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The effect of sewage sludge application on the growth and absorption rates of Pb and As in water spinach

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Abstract: This paper investigated the effect of the application of sewage sludge on the growth rates and absorption rates of Pb and As in potted water spinach. Our results indicated that application of sewage sludge promoted vegetable growth, and the dry weight of water spinach reached a maximal value (4.38 ± 0.82 g) upon 8% sludge application. We also found that the dry weights of water spinach after treatment were all greater than those of the control systems (CK). Treatment with sludge promoted the absorption of Pb and As in water spinach, with a significant ($p < 0.05$) increase of absorbed Pb following treatment concentrations above 10%, and a peak absorption of As at 8%. Finally, we found that concentrations of Pb and As were higher in rhizosphere-attached soil than in free pot.

Keywords: sludge, heavy metal, red soil, migration, enrichment

1 Introduction

Red soil is one of the most important types of soils in south China, accounting for about 11% of the national soil area [1]. In Jiangxi, where red soil area is the most widely distributed, red soil area accounts for 64% of the province's total land area [2]. Previous data indicate that red soil in in this area exhibit strong acidity, heavy soil hardening, low organic matter content, poor fertilizer conservation and water retention capacity [3]. In recent years, red soil acidification and hardening have negatively affected the development of sustainable agriculture [4]. Urban sewage sludge contains high concentrations of organic matter required for plant growth, including nitrogen and phosphorus [5]. Its application in red soil has been shown to significantly increase the soil's aggregate stability ratio [6], reduce soil bulk density [7], improve cation exchange capacity [8], improve soil pH, and enhance soil fertility, achieving an equivalent effect as compared to commercially available compound fertilizer [9]. Accordingly, quantifying and understanding the application of sewage sludge to red soil can help achieve sustainable economic and environmental growth [10]. However, the presence of heavy metals in sewage sludge becomes a major factor limiting its application [11]. Studies have shown that excessive application of sewage sludge will inhibit the growth of crops due to heavy metal poisoning [12]; moreover, the soil applied with sewage sludge will directly interact with the human food chain, currently there is a lack of detailed data regarding the risk of the agricultural utilization of sewage sludge in China [13]. Therefore, determining the absorption and enrichment patterns of heavy metals in sewage sludge-treated crops is a key issue that requires further attention [14-17].

It is known that various environmental factors affect heavy metal entry into soil [18-19]. Pb and As are two common elements found in urban sewage sludge [20]. Following soil treatment with sewage sludge, Pb and As accumulate in different plant structures [21-22]. Presently, there is little in this field. By conducting experiments where varying concentrations of sludge is applied to individual potted plants, the effects on growth rates and absorption rates of PB and As can be determined and applied as a reliable theoretical basis for application of sewage sludge in red soil.

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2 Materials and Methods

2.1 Tested materials

Bare acidic red topsoil (0 ~ 20cm) was obtained from of from Jiangxi Agricultural University. Sludge was obtained from the dewatering workshop of Nanchang Qingshan Lake Sewage Treatment Plant. The moisture content of the sludge was 78%, with other properties shown in Table 1. Soil and sludge samples were placed on plastic film to dry, crushed and filtered for foreign bodies with 2 mm nylon mesh. Treatment subjects (water spinach) and seeds were purchased from Jiangxi Academy of Agricultural Sciences.

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

Item	pH	Pb	As
Soil	5.22 ± 0.16	23.07 ± 0.56	3.86 ± 0.15
GB15618-2008	≤5.5	50	35
	5.5~6.5	50	30
Sludge	8.05 ± 0.26	20.57 ± 3.36	20.25 ± 1.33
GB4294-84		300	75

2.2 Experimental method

Sludge and soil were mixed in accordance with a mass ratio (air-dried) of 0% (CK), 2% (T1), 4% (T2), 6% (T3), 8% (T4), 10% (T5), 12% (T6), 14% (T7), 16% (T8) Two plants with seedling emergence lengths of 10cm were placed in 20cm diameter plastic pots with 1.5 kg treated soild. Plants were watered daily with deionized water to maintain a water retention rate of 60%. After a growing period of 60 days, the above-ground plant structures were harvested along the soil surface. Enzymes were deactivated by heating for 30 minutes at 105°C after washing with deionized water, then dried at 60°C. Rhizosphere soil was also collected for analysis.

2.3 Determination method

Heavy metals were extracted from soil and sludge by perchloric acid - nitric acid digestion, and from dry plants with concentrated nitric acid digestion. ICP-AES analysis was performed on the extracts. Two parallel samples for each extract were used, and repeated twice. Results give the arithmetic mean value of the 4 data ± standard deviations.

2.4 Data processing

SPSS19.0 and origin8.0 were employed to conduct statistical analysis and graphing for the resulting data.

3 Results and Discussion

3.1 Effect of different treatments on water spinach biomass

Changes in biomass represent the most direct response of crops to the impact of heavy metals [23]. The dry mass variation of water spinach resulting from different treatment levels is shown in Fig. 1.

Fig. 1 shows that with the increase of the sludge application, the dry mass of water spinach plants tends to increase and then decrease. The dry mass (4.38 ± 0.82g) of water spinach at T4 (8%) is significantly higher than that at other concentrations ($p < 0.05$). Except at treatments T2 and T3, differences among the remaining treatment levels are significant ($p < 0.05$). a comparison of soil with and without sludge reveals that dry mass of water spinach plants at sludge treatment level (T1 ~ T7) is significantly higher than that without sludge treatment CK.

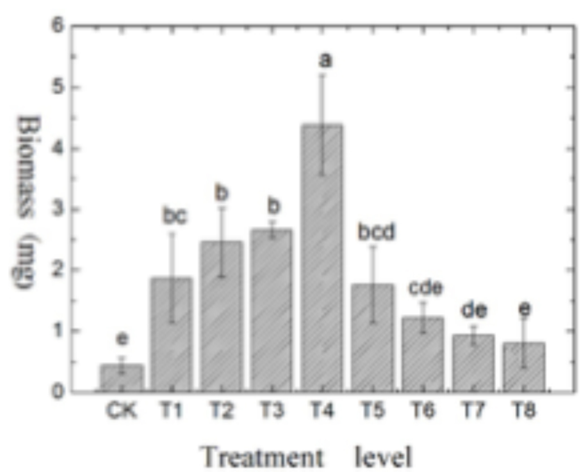

Figure 1. Biomass variation of water spinach at different sludge treatment levels. Note: Data in the table are mean ± standard deviation. Duncan’s multiple range tests were performed for analysis. Different letters on the same column indicate significant differences ($p < 0.05$, $n = 4$) (as in other tables).

Results show that appropriate application concentrations of sludge can improve red soil properties, promote growth, and improve biomass However an excessive application will inhibit growth, which is consistent with existing data [24-25]. This is mainly because sludge is rich in nutrients and can significantly improve the soil nutrient content after being applied [26-27]. However, if the concentration of heavy metals in the sludge exceeds the upper limit that can be tolerated, plant growth will exhibit different degrees of inhibition [18]. Through experimental observation, we found that when the sludge application ratio reaches 14% in the later period of experiment (about 40d later), the water spinach showed growth retardation and chlorotic leaves. The highest treatment level designed in the experiment was 16%, under which dry mass was higher than that of the CK group. This is mainly because sludge is rich in organic matter and nutrients, while in the CK control group, no nutrients or fertilizers were added. Sludge application of 20% -30% is generally agreed to be a limit that will significantly inhibit crop growth [27-28].

3.2 Content change of Pb and As in the soil applied with sludge before and after water spinach planting

In order to study the impact of water spinach planting on the absorption of Pb and As in soil, we measured Pb and As contents in original soil samples before water spinach planting, as well as in basin and root soil after planting.

3.2.1 Pb content changes in the soil applied with sludge before and after water spinach planting

Changes in soil Pb content before and after planting under different treatment levels are shown in Fig. 2. As can be seen from Fig. 2, with the increase in treatment concentration, changes of Pb content in soil before planting is insignificant, and differences among all treatment groups is ($p < 0.05$). This is mainly because Pb content (20.57 ± 3.36mg / kg) in the sludge applied was slightly lower than that (23.07 ± 0.56mg / kg) in the experimental red soil. Therefore, sludge application plays an insignificant role in changing the Pb content in the soil. After planting, the Pb content of soil shows a downward trend with the applied sludge. When the application amount of sludge accounts for less than 10% (at T5 treatment), the Pb content in soil shows an insignificant decrease, and the difference among all treatment levels is insignificant ($p < 0.05$). However, when the application amounts account for more than 10%, Pb content in soil decreases significantly while thedifference among all treatment levels was significant ($p < 0.05$). Compared with the situation before planting, the Pb content in soil after planting declines by varying degrees. The decrease in Pb contents for the former six treatments (CK ~ T5) are similar, varying from 0.20 to 0.27. Higher treatment concentrations lead to a downward trend of Pb, with a difference of 0.58 at T8. Pb content in root soil decreased with the increase of sludge. Treatment T1-T4 showed no effect, but T5-T9 showed significant changes ($p < 0.05$). This observation is consistent with the changes of Pb contents in soil observed after water spinach planting, although the Pb content of each treatment was higher than that of the soil after planting.

The above results show that sludge application in this experiment exerted little effect on Pb content in soil. The Pb contents in root soil and pot soil after planting were less than that those prior to planting, indicating that water spinach can absorb and concentrate Pb to higher levels than in the surrounding soil. Moreover, when the sludge application amount accounted was above 10%, the absorption and

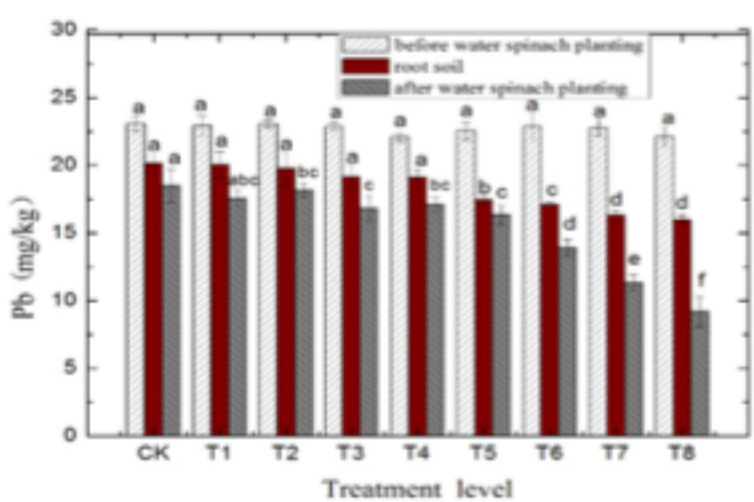

Figure 2. The Pb content in soil under different treatment levels before and after planting.

concentration capacity grew. The Pb content in applied sludge was slightly lower than that in pot soil. The applied sludge exerted no significant effect on the Pb content in soil, but did affect plants' absorption and concentration of Pb. This is very likely due to the synergistic effort of other heavy metals such as Cu and Zn in sludge, which may accelerate Pb absorption by through roots. As compared to the soil after planting, the root soil concentrated more Pb, indicating that various organic matter secreted by the plant roots play a vital role in chelating, fixing and accumulating Pb in soil. The research result coincides with the research findings [29-30].

3.2.2 Change of As content in the soil applied with sludge before and after water spinach planting

Fig. 3 shows the changes of As content in soil before and after planting and at different amounts of sludge. As can be seen from Figure 3, with an increasing treatment concentration, the As content in soil before planting shows an continuous upward trend. Except for T7, T8, the difference among the remaining treatment levels is significant ($p < 0.05$), increasing from 3.86 ± 0.15mg / kg (CK) to 15.12 ± 1.03mg / kg at T8. After planting, the As content in soil only declined at T4, while others showed an increasing trend. The differences among CK ~ T3 are insignificant ($p < 0.05$), but significant ($p < 0.05$) among T5 ~ T8 treatments. Compared with the situation before planting, As content in soil is subjected to different degrees of decline after planting. The decline at T4 was the most significant, reaching 42.7%. As contents in root soil and after-planting share the similar change rates, showing an overall upward trend with only a decline at T4 treatment. Compared with As content in plants after planting, As content in root soil was generally higher. This change coincides with that observed for Pb.

The results show that sludge application increases As content in the soil, which is consistent with previously reported data [25-26]. The As content in soil after planting and in root soil was lower than that before planting, indicating that water spinach can absorb and concentrate As, reaching a maximum at T4. This is very likely because water spinach reaches maximum biomass at T4 and growth accelerates As absorption in soil. Compared to the situation after planting, root soil contains relatively more As, similar to that observed for Pb.

3.3 Impact of different treatments on water spinach's BCFs of Pb and As

In order to better study the absorption rates observed for Pb and As in –plants and soil, this paper applies the bioconcentration factor (BCF) which is the concentration ratio of heavy metals in vegetable plants and rhizosphere soil [18]. A larger BCF indicates plants' higher absorption capacity of heavy metal [22]. Tables 2 and 3 show the water spinach's BCFs of Pb and As under different treatment levels.

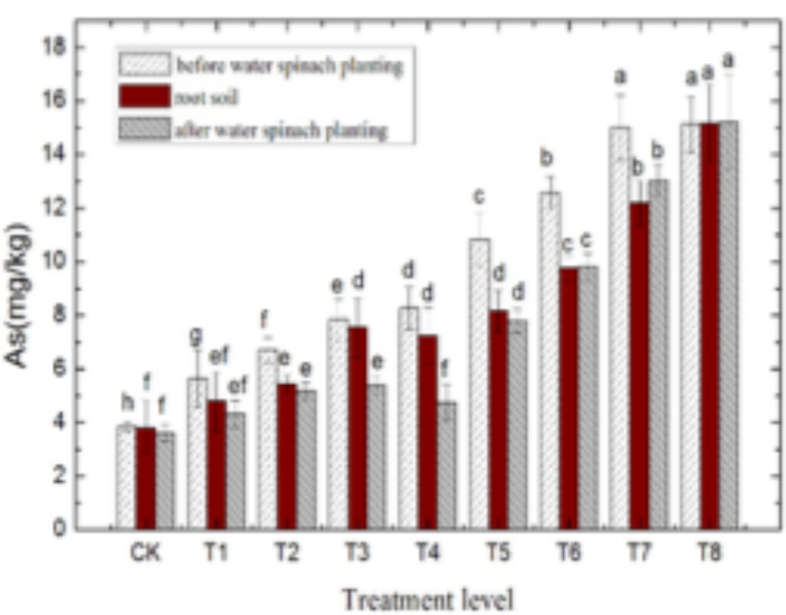

Figure 3. As content in soil before and after planting at different treatment levels.

Table 2. The BCFs of Pb in different treatment levels (unit: mg/kg; the BCFs have no dimensions).

Treatment Level	Root soil	Dry plant	BCF
CK	20.15 ± 0.63a	0.86 ± 0.11b	0.043 ± 0.005a
T1	20.03 ± 0.94a	0.27 ± 0.02c	0.013 ± 0.001c
T2	19.11 ± 0.55a	0.44 ± 0.05b	0.023 ± 0.002c
T3	19.15 ± 0.88a	0.45 ± 0.04b	0.023 ± 0.001c
T4	19.78 ± 1.21a	0.66 ± 0.02a	0.033 ± 0.001c
T5	17.44 ± 0.29b	0.13 ± 0.02d	0.007 ± 0.001e
T6	17.09 ± 0.16bc	0.25 ± 0.03c	0.015 ± 0.002d
T7	16.28 ± 0.38cd	0.14 ± 0.00d	0.009 ± 0.000e
T8	15.94 ± 0.37d	0.05 ± 0.02e	0.003 ± 0.001f

Table 3. BCFs of As at different treatment levels in water spinach (Unit: mg/kg; the BCFs have no dimensions).

Treatment Level	Root soil	Dry plant	BCF
CK	3.82 ± 1.01f	4.01 ± 0.63a	0.88 ± 0.21a
T1	4.80 ± 1.09ef	1.33 ± 0.49e	0.28 ± 0.09c
T2	5.43 ± 0.34e	1.57 ± 0.27de	0.29 ± 0.04c
T3	7.54 ± 1.11d	2.58 ± 0.64cde	0.35 ± 0.13c
T4	7.24 ± 1.044d	4.15 ± 0.56ab	0.58 ± 0.10b
T5	8.16 ± 0.77d	2.94 ± 1.43bc	0.35 ± 0.15c
T6	9.75 ± 0.47c	3.01 ± 1.24bc	0.31 ± 0.14c
T7	12.18 ± 0.83b	2.61 ± 0.72cde	0.21 ± 0.05c
T8	15.16 ± 1.46a	2.71 ± 0.69cd	0.18 ± 0.04c

As can be seen from Table 2, after application of sewage sludge, the plants' BCF of Pb increases and then decreases, reaching the inflection point at T4. With the sludge application growing from 2% to 8%, water the BCF of Pb increases from 0.013 to 0.033. Afterwards, as the sludge application amount continues to increase, BCF drops significantly. This observation coincides with change law of water spinach biomass. In terms of BCF of Pb, the control treatment CK without sludge application is higher than all treatments with sludge application, and significant differences are shown ($p < 0.05$).

As it can be seen from Table 3, following sludge application BCF of As increases and then decreases, reaching the maximum of 0.58 ± 0.10 at T4. Among all treatments, there is a significant difference ($p < 0.05$) between BCF of CK, T4 treatment and that of other treatments, while differences among other groups are insignificant. The control treatment CK shows a significantly ($p < 0.05$) larger BCF than all treatment groups.

These results indicate that after applying sludge to soil, water spinach plants present similar absorption rates of Pb and As, reaching a maximum at T4. This is mainly because as plant growth stops at T4, absorption rates decline after this point. Pb and AsBCF of Pb andAs BCFs were higher for control groups than treatment groups. Although sludge application increases water spinach's absorption capacity of Pb and As, water spinach plants' BCFs of Pb and As do not increase with increasing treament amounts. This is likely because in an environment with high concentrations of heavy metals, plants can produce phytochelatin through translocation proteins as well as concentrating complex heavy metals in vacuoles, thus limiting transportation from root to plant [31]. The specific mechanisms for this require further study.

4 Conclusions

1. Appropriate amounts of sludge application in red soil can promote growth of water spinach. Dry mass of water spinach reaches a maximum of 4.38 ± 0.82g when sludge application amount accounts for 8%. Afterwards, water spinach growth declines with

increasing sludge application amount

2. Sludge application can promote water spinach's absorption of Pb and As. With the increase of sludge application amount, water spinach's absorption rates of Pb increases significantly ($p < 0.05$) after application with 8%, while water spinach's absorption of As achieves a maximum at 8%. Compared with the soil after planting, root soil concentrates relatively more Pb and As.
3. After sludge application, water spinach plants' BCFs of Pb and As increase and then decrease, reaching a maximum of 0.033 ± 0.001 and 0.58 ± 0.10 respectively when sludge application amount accounts for 8%. Pb and As BCFs of control treatment without sludge application are higher than those of treatments with sludge application, reaching 0.043 ± 0.005 and 0.88 ± 0.21, respectively.

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Conflict of interest: The authors report no conflicts of interest and have nothing to disclose.

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