sewage sludge exceeds the growth limit of crops, the growth and development of crops would be inhibited to different degrees (Ashraf et al., 2013b). Studies also showed that when the percentage of applied sewage sludge reached 14%, water spinach and Chinese chive at the late stage of experiments (after approximately 40 d) had symptoms of slow growth and leaf chlorosis in plants. However, biomass was not significantly decreased. The highest treatment level in the present experimental design was 16%. In this level, the dry weights of these two types of tested vegetables were both higher than that in the CK treatment. The pattern likely occurred because sewage sludge contained rich organic material and nutrient elements, whereas the CK control group in this experiment did not have any added nutrient element or fertilizer, and the application amount of sludge did

Table	2	Dried	weights	of	vegetable	plants	in	different
treatm	ent	levels						

Treatment levels	Dried weight of water spinach (g)	Dried weight of Chinese chives (g)
CK	$0.44 \pm 0.13e$	$0.07 \pm 0.01 f$
T1	$1.87 \pm 0.73 bc$	$0.11 \pm 0.02 ef$
T2	$4.38 \pm 0.82a$	$0.38 \pm 0.11$ de
T3	$2.66 \pm 0.13b$	$0.70 \pm 0.06c$
T4	$2.45~\pm~0.57b$	$1.25 \pm 0.20b$
T5	$1.76 \pm 0.62 bcd$	$1.56 \pm 0.31a$
T6	$1.22 \pm 0.25$ cde	$0.59 \pm 0.16$ cd
T7	$0.93 \pm 0.15$ de	$0.52 \pm 0.09$ cd
T8	$0.80 \pm 0.40e$	$0.36 \pm 0.09$ de

*Note:* Data in the table are all mean  $\pm$  standard deviation. Duncan's multiple range tests were performed for analysis. Different letters on the same column indicate significant differences (p < 0.05, n = 4) (as in other tables).

not reach the limiting value of 20–30% that is generally recognized by most scholars to be able to significantly inhibit crop growth (Dai et al., 2012; Chu et al., 2013; Balkhair and Ashraf, 2016).

# 3.2. Effects of different treatments on Cu and Zn in the soil before and after planting vegetables

Cu and Zn concentrations in the soil after the application of different ratios of sewage sludge are shown in Table 3. Cu and Zn concentrations in the soil for the two types of vegetables increased with increasing applied amounts of sludge (Table 3). The peak value was not reached under the treatment levels designed in this experiment, and the differences between the concentrations of Cu and Zn were significant (p < 0.05). Due to the difference of heavy metal concentrations in applied sludge in the tested soil for the two types of vegetables, the degree of the increase of the concentrations of Cu and Zn in the soil for the tested Chinese chive was significantly higher than that for the tested water spinach. Considering the "Environmental quality standard for soils" (GB15618-2008), when the application ratio of sludge in tested soil for water spinach was higher than 8%, Cu was over the limit, whereas Zn did not exceed the limit. When the application ratio of sewage sludge in the tested soil for Chinese chive was higher than 4%, the Cu concentration was over the soil standard level; when the application ratio of sludge was greater than 12%. Zn was over the standard.

Changes in the concentrations of Cu and Zn in the soil used for the two tested vegetables in different treatment levels before planting are shown in Fig. 1.

After planting the two types of vegetables, the concentrations of Cu and Zn had different degrees of decrease (Fig. 1). In the soil for planting water spinach, the degree of decrease of the concentration of Zn was significantly higher than that of Cu. Under different treatment levels, the degrees of decrease of Cu and Zn were the largest in CK, reaching 39.86% and 47.53%, respectively. In the soil for planting Chinese chives, in treatments with less than 8% sewage sludge (T4), the degrees of decrease of Cu and Zn concentrations were similar and were between 4% and 6%. When treated with the amounts of applied sludge equal to or greater than 8% (T4), the degree of decrease of the Zn concentration began to exceed that of the Cu concentration. When the applied amount of sludge reached 16%, the degrees of decrease of Cu and Zn concentrations were 28.45% and 40.94%, respectively.

Results indicated that an increase of the applied amount of sewage sludge would increase the concentrations of Cu and Zn in red soil. After the crops were planted, heavy metals in the red soil had different degrees of absorption and accumulation functions. In different treatment levels, the degrees of reduction of Zn were generally higher than those of Cu, due to the stronger migration and plant absorption ability of Zn in the soil (Ashraf et al., 2013c; Zhou and Hu, 1999; Chen, 1996).

# 3.3. The effect of different treatments on the enrichment coefficients of Cu and Zn in vegetables

To better study the patterns of migration and transformation of Cu and Zn in vegetable-supporting soil, this study introduced the bioconcentration factor (BCF). BCF was the ratio

Table 3	Concentrations of	Cu and Zn in	the different treatment	levels, in	the soil before pl	anting.
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Treatment level	Water spinach soil (mg	g/kg)	Chinese chives soil (mg/kg)		
	Cu	Zn	Cu	Zn	
CK	$17.51 \pm 0.43 f$	$33.83 \pm 1.07 g$	$21.40 \pm 1.32e$	$39.26 \pm 2.07 f$	
T1	$23.97 \pm 5.05 f$	$46.03 \pm 6.32f$	$38.45 \pm 3.77d$	$59.00 \pm 6.14e$	
T2	$36.16 \pm 1.46e$	$56.02 \pm 3.90e$	$49.01 \pm 1.90d$	$69.47 \pm 5.24e$	
T3	$41.10 \pm 2.72 de$	$62.29 \pm 3.52$ de	$63.87 \pm 5.45c$	$92.16 \pm 4.98d$	
T4	$44.66 \pm 5.41$ cd	$63.25 \pm 2.28$ cd	$71.73 \pm 3.30c$	$104.39 \pm 6.92cd$	
T5	$51.15 \pm 6.87c$	$66.82 \pm 4.15$ cd	$72.38 \pm 4.10c$	$115.05 \pm 8.01c$	
T6	$65.12 \pm 2.17b$	$70.20 \pm 6.84 bc$	$93.71 \pm 4.94b$	$144.21 \pm 5.52b$	
T7	$65.91 \pm 3.41b$	$75.60 \pm 2.84ab$	$100.50 \pm 8.54b$	$155.26 \pm 5.49b$	
Т8	$72.10 \pm 7.82a$	$81.36 \pm 7.44a$	$114.65 \pm 18.02a$	$196.46 \pm 12.69a$	
GB15618-2008	50	150	50	150	



**Figure 1** Changes in the concentrations of Cu and Zn before and after planting the two types of tested vegetables in different treatment levels. ((a, b) Changes in the concentrations of Cu and Zn before and after planting water spinach. (c, d) Changes in the concentrations of Cu and Zn before and after planting changes chive.)

of the heavy metal concentration in dry plants of vegetables to that in rhizosphere soil (Yu et al., 2011; Noor et al., 2014). A greater BCF indicates greater enrichment and migration ability of plants for heavy metals. Tables 4 and 5 show the enrichment coefficient of Cu and Zn in the two tested vegetables in different treatment levels.

With an increase of the amount of applied sewage sludge, the Cu concentration in the soil of the water spinach roots showed a continuously increasing trend and did not reach the peak value under the treatment levels designed in this experiment (Table 4). Except for T6 and T7, the other treatment levels all had significant differences (p < 0.05). The Cu

Table 4 Enrichment coefficient of Cu in different treatment levels (unit: mg/kg; the enrichment coefficient did not have dimensions).

Treatment sample	Water spinach			Chinese chives			
	Root soil	Dried plant	Enrichment coefficient	Root soil	Dried plant	Enrichment coefficient	
СК	$17.65 \pm 0.66g$	$15.24 \pm 1.79c$	$0.86\pm0.08a$	$21.99 \pm 2.39e$	$54.42 \pm 8.17a$	$2.47~\pm~0.38a$	
T1	$23.89 \pm 1.72 f$	$21.99~\pm~4.38b$	$0.93 \pm 0.18a$	$41.98 \pm 5.78d$	$21.08 \pm 1.51b$	$0.48~\pm~0.06b$	
T2	$34.44~\pm~4.38e$	$31.57~\pm~5.09a$	$1.04\pm0.38a$	$50.71 \pm 7.76$ cd	$13.11 \pm 1.18 bc$	$0.26~\pm~0.03c$	
T3	$40.64 \pm 1.12d$	$17.22 \pm 1.26 bc$	$0.42\pm0.03b$	$59.66 \pm 7.72c$	$12.79 \pm 3.05 bc$	$0.22~\pm~0.07c$	
T4	$53.44 \pm 1.37c$	$11.25 \pm 1.12c$	$0.21 \pm 0.02b$	$78.57~\pm~5.28b$	$10.81 \pm 1.42 bc$	$0.14 \pm 0.02c$	
T5	$55.82~\pm~3.53bc$	$12.36\pm0.58c$	$0.22\pm0.00b$	$77.36 \pm 5.09b$	$12.47~\pm~2.59bc$	$0.17~\pm~0.03c$	
T6	$61.27 \pm 6.37b$	$12.06 \pm 1.52c$	$0.20\pm0.04b$	$108.29 \pm 8.11a$	$17.76 \pm 11.53 bc$	$0.16~\pm~0.08c$	
T7	$61.27 \pm 6.37b$	$11.81 \pm 0.66c$	$0.20\pm0.03b$	$108.61 \pm 8.30a$	$7.53~\pm~0.12c$	$0.07~\pm~0.00c$	
<u>T8</u>	$68.53\pm0.81a$	$12.86~\pm~3.75c$	$0.19\pm0.05b$	$106.04 \pm 9.16a$	$8.65~\pm~5.69 bc$	$0.08~\pm~0.05c$	

Table 5 Enrichment coefficient of Zn in different treatment levels (unit: mg/kg; the enrichment coefficient was dimensionless).

Treatment sample	Water spinach			Chinese chives			
	Root soil	Dried plant	Enrichment coefficient	Root soil	Dried plant	Enrichment coefficient	
СК	$37.01 \pm 1.15g$	$16.73 \pm 1.71d$	$0.45 \pm 0.03 bcd$	$44.23 \pm 2.62 f$	$274.35 \pm 95.44a$	$6.25 \pm 1.98a$	
T1	$46.11 \pm 3.43f$	$17.99 \pm 7.56cd$	$0.39 \pm 0.12d$	$62.49 \pm 8.64e$	$167.16 \pm 4.31 ab$	$2.71~\pm~0.36b$	
T2	$52.72~\pm~5.73e$	$20.25 \pm 3.31c$	$0.38~\pm~0.04d$	$84.44 \pm 13.61d$	$150.31 \pm 20.10b$	$1.79 \pm 0.15 bc$	
T3	$58.88 \pm 2.91d$	$27.92 \pm 6.96cd$	$0.40 \pm 0.21d$	$82.67 \pm 11.52d$	$119.63 \pm 1.47b$	$1.47 \pm 0.22$ cd	
T4	$68.09~\pm~5.78c$	$30.08~\pm~4.15c$	$0.44~\pm~0.04cd$	$105.38 \pm 7.12c$	$136.39 \pm 26.13b$	$1.29 \pm 0.19$ cd	
T5	$68.63\pm2.87bc$	$32.51~\pm~1.53bc$	$0.47~\pm~0.04bc$	$103.54 \pm 1.14c$	$170.52 \pm 47.39 ab$	$1.64 \pm 0.36 bcd$	
T6	$70.29~\pm~3.09a$	$42.25~\pm~7.17ab$	$0.60\pm0.11ab$	$146.60 \pm 7.56 ab$	$160.85 \pm 46.17b$	$1.09 \pm 0.23$ cd	
T7	$67.04 \pm 2.54b$	$48.47~\pm~3.93a$	$0.72 \pm 0.07a$	$161.75 \pm 20.04a$	$86.16 \pm 12.82b$	$0.54 \pm 0.13d$	
T8	$69.80~\pm~1.94a$	$50.88~\pm~4.32cd$	$0.73\pm0.06a$	$143.20\pm12.96b$	$182.41\ \pm\ 54.94ab$	$1.29~\pm~0.38cd$	

concentration in dry plants of water spinach showed a trend of an initial increase followed by a decrease. In all treatment levels, the difference between CK and 7 treatments (T3-T8) was not significant (p < 0.05), and T1 and T2 were significantly different from other treatment levels (p < 0.05). The enrichment coefficient of water spinach on Cu overall showed a trend of initial increase followed by decrease. In all treatment levels, the coefficients for CK, T1 and T2 treatment levels were significantly higher than those of the other treatment levels (p < 0.05), and there was a trend of T2 > T1 > CK. When the amount of applied sewage sludge was greater than 4% (T2), the enrichment coefficient of water spinach on Cu significantly decreased and reached the minimal level under the highest treatment level T8 designed in the experiment. These results showed that when the amount of applied sewage sludge was higher than a certain level, the enrichment coefficient of water spinach on Cu decreased. This pattern occurred because with the increase of heavy metal concentrations in the soil, the growth of plants was inhibited to a certain degree, and Cu absorption was also inhibited accordingly, consistent with the conclusion regarding Cd in Brassica campestris L. by Wu et al. (2012).

With increasing amounts of applied sewage sludge, the Cu concentration in the soil of the Chinese chive roots showed a continuously increasing trend and peaked in the T7 treatment. Among the treatment levels, the differences in the CK-T6 levels were significant, whereas the differences in the T6–T8 treatment levels were not significant (p < 0.05). After sludge application, the enrichment coefficient for CU of Chinese chives in all treatment levels did not significantly differ except

for T1 (p < 0.05), and the enrichment coefficient was lower than that in the CK treatment level without sludge application. These results indicated that after sludge application to the soil, the Cu-enrichment coefficient of Chinese chives was substantially lower than that without sludge application. It was possible that the sewage sludge contained a large amount of organic material that had chelating fixation and accumulation functions on heavy metals in rhizosphere soil and inhibited their transport into plants, similar to the organic substances secreted by the root system of crops (Wang et al., 2010; Gharibreza et al., 2013). However, the specific mechanism requires further study.

With increasing amounts of applied sewage sludge, the Zn concentration in the soil of the water spinach and Chinese chive roots gradually increased (Table 5). The Zn concentration in water spinach soil did not reach the peak value, whereas the concentration in Chinese chive soil reached the peak value (T7 levels). With increasing amounts of applied sludge, the Zn concentration in dry plants of water spinach showed a gradually increasing trend, and the difference between two adjacent treatment levels was not significant (p < 0.05). After sewage sludge was applied to Chinese chive soil, the pattern of enrichment of Zn by Chinese chive was not obvious, and the differences among all treatment levels were not significant (p < 0.05). However, the enrichment coefficient after sludge application was significantly lower than that without sludge application.

Comparing Tables 4 and 5, when the amount of applied sludge was less than 6% (T3), the enrichment coefficient of Cu was higher than that of Zn in water spinach. However,

when the amount of applied sludge further increased, the enrichment coefficient of Zn began to exceed that of Cu in water spinach. Among all treatment levels designed in this experiment, the enrichment coefficients of Cu were all lower than those of Zn in Chinese chives. These results indicated that Zn had a stronger migration and plant-absorption ability than Cu in the soil, corroborating the conclusions of Zhou and Hu (1999).

### 4. Conclusion

- (1) Application of appropriate amounts of sewage sludge to red soil was conducive to the growth of crops. Maximal biomass was achieved when the application amounts of sewage sludge to the soil for water spinach and Chinese chive were 4% and 10%, respectively. With greater percentages, the biomass gradually decreased. However, during the range of treatment levels designed in this experiment, the growth of water spinach and Chinese chive after the application of sludge was better than that in the control.
- (2) Concentrations of Cu and Zn in red soil treated with sewage sludge showed a continuously increasing trend with increasing amounts of sewage sludge and did not reach the peak value under the treatment levels designed in this experiment. After water spinach and Chinese chive were planted, the concentrations of Cu and Zn in the soil decreased in different degrees. In the soil for water spinach, the degree of reduction of the concentration of Zn was significantly higher than that of Cu. In the soil for planting Chinese chive, when the amount of applied sewage sludge was lower than 8%, the degrees of reduction of the concentrations of Cu and Zn were similar. When the amount of applied sewage sludge was higher than 8%, the degree of reduction of the concentration of Zn began to exceed that of Cu.
- (3) With increasing amounts of applied sewage sludge, the enrichment of Cu and Zn by the tested vegetables showed the following pattern. The enrichment coefficient of Cu by water spinach showed a trend of initial increase followed by a decrease and reached the peak value when the amount of applied sewage sludge was 4%. The enrichment coefficient of Chinese chive on Cu overall showed a decreasing trend, and the difference was not significant when the amount of applied sewage sludge was higher than 4% (p < 0.05). The enrichment coefficient of Zn of water spinach gradually increased and did not reach the peak value under the treatment levels designed in this experiment. The pattern of enrichment of Zn by Chinese chive was not obvious, and the difference among all treatment levels was not significant (p < 0.05). However, the enrichment coefficient after sewage sludge application was significantly lower than that without sludge application.

#### Acknowledgments

Natural Science Fund Project (20114BAB203022).

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