Effect of Humic Acid and Bacterial Manure on Distribution of Heavy Metals in Different Organs of Maize

Tao Li, Hongyan Cheng, Kokyo Oh, and Shigeo Hosono

***Abstract*—Heavy metal contamination of soil may pose risks to human health and ecosystem environment. Phytoremediation is a low-cost and ecologically sustainable way to remediate heavy metal contaminated soils. As most of the heavy metal accumulator plants are low biomass producers, we selected maize as our experimental phytoremediation plant, which can both produce large useful biomass and remediate heavy metal contaminated soils. The effect of humic acid and bacterial manure on heavy metal accumulation in different organs of maize was studied in this paper, in order to investigate whether the fertilizer application has an effect on phytoremediation efficiency. The results showed that Cu, Pb and Zn contents in the organs of maize generally followed the order root>stem≈leaf>grain. Application of humic acid and bacterial manure improved the contents of Cu, Zn and Pb in different organs compared with those without fertilizer application. Maize with humic acid application generally had higher contents of Cu, Zn and Pb in the organs than that with bacterial manure application. This study indicated that fertilizer application was possibly one of the efficient ways to enhance the efficiency of soil phytoremediation.**

***Index Terms*—Soil contamination, heavy metal, phytoremediation, humic acid, bacterial manure, maize.**

I. Introduction

Soil pollution with heavy metals such as Cd, Cu, Zn and Pb is a universal problem because these metals are indestructible and most of them have toxic effects on living organisms. The heavy metals in environment affect human health are mainly through the food chain [1]. In Japan, the itai-itai disease was aroused in the late 1960s as the people ate the rice grown in the Cd polluted area, arousing worldwide concerns [2].

Phytoremediation is a biological technology process that utilizes the green plants or specially selected and engineered metal-accumulating plants to enhance degradation and removal of organic or inorganic contaminants in contaminated soil, groundwater or sediment [3], [4]. Phytoremediation takes advantage of the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues. Many studies have shown that phytoremediation is a low cost and eco-friendly technology compared with the conventional physicochemical methods [5], [6].

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Hyperaccumulator plants are extensively studied for application in phytoremediation. Hyperaccmulators can have heavy metal concentrations in their dry biomass that are 100 times higher than non-hyperaccumulators growing in the same soil [4]. These plants have several beneficial characteristics for phytoremediation, but they usually grow slowly and produce low biomass. For example, *thlaspi caerulescens*, one of the best-known metal hyperaccumulator, only produces 2-5 Mg ha^{-1} [7], [8]. It is possibly a approach way to use high biomass crops like maize as phytoremediation plants to overcome these shortcomings. It is well known maize (*Zea mays* L.) is one of the most widely grown cereal crop in the world that grows rapidly in a large rang of soil and clime types. It grows quickly, produces extensive root system as well as large shoot biomass and very high grain yield. At the same time, maize roots have higher enrichment of various heavy metals [9]. It is not only used as cereal foods, but also used as a feedstock for the production of bioethanol [10], [11]. In the United States, about 332 million metric tons of maize was grown annually, in which approximately 40% of them-130 million tons-is used for bioethanol [12]. Therefore, over the recent years, maize has also been consideration for utilization in phytoremediation of contaminated soil.

Humic acid widely exists in soil, lakes, rivers and oceans, and is the main component of soil organic matter. It is also a natural soil conditioner, which contains a large number of carboxyl, phenolic hydroxyl and other active groups to improve the formation of soil microbial community. It increases the utilization rate of N, P, K and trace elements to stimulate the growth of the crops and enhance the resistance of crops [13], [14]. Bacterial manure is an environmental friendly fertilizer, which has been widely used for promotion of crop production and conservation of soil quality [15], [16]. Application of humic acid and bacterial manure not only effectively improve soil physical and chemical properties, to provide a more suitable environment for plant growth, but also significantly reduce the use amount of chemical fertilizers and pesticides in soils [17]. As an important way for increasing yield in agricultural production, fertilization of humic acid and bacterial manure also can accelerate remediation of contaminated soil by heavy metals [18]. Studies have shown that in heavy metal stress, mycorrhizal fungi through secretions change the rhizosphere environment, and affect the bioavailability of heavy metals and toxicity of heavy metal to plants [19]. On the other hand, by changing the distribution of heavy metals in plants, mycorrhizal fungi can improve the tolerance of plant to heavy metals, and promote the heavy metal transfer from root to aboveground [20], [21].

There are many researches about effects of nitrogen,

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phosphorus and potassium fertilizations on plant heavy metal uptake, however, the researches about effect of humic acid and bacterial manure on absorption and distribution of heavy metals in plants are still few. Therefore we carried out a pot experiment to study the humic acid and bacterial manure on distribution of heavy metals (Pb, Cu, Zn) in different organs of maize.

II. Materials and Methods

A. Soils and Plant

The soil was collected from Shanxi Agricultural University experiment station, which was a calcareous cinnamon according to Classification and codes for Chinese soil (GB/T17296-2000) [22]. The physic-chemical properties of the soil are shown in Table I. The maize variety Changyu 16 was used in the experiment.

TABLE I: Physic-Chemical Properties of the Experimental Soil

pH	Organic matter (g kg^{-1})	Available phosphorus (mg kg^{-1})	Available nitrogen (mg kg^{-1})	Available potassium (mg kg^{-1})
7.92	3.51	60.93	2.06	126.2

B. The Modulation of Contaminated Soil

The soil contamination was made by the chemicals with addition of Pb, Cu, and Zn, and mixed thoroughly. Each pot (10 kg soil) was added 4.991g $(CH_3COO)_2Pb•3H_2O$ (Tianjin Fucheng Chemical Reagent Factory production, AR, contain not less than 99.0%,molecular weight 331.21) for Pb contaminated treatment, 2.539g $CuSO_4•5H_2O$ (Tianjin Fucheng Chemical Reagent Factory production, AR, contain not less than 99.0%,molecular weight 249.68) for Cu contaminated treatment, and 7.339g $ZnSO_4•7H_2O$ (Tianjin Fucheng Chemical Reagent Factory production, AR, contain not less than 99.5%,molecular weight 287.56) for Zn contaminated treatment, respectively. The final concentrations of the experimental soil were 350 mg kg^{-1} for Pb, 100 mg kg^{-1} for Cu and 300mg kg^{-1} for Zn.

C. Pot Culture and Treatments

The pot culture experiment was conducted in Shanxi Agricultural University (37 °12'~37 °32' N, 111 °28'~111 °111' E). Initially the seeds of the maize were grown in pots in a green house. Each pot was filled with 10 kg contaminated soil. The average temperature of green house was approximately 28 ℃ in the day time and 20 ℃ at night.

TABLE II: The Treatments in the Experiment

Pb contaminated soil	Cu contaminated soil	Zn contaminated soil
Pb (CK)	Cu (CK)	Zn (CK)
Pb+J	Cu+J	Zn+J
Pb+F	Cu+F	Zn+F

As shown in Table II, for each heavy metal the experimental treatments were CK (contaminated soil), CK + F (10 kg contaminated soil mixed with 5g humic acid), CK + J (10 kg contaminated soil mixed with 5g bacterial manure). The experiment was randomly arranged with three replicates for each treatment.

D. Heavy Metal Analysis

Each plant sample was weighted 0.5 g and put it into porcelain crucible, then placed into a muffle furnace at 525 ℃ for ashing 3 hours, and make the color to gray. After cooling, the sample was dissolved with 1:1 HNO_3, filtered with filter paper, and transfer to the 50 ml flask. Heavy metal detection was made using ICP (PE5300DV America) [23].

E. Data Analysis

Fig. 1. Pictures of the pot experiment in the greenhouse.

All experiments were conducted using a completely randomized design. Microsoft Excel 2003 and Date Processing System (DPS) were used for data analysis. Means with different letters in the same column differ significantly at $P<0.05$ (see Fig. 1).

III. Results and Disscussion

A. Distribution of Zn in Different Organs of Maiz

Zn concentrations in different parts of plants were shown in Table III. Quite different concentration was observed under Zn (300mg kg^{-1}) stress with different application of the fertilizers. The high-to-low trend of Zn content in each fertilizer was root >shoot or leaf > grain. The Zn+F treatment showed the highest Zn concentration in root, shoot and leaf, which was increased about 19.89%, 66.42%, 22.35% than Zn CK, respectively. In Zn+J treatment, Zn concentration in root, shoot, leaf and grain increased 15.14%, 62.42%, 32.51%, 32.87% than Zn CK, respectively. The Zn CK treatment showed the lowest Zn contents in root, shoot and grain.

TABLE III: Zn Concentration in Different Parts of Plants (mg kg^{-1})

	Root	Shoot	Leaf	Grain
Zn CK	509.2 b	72.49 de	126.7 cde	28.59 e
Zn+J	600.0 ab	192.9 cd	187.7 cd	42.59 e
Zn+F	635.6 a	215.9 c	241.7 c	31.79 e

Means with same letter are not significantly different at the 5% level.

The total Zn contents in maize in different treatments also showed that the Zn+F treatment was the highest content (173.5 mg kg^{-1}), while those of Zn CK was the lowest (Fig. 2).The increase rates of total Zn contents for Zn+F and Zn +J were 23.8% and 13.6% compared to that of Zn CK, respectively.

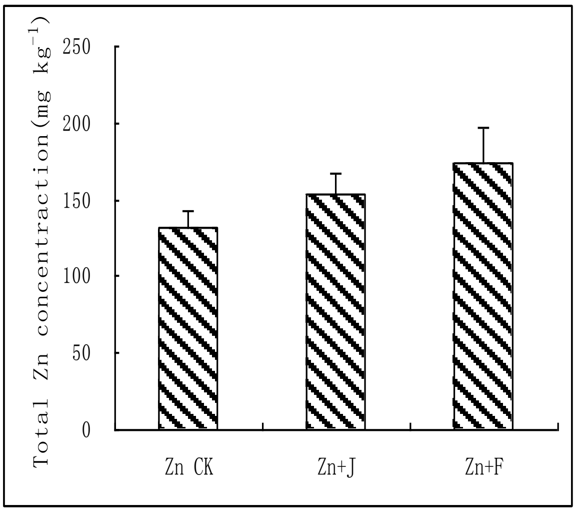

Fig. 2. Zn concentration in the whole plant under different treatments.

This is possibly caused by influenced by the increase of mobility of heavy metals in the soil with application of HA and BM, as Tian reported that mycorrhizal inoculation with application of chelating agents, can promote the remediation efficiency of maize to heavy metals [24]. Humic acid affect many aspects of plant physiological activities, including crop growth, uptake of nutrient elements, photosynthesis, respiration and activities of enzymes [17].

B. Distribution of Cu in Different Organs of Maize

Cu concentrations in different parts of plants are given in Table IV. The distribution of Cu content in different organs is root>leaf>shoot>grain. The Cu+F treatment showed the highest Cu concentration in root, shoot and leaf, which was increased 62.36%, 9.85%, 27.87% than Cu CK , respectively. The Cu+J treatment showed the highest Cu concentration in grain, which increased about 11.95% than Cu CK.

The increase rates of Cu+F and Cu+CK ranged from 0.67% to 62.4% against Cu CK. The total Cu content in different treatments also showed the same trend with Zn content (Fig. 3). A series of physical, chemical and biological reactions occur after applied fertilizer, leading to morphological change of heavy metals [25]-[28]. The reason of humic acid and bacterial manure fertilization increased the content of Cu in maize possibly due to that humic acid and bacterial manure increased mobility of soil Cu.

TABLE IV: Cu Concentration in Different Parts of Plant (mg kg^{-1})

	Root	Shoot	Leaf	Grain
Cu CK	14.07 bc	8.88 cd	11.26 cd	5.01 d
Cu+J	19.82 b	8.94 cd	10.01 cd	5.69 d
Cu+F	37.38 a	9.85 cd	15.61 bc	5.43 d

Means with same letter are not significantly different at the 5% level.

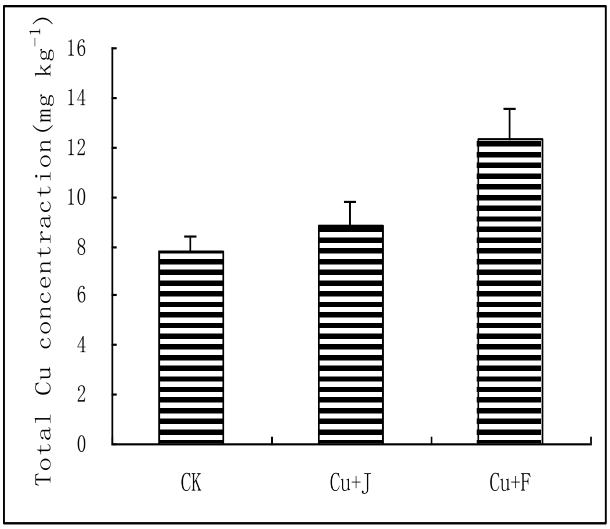

Fig. 3. Cu concentration in the whole plant under different treatments.

C. Distribution of Pb in Different Organs of Maize

The results indicated that under Pb (350mg kg^{-1}) stress, the soil with application of humic acid and bacterial manure promoted the Pb content in maize (Table V). The increase rates ranged from 0.78% to 61.96% against Pb CK. The Pb contents of different parts of maize increased compared with Pb CK. The Pb+F treatment showed the highest Pb content, and Pb CK showed the lowest Pb content. The total Pb content in different treatments also showed the same trend with that of Zn and Cu content as mentioned above (Fig. 4). The Pb+F treatment showed the highest total Pb content, which was increased 16.91% and 4.45% than Pb CK.

TABLE V: PB CONCENTRATION IN DIFFERENT PARTS OF PLANTS (MG KG^{-1})

	Root	Shoot	Leaf	Grain
Pb Ck	774.8 b	6.93 de	6.74 de	3.58 e
Pb+J	780.9 b	9.53 d	7.19 d	6.37 de
Pb+F	905.1 a	23.69 c	23.17 c	9.41 d

Means with same letter are not significantly different at the 5% level.

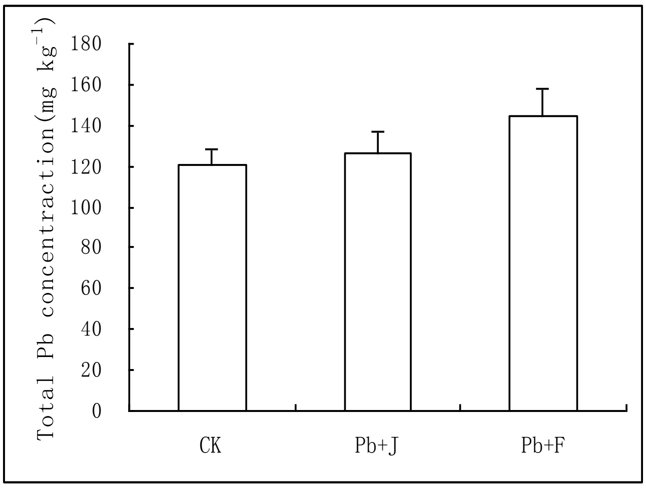

Fig. 4. Pb concentration in the whole plant under different treatments.

In summary, the application of humic acid and bacterial manure increased the concentrations of Zn, Cu and Pb in maize. This possibly due to bacterial manure and the humic acid increased the availability of Zn, Cu and Pb, thus increased their uptake and concentration in maize.

IV. CONCLUSIONS

The results showed that contents sequence of Cu, Pb and Zn in the different organs of maize was root>stem≈leaf>grain. Application of humic acid and bacterial manure improved the content of Cu, Zn and Pb in different maize organs. The humic acid application showed larger contents of Cu, Zn and Pb in different organs than bacterial manure application. The uptake to Pb was more easily promoted with application of humic acid. According to these results, the humic acid and bacterial manure application is possibly a way to enhance the efficiency of phytoremediation. Phytoremediation efficiency may be promoted through selection of fertilizer types. Our further study will focus on finding more environmental friendly fertilizers and study their suitable concentration for plant growth in contaminated soils, through which to improve the efficiency of phytoremediation.

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