

EFFECTS OF BACTERIAL FERTILIZER ON THE GROWTH AND ABSORPTION OF CADMIUM AND LEAD IN MAIZE

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Abstract

This study involved a simulated pot experiment to examine the effects of different levels of bacterial fertilizer application on the growth of maize. As per the results stated, SJ-1 or 0.35 g/kg should be the Maximum and SJ-4 or 2.25 g/kg should be the Minimum but both data points are being referred to as Maxima. As per the results CK and SJ-1 showed no difference and SJ-3 had the lowest but both SJ-1 and SJ-3 are stated to be the lowest. The bioconcentration factor (Pb) and transfer factor (Cd and Pb) in maize were reduced the most following the 2.25 g/kg treatment. The application of the bacterial fertilizer was found to reduce the pH and the amounts of EDTA-Cd and EDTA in the soil. The amount of the bacterial fertilizer applied was found to be positively correlated with the Cd and Pb contents in the underground parts. The results suggested that the correct application of the bacterial fertilizer could promote the growth of maize.

Introduction

As shown in the survey by the Ministry of Land and Resources in China in 2014, 16.1% of the soil in China exceeds the standard point for pollution, and 7.0% and 2.1% of soil exceeds the standard points for Cd and Pb pollutants, respectively. Cd and Pb are a serious cause of pollution in China.

Gejiu is a traditional polymetallic mining area in Yunnan province, China. Heavy metal pollution in farmland soil around the mining area has become serious with the development of the mining and smelting industries, and now affects the yield and quality of local agricultural products (Qiang *et al.* 2017). Therefore, the restoration and treatment of heavy metal-contaminated farmland in this area are urgently needed.

A bacterial fertilizer is a type of microbial fertilizer, which does not directly provide nutrition for crops. However, bacterial fertilizer promotes the transfer of nutrients in the soil through microbial metabolism, improve the availability of nutrients in soil, improve plant nutrition conditions, and stimulate plant growth, thus increasing crop yield and quality (Liu *et al.* 2019, Arriola *et al.* 2015).

As the third-largest grain crop in China, maize is cultivated in many areas of western China, and the cultivation area is quite large. However, the soil in the area of maize cultivation is threatened by heavy metal pollution to varying degrees, with many areas being seriously polluted. The soils and edible crops in these areas now pose a threat to the health of local residents (Zhan *et al.* 2016).

Different varieties of maize take up heavy metals differently. This study aimed to investigate the effect of different amounts of microbial fertilizers on the growth of maize and on the absorption of the heavy metals cadmium and lead by the crop. The study had two main goals: (1) to investigate the effect of a bacterial fertilizer on the biomass of maize roots, stems, leaves, and seeds; (2) to investigate the effects of a bacterial fertilizer on maize roots, stems leaves, and seeds on the absorption of the heavy metals cadmium and lead.

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Materials and Methods

The test soil was a yellow-brown soil collected from a farmland at a depth of 0–20 cm from a tail mining area in Gejiu city, Yunnan province. The soil was dried and passed through a 2-mm sieve ready for use. The basic agrochemical properties of the soil were as follows: full nitrogen, 1.033 g/kg; full phosphorus, 0.683 g/kg; full potassium, 5.194 g/kg; alkali nitrogen, 60.90 mg/kg; quick-effect phosphorus, 16.53 mg/kg; quick-effect potassium, 49.95 mg/kg; organic matter 1.41%; pH value, 7.8; Pb, 81.7 mg/kg; and Cd, 4.56 mg/kg.

The maize variety used in the experiments was the low heavy metal-accumulating variety “red single 3”. The bacterial fertilizer used in the experiments was the “Beautiful” Biological Organic Fertilizer III, produced by Nanjing Beauty Ecological Engineering Co., Ltd, which had the following properties: N + P + K \geq 5%; organic matter \geq 50%; moisture \leq 30%; trace elements \geq 1%; and active bacteria $\geq 5 \times 10^9$ CFU/g. The fertilizer also contained urea (including N 45%), calcium superphosphate (effective P₂O₅ 16.0%), and potassium sulfate (including K₂O 50%).

The experiment was conducted in the Honghe College greenhouse in January 2018. Polyethylene plastic basins were employed. Basins were 30 × 27 cm² in size, and were filled with 10 kg dried soil per basin. The maize variety was cultivated in basins. After growing two true leaves, the plants were transplanted into the basins. Each basin was planted with two plants and given one treatment. The experiment comprised five treatments: (1) no bacterial fertilizer (CK), (2) 0.35 g/kg fertilizer (SJ-1), (3) 0.75 g/kg fertilizer (SJ-2), (4) 1.5 g/kg (SJ-3) fertilizer, and (5) 2.25 g/kg fertilizer (SJ-4). Calcium persulfate and potassium sulfate were administered as base fertilizers to all treatments equally. As for the base fertilizer, it contained urea (1.3 g/kg), superphosphate (0.8 g/kg), and potassium sulfate (0.228 g/kg). Urea was also applied equally to all treatments, but both as a base fertilizer and subsequently as a topdressing fertilizer. The treatments were arranged randomly within the greenhouse, and the experiment was repeated eight times.

Plant and soil samples were collected during the maize harvest in January 2018. All the plant samples were harvested. From each basin, the maize roots, stems, leaves, and seeds were taken from every plant, segregated into different mesh bags, and taken back to the laboratory. The samples were then washed with clean water and rinsed with deionized water. They were subsequently placed in an oven at 150°C for 15 min, after which they were dried to a constant weight at 80°C. Each plant part (leaves, roots, stems, and seeds) was then weighed. Besides, a sieve with 0.25-mm aperture was prepared for the samples to be tested.

The soil was prepared for sampling in the following way. It was spread in the same basin and mixed evenly. Then, 1 kg soil was taken. The samples were placed into self-sealing bags and labeled. In the laboratory, the soil samples were air dried naturally and then sieved through sieves with apertures of 1 mm, 0.25 mm, and 0.15 mm. The samples were ready for testing.

The Cd and Pb contents of the plant samples were determined using microwave digestion–ICP–OES (6 ml HNO₃ + 2 ml H₂O₂) on an OPTIMA 2000 spectrometer (Perkin Elmer Co. MA, USA) (Sparrow, 1996). The concentrations of EDTA–Cd and EDTA–Pb in the soil samples were determined by the diethanolamine pentacetate–triethanolamine method using ICP–OES. The amount of organic matter in the soil was determined using the K₂Cr₂O₇–H₂SO₄ external heating method. The pH value of the soil was determined in a 1:2.5 soil:water mixture using a pH acid meter. The levels of alkaline nitrogen were determined using the alkaline hydrolysis diffusion method; the levels of fast-acting phosphorus at pH = 8.5 0.5 mol/l NaHCO₃ using the molybdenum blue color method, and levels of active potassium using NH₄OAC extraction–flame photometry (Bao 2000).

Enrichment coefficient (BCF) was calculated using the following equation:

BCF = Aboveground heavy metals/heavy metals in soil.

Transfer factor (TF) was calculated using the following equation:

$$TF = \text{Aboveground partial heavy metal content} / \text{Underground partial heavy metal content}.$$

Microsoft Excel was used for data processing and drawing, and data were analyzed using the SPSS 19.0 statistical software. Differences in significance levels were determined ($p < 0.05$) using the least significance difference method (LSD), and the Pearson correlation coefficient was adopted for correlation analysis.

Results and Discussion

As shown in Fig. 1, the bacterial fertilizer can increase the biomass of roots, stems, leaves and grains in maize. In maize roots, stems, and leaves, the biomass was best treated with applied bacterial fertilizer of 0.35 g / kg, increasing by 28.3, 41.7 and 63.9% over CK, respectively. The biomass of maize kernels was highest by applying bacterial fertilizer at 1.5g / kg, which was 14.4% higher than CK. The results were consistent with the observed effects of the substitution of the biological fertilizer with nitrogen and the effects of a phosphate–potassium compound fertilizer on the production of winter wheat and summer maize and on soil fertility. The biological fertilizer could increase the number of corn kernels and yield by 1000 times in summer (Xu *et al.* 2019).

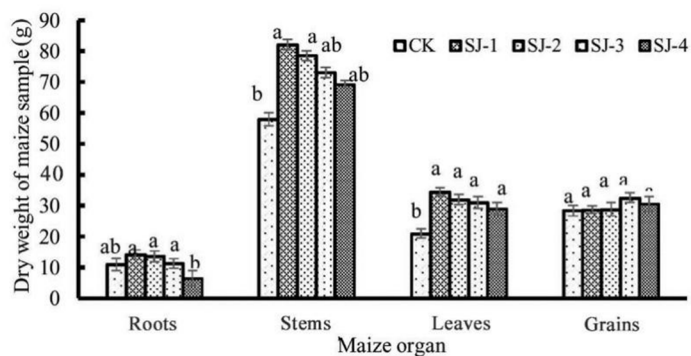


Fig. 1. Effects of bacterial fertilizer on maize growth. CK = no bacterial fertilizer; SJ-1 = 0.35 g/kg fertilizer; SJ-2 = 0.75 g/kg fertilizer; SJ-3 = 1.5 g/kg fertilizer; SJ-4 = 2.25 g/kg fertilizer. Means \pm standard errors followed by different letters within columns are significantly different ($P < 0.05$).

As can be seen from Figure 2, bacterial fertilizer can promote the absorption of Cd in maize roots, leaves and grains, with the highest content of SJ-4, SJ-1 and SJ-4 treatment, respectively, which increased by 83.0, 28.0 and 84.0% over CK, showing significant difference from CK by analysis of variance; the content of Cd in SJ-1 and SJ-2 maize stems was increased compared to CK, and SJ-3 and SJ-4 treatments were decreased compared to CK. The amount of Cd found in the various maize organs was stem > grain > leaf > root, which was consistent with the results of other studies investigating the effects of the bacterial fertilizer on different varieties of maize in Cd-contaminated soil (Liu *et al.* 2020). This indicates that bacterial fertilizer promotes Cd absorption in maize and thus reduces the soil Cd content. Application of bacterial fertilizer can promote Pb absorption in maize roots, with the highest content of SJ-4 treatment, which is increased by 2.8 times over CK, which is significantly different from CK. Except for SJ-1 treatment, maize stems and leaves were all lower than CK, with the lowest SJ-4, reducing 58.6% and 8.25% than CK, respectively. Pd in maize kernels was lower than CK, and the least treatment with SJ-3, 48.7% less than CK, reaching a significant difference. It indicates that bacterial

fertilizer can reduce Pb absorption in aboveground parts, which is consistent with the application of bacterial fertilizer on lead-contaminated soil to effectively reduce Pb content in maize (Guo *et al.* 2018). As the absorption of pb into the plant, the content showed as roots>stems>leaves (Jiang *et al.* 2002; Guo *et al.* 2018). Studies have also shown that the content of Pb in ground part is higher than underground part in some Pb-resistant plants, which shows as leaves> stems> roots. However, the content of Pb in grains is the lowest regardless of plant (Shi *et al.* 2017, Tang *et al.* 2020). In this study, the content of Pb in all maize organs showed as stem> grain> leaf> roots. After the application of bacterial fertilizer, the content of Cd in the edible grains of maize was 0.331~0.593mg/kg, and the content of Pb was 2.400~4.120mg/kg, which exceeded the limits of Cd and Pb content in the national food hygiene standard (GB2762-2017) (0.2mg / kg), which was not edible, but did not exceed the requirements of the national feed hygiene standard (GB13078-2017) (Cd 1 mg / kg, Pb 5mg / kg), and still had high feeding value. The bacterial fertilizer can promote Cd enrichment and reduce the transfer coefficient of Pb enrichment, Cd and Pb (Fig. 2). The enrichment coefficient of Cd in SJ-4 was 0.6118, while the enrichment coefficient of Pb and the transfer coefficient of Pb and Cd were lowest by SJ-4, which was 17.5, 46.5 and 78.4% lower than the control, respectively. The enrichment coefficient of bacterial fertilizer on Changyu was improved, and the optimal application amount of bacterial fertilizer was 50g / basin. It shows that the bacterial fertilizer has achieved a certain remediation effect on the soil contaminated with heavy metals.

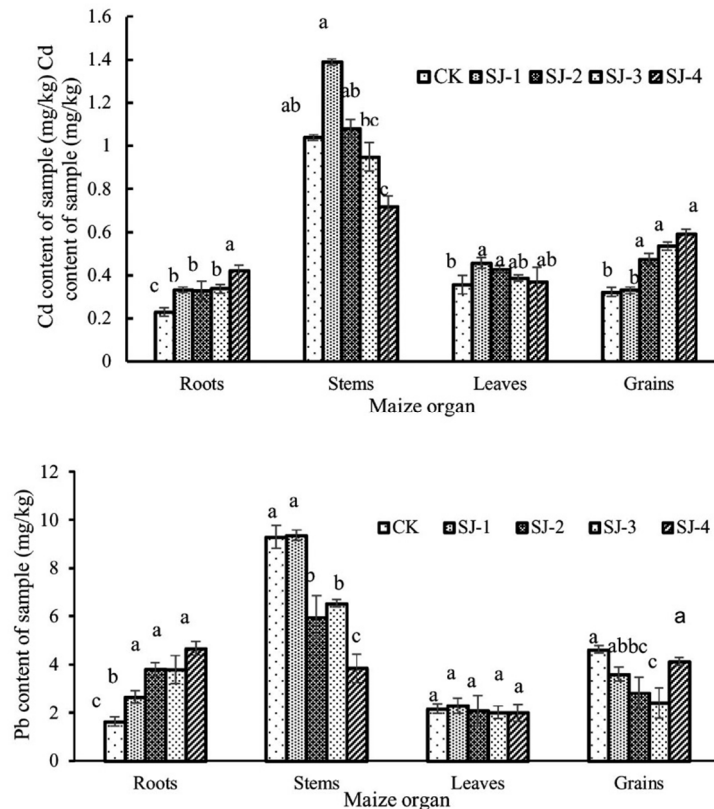


Fig. 2. Effects of bacterial fertilizer on the absorption of the heavy metals Cd and Pb by maize. CK = no bacterial fertilizer; SJ-1 = 0.35 g/kg fertilizer; SJ-2 = 0.75 g/kg fertilizer; SJ-3 = 1.5 g/kg fertilizer; SJ-4 = 2.25 g/kg fertilizer. Means \pm standard errors followed by different letters within columns are significantly different ($P < 0.05$).

As shown in Fig. 3, the soil pH value is maintained at 7.58-7.78 after the application of bacterial fertilizer, which has no significant effect on soil pH, but further study is needed. The content of effective Cd and effective Pb was the lowest with SJ-1 and SJ-4, respectively, reduced by 14.5 and 13.6%. This shows that the bacterial fertilizer reduced the proportion of strong biological activity in soil, weakened the heavy metal activity in soil, transformed part of it into the residual state with weak biological availability, and increased the proportion of soil insoluble binding state (Wang *et al.* 2019). The results suggested that the bacterial fertilizer could significantly promote the enrichment of Cd in maize tissues, but it controlled the enrichment of heavy metal Pb in maize.

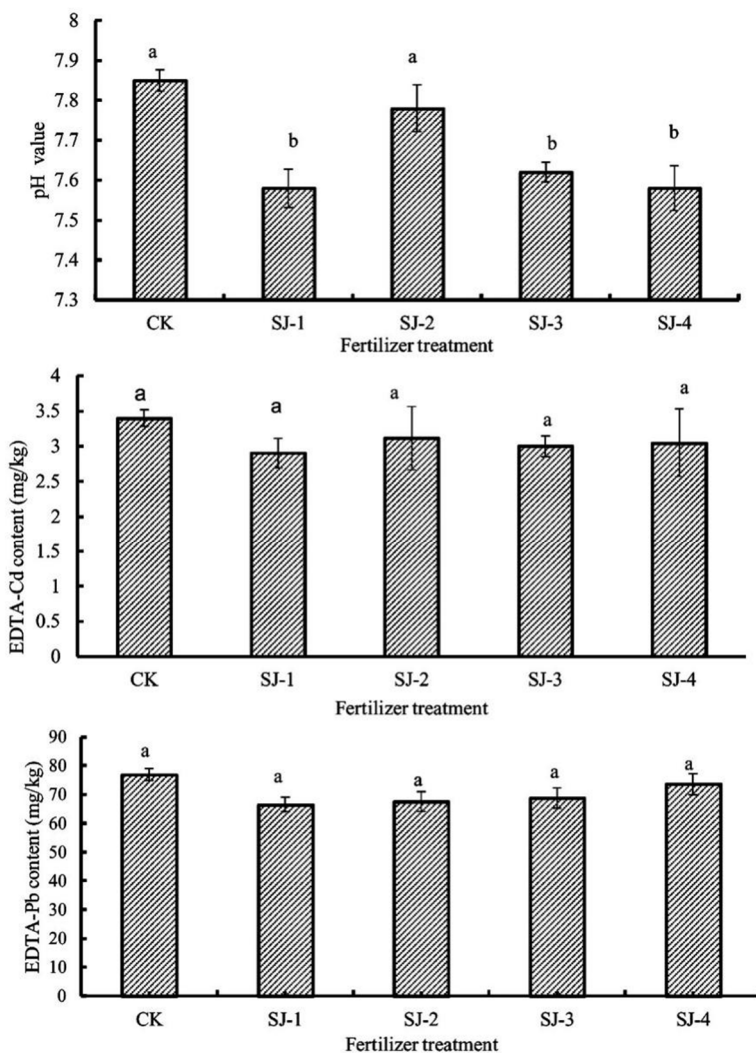


Fig. 3. Effects of bacterial fertilizer on soil pH, soil EDTA-Cd content, and soil EDTA-Pb content. CK = no bacterial fertilizer; SJ-1 = 0.35 g/kg fertilizer; SJ-2 = 0.75 g/kg fertilizer; SJ-3 = 1.5 g/kg fertilizer; SJ-4 = 2.25 g/kg fertilizer. Means \pm standard errors followed by different letters within columns are significantly different ($P < 0.05$).

As the increasing application of biological fertilizer and bacterial fertilizer in the soil, the organic matter increased significantly (Sun *et al.* 2015). According to Table 1, the application of bacterial fertilizer could increase the organic matter content in the soil with SJ-1 treatment by 1.81, 16.8% over CK, but not a significant difference ($p < 0.05$). Previous studies have shown that bacterial fertilizer can improve the effectiveness of soil nutrient elements, increase soil fertility and improve the soil microbial environment (Xu *et al.* 2018). With the increase of biological fertilizer, the content of quick nutrients in the soil of blueberry seedlings can be increased (Wang *et al.* 2020). In this study, it was found that the content of alkali nitrogen, quick phosphorus and effective potassium in soil was increased by SJ-3, SJ-4 and SJ-4, respectively, which are 15%, 3.1 times and 1.5 times higher than CK. Application of bacterial fertilizer can increase the number of bacteria in the soil (Li *et al.* 2019; He *et al.* 2017). This study also found that bacterial fertilizer increased the number of bacteria in the soil, with the highest SJ-4 treatment, increasing by 64.9% over CK. It shows that bacterial fertilizer can increase the content of organic matter, quick nutrient and the number of bacteria in soil, improve soil nutrient status, and play a certain role in fertilizer.

Table 1. Effects of bacterial fertilizer on effective nutrients and the number of bacteria in the soil.

Treatment	Organic matter (%)	Alkali-hydrolyzable nitrogen (mg/kg)	Available P (mg/kg)	Available potassium (mg/kg)	Number of bacteria (10^5 CFU/g)
CK	1.55 ± 0.016a	134.4 ± 5.692a	23.78 ± 1.369b	125.4 ± 5.691b	40.17 ± 10.2b
SJ-1	1.81 ± 0.048a	132.8 ± 4.985a	46.85 ± 4.259b	144.9 ± 3.264a	43.83 ± 8.26b
SJ-2	1.60 ± 0.058a	137.8 ± 2.598a	34.62 ± 1.498b	147.5 ± 4.597a	41.82 ± 12.69b
SJ-3	1.75 ± 0.036a	154.6 ± 2.498a	67.48 ± 2.752a	172 ± 2.234a	49.80 ± 9.24ab
SJ-4	1.74 ± 0.092a	146.6 ± 6.264a	74.83 ± 2.635a	185.6 ± 6.521a	66.25 ± 11.25a

CK = no bacterial fertilizer; SJ-1 = 0.35 g/kg fertilizer; SJ-2 = 0.75 g/kg fertilizer; SJ-3 = 1.5 g/kg fertilizer; SJ-4 = 2.25 g/kg fertilizer. Means ± standard errors followed by different letters within columns are significantly different ($P < 0.05$).

Table 2. Cd and Pb enrichment and transfer coefficients in maize following bacterial fertilizer application.

Treatment	Enrichment coefficient (BCF)		Transfer factor (TF)	
	Cd	Pb	Cd	Pb
CK	0.4274 ± 0.059a	0.2170 ± 0.017a	7.4739 ± 0.9826a	9.8773 ± 0.257a
SJ-1	0.5507 ± 0.076a	0.2191 ± 0.052a	6.5405 ± 0.267a	5.7547 ± 0.526b
SJ-2	0.5072 ± 0.032a	0.1796 ± 0.042a	6.0304 ± 0.754a	2.8605 ± 0.826c
SJ-3	0.4846 ± 0.027a	0.1804 ± 0.036a	5.5385 ± 0.523ab	2.8892 ± 0.262c
SJ-4	0.4612 ± 0.038a	0.1790 ± 0.058a	3.9952 ± 0.269b	2.1328 ± 0.498c

CK = no bacterial fertilizer; SJ-1 = 0.35 g/kg fertilizer; SJ-2 = 0.75 g/kg fertilizer; SJ-3 = 1.5 g/kg fertilizer; SJ-4 = 2.25 g/kg fertilizer. Means ± standard errors followed by different letters within columns are significantly different ($P < 0.05$).

This study adopted a pot experiment in a glasshouse to explore the influence of the bacterial fertilizer on maize growth in Gejiu, Yunnan province, China. In this area, heavy metal pollution in farmland soil around the mining area has become serious. The findings were as follows: (1) The bacterial fertilizer could promote the growth of maize roots, stems, leaves, and kernels, with the maximum increases in the biomass of maize roots, stems, and kernels at 1.5 g fertilizer/kg. (2) The Cd and Pb contents in each part of the maize plant showed as stem > kernel > leaf > root. The fertilizer application decreased BCF (Pb) and TF (Cd and Pb), with the best results at 2.25 g/kg. Meanwhile, the bacterial fertilizer was found to increase BCF (Cd), with a maximum increase seen at 2.25 g/kg. (3) The treatment with the bacterial fertilizer reduced the contents of EDTA-Cd and EDTA-Pb in the soil, as well as the soil pH, with maximum reduction seen at 0.35 g/kg.

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