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Full Length Article

Houttuynia cordata Hyperaccumulates Lead (Pb) and its Combination with *Bacillus subtilis* wb600 Improves Shoot Transportation

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Abstract

Phytoremediation can play an important role in remediating the lead (Pb)-contaminated soils. Thus, capability of the plant species to absorb and transport Pb from root to the shoot is vital for successful phytoremediation. This study focuses on exploring the potential of *Houttuynia cordata* to treat Pb in soil. The results indicate that *H. cordata* could tolerate a high concentration of Pb (up to 1000 mg kg^{-1}) and the main part of Pb accumulation is plant root. Furthermore, the inoculation of common soil bacteria *Bacillus subtilis* wb600 can facilitate Pb absorption by both root and shoot of *H. cordata*. The inoculation of *Bacillus* improves the transportation of Pb from the root to the shoot, suggesting that the combination of *H. cordata* and *Bacillus* might be used for treating Pb in the future. © 2018 Friends Science Publishers

Keywords: *Bacillus*; *Houttuynia cordata*; Pb; Hyperaccumulation; Shoot transportation

Introduction

Lead (Pb) is among the top harmful lists of heavy metals. Pb can seriously damage every organ of humans including liver and immune system and is especially toxic to the neurons. Crops as one of the most important natural resources for human survival are always contaminated by heavy metals, especially by Pb Malone *et al*. (1974). Pb accumulates in the soil which cannot be degraded and can contaminate the soil and environment in a long term. Pb as one main heavy metal coming from mining and automobile exhausts greatly impacts human health and food safety. Therefore, it is urgent to solve the problem of heavy metal pollution and many efforts should be urgently taken to solve the heavy metal contamination (Tang *et al.*, 2008; Jin *et al*., 2010; Jinhui *et al.*, 2011). The remediation of heavy metal pollution has become one of the hottest topics worldwide. For treating Pb contamination in the soil, phytoremediation is the main way (Kramer *et al*., 1997; Schwitzguebel *et al.*, 2011).

Hyper-accumulators are plants accumulating 100 times more heavy metals such as Cr, Ni, Cu and Pb than common plants. The capability of absorbing heavy metals by them is stronger than common plants and they can transport heavy metals to the above organs including shoots and leaves (Papoyan and Kochian, 2004; Kramer, 2010). Phytoremediation is making use of the plant to transform and absorb heavy metals such as cadmium and organic pollutes such as pesticide (Romeh, 2010). Another research indicated that some planta such as *M. flavida* was recognized as the candidate for treating both Cd and Zn contamination (Jamali *et al*., 2014). This way is very cheap and without second contamination (Lombi *et al*., 2001; Kramer, 2010; Schwitzguebel *et al.*, 2011). Most of the plants can be seriously damaged by Pb. Nevertheless there are still some plants which can accumulate certain internal higher concentrations of Pb and therefore have great potential for phytoremediation. However, only few plants were further explored for treating Pb by now. It was confirmed in another research that *S. alfredii* can accumulate a Pb concentration of 136.8 and 893.7 mg kg^{-1} dry weight in the shoot and root respectively in the accumulating Pb ecotype of *S. alfredii* (Luo *et al*., 2017).

The efficiency of phytoremediation is limited by the toxicity of Pb to plants, the impaired metabolism and reduced plant growth. Many soil modifiers including the chemical compounds (Cui *et al*., 2007; Liu *et al.*, 2008; Okem *et al.*, 2015) were added to improve phytoremediation. Even biowastes including tea leaves, soy cake, and potato skin were explored to improve the absorption of Pb by *Dracaena reflexa* (Dadrasnia and

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Agamuthu, 2013; Dadrasnia and Pariatamby, 2016). Former research suggested that heavy metals such as Chromium and cadmium had toxicity on common soil microbial strains (Pavel *et al.,* 2012). However, the bacterial such as *Pseudomonas* was added to improve the ability of phytoremediation such as in the degradation of naphthalene (Germaine *et al*., 2009). The interaction between soil-contained microorganisms and plants has been thought to greatly impact the development of the plants. Soil-contained microorganism is very important because they can provide nutrients to plants and the metabolites produced by them can facilitate the bioavailability of the heavy metals (Becerra-Castro *et al*., 2013). For instance, the research was carried out to explore whether the microbial community composition affect cadmium and zinc absorption by *Arabidopsis halleri* (Muehe *et al*., 2015). The combination of plant and microorganism has low cost and low environmental impact. It can not only repair contaminated soil, but also improve soil ecological environment.

Houttuynia cordata was reported to grow around the mining areas accumulating high Pb and arsenic, indicating at least that *H. cordata* can tolerate Pb. Though *H. cordata* prefers arsenic to Pb, Pb concentration is considerately high in it (Nguyen *et al*., 2011). Therefore, *H. cordata* might have the potential for phytoremediation as well. *Bacillus* is the common bacteria strain in the soil. It was reported by many papers that *Bacillus* could detoxify heavy metals by absorbing heavy metals. The study from Shameer Syed compared the capability of detoxifying heavy metals by different *Bacillus* Species isolated from Solar Salterns and showed a maximum of around 90% Pb biosorption by *Bacillus cereus* NSPA8 (Syed and Chinthala, 2015), suggesting that *Bacillus* has the potential of absorbing Pb directly and facilitates the phytoremediation. However the removal of heavy metal by microorganisms is so limited by the growth of them, which are commonly so sensitive to the environment, resulting in more efforts to improving phytoremediation of Pb. The previous study indicated that *B. megaterium* MCR-8 improve the biological stress tolerance in tomato. Furthermore, *B. megaterium* MCR-8 can induce the pytoextraction of nickel in *Vinca rosea* (Khan *et al*., 2017). Moreover, it was reported that the inoculation of *Bacillus* facilitates the uptake of Cd, Cr, and Ni by *Brassica juncea* (Ndeddy Aka and Babalola, 2016), suggesting that *Bacillus* can improve the capability of absorbing heavy metals by plants. Here we would like to explore the ability of *H. cordata* to absorb Pb and the effect of *Bacillus* on the capability of *H. cordata* to absorb the Pb. This study indicates that the combination of *H. cordata* and *Bacillus* has the potential of treating Pb.

Materials and Methods

Plant Materials and Pb Application

The young seedlings of *H. cordata* were first germinated in the soil. After the growth of ten days, the young seedlings were transferred to the prepared soil with a certain concentration of Pb. The soil was prepared with 3 parts of nutrient soil with one part of saw dust and applied with Pb $(CH3COO)_2$ to make three certain concentrations of Pb (500, 5000 and 10000 mg kg^{-1}). At each concentration of Pb, two groups of *H. cordata* was cultivated. One group has the inoculation of *Bacillus* and the other group has no *Bacillus*. The initial concentration of Pb in the mixed soil is 180 mg kg^{-1}.

Bacillus Inoculation

Bacillus subtilis WB600 was got from Si Chuan University in China. The strain was cultivated at the speed of 190 r min^{-1} at 37℃ in LB broth until OD=1. Then the bacterial cells were harvested by centrifugation at 4000 rpm for 15 min. The cells were re-suspended in sterile distilled water and adjusted to 108 CFU mL^{-1} by using spectrophotometer (OD 1.0 at 600 nm). Then *Bacillus* was applied to each pot of *H. cordata* by watering. The first inoculation of *Bacillus* was done to *H. cordata* 17 days later after the transplant from the normal soil and the second inoculation was done 31 days later after the transplant.

Measurement of Pb

Before the measurement, the whole of *H. cordata* was collected including the root was taken out from the soil and rinsed with distilled water. Then it was separated to shoot sample and root sample and then dried in drying oven at 100℃ for 24 h. Then the sample was taken out and grinded to powder. The measured powder with 0.3 g was digested in the digestion furnace with 5 mL of prepared mixture liquid (1 portion of acid and 4 portions of nitric acid). Later the digested sample was precooled to be dry and dissolved in 25 mL distilled water. Then the sample was measured by the atomic absorption spectrometer (the module is SpectrAA 220FS/220Z). The quantity of Pb was calculated by the formula W (mg kg^{-1})=(C*V)/m ("C" stands for the concentration of Pb, calculated from the standard curve according to the absorbance. "V" stands for the volume and "m" stands for the weight of the plant powders), (Eq. 1).

The soil sample was sieved and measured to 0.2–0.3 g in furnace. Five mL of 1.19g mL^{-1} HCl was added to the furnace and heated until 2–3 mL of the liquid remains. The liquid was cooled to the normal temperature, added with 5 mL nitric acid, 4 mL hydrofluoric acid and 2 mL perchloric acid and covered with the lid to be heated for around one hour until the black organic materials disappear. Then 1 mL of nitric acid was added into the furnace to dissolve the residues. Finally the liquid was added with distilled water and 3 mL of $(NH_4)_2HPO_2$ to 25 mL volume. Then the sample was measured by the atomic absorption spectrometer. The quantity of Pb was calculated by the formula W (mg kg^{-1}) =(C*V)/m*(1-f) ("C" stands for the

Table 1: Comparing of height of *H. cordata* before and after the inoculation of *Bacillus.* The height of *H. cordata* was measured different days later after being transferred to different concentrations of Pb (500 mg kg^{-1}, 5000 mg kg^{-1} and 10000 mg kg^{-1}). The soil was mixed with Pb acetate at a required Pb concentration in advance then planted with 10-day seedling of *H. cordata*. The soil was inoculated with *Bacillus* 14 days before the 30th day and 45th day after being transferred to Pb. Values represent the means and standard deviations in the bracket were obtained from six individual plants as biological replicates

Concentration of lead/	mg kg^{-1}		
Age with or without *Bacillus*	500	5000	10000
0 d (-) *Bacillus*	3.3 (±0.44) cm	3.3 (±0.24) cm	3.8 (±0.21) cm
0 d (+) *Bacillus*	3.5 (±0.21) cm	3.1 (±0.37) cm	3.7 (±0.40) cm
30 d (-) *Bacillus*	6.7 (±0.40) cm	5.4 (±0.18) cm	4.9 (±0.25) cm
30 d (+) *Bacillus*	6.5 (±0.24) cm	5.5 (±0.29) cm	5 (±0.50) cm
45 d (-) *Bacillus*	8.2 (±0.57) cm	7 (±0.33) cm	6.2 (±0.46) cm
45 d (+) *Bacillus*	8 (±0.50) cm	7.1 (±0.37) cm	6.3 (±0.50) cm

concentration of Pb, calculated from the standard curve according to the absorbance. "V" stands for the volume, "m" stands for the weight of the plant powders and "f" stands for the water content in the soil), (Eq. 2).

Statistical Analysis

The values obtained were the mean ± standard deviation for six replicates in each treatment.

Results

The Growth of *H. cordata* is Hindered by Pb However Without Influence from the Inoculation of *Bacillus*

To study the effect of Pb on the growth of *H. cordata*, different concentrations of Pb were applied to the soil planted *H. cordata*. The concentrations (500, 5000 and 10000 mg kg^{-1}) were applied to the soil based on the former measurement of Pb in soil from other researches (Wang *et al*., 2016). It was indicated by others that 300 mg kg^{-1} of Pb is already toxic to the plant. As shown in Fig. 1, *H. cordata* still can grow bigger as days go by in the soil with concentrations of Pb with 500, 5000 and 10000 mg kg^{-1}, suggesting the tolerance of Pb by *H. cordata*. Furthermore the height of *H. cordata* becomes much smaller as the concentration of Pb increases from 500 to 10000 mg kg^{-1}, suggesting that Pb can hinder the growth of *H. cordata*.

In order to investigate the influence of *Bacillus* on the growth of *H. cordata*, the length of *H. cordata* was measured and compared before and after inoculation of *Bacillus*. As shown in Table 1, there is no clear difference of the height of *H. cordata* before and after the inoculation of *Bacillus*. This suggests that *Bacillus* could not impact the growth of *H. cordata*.

H. cordata Absorbs more Pb in the Root Part than in the Shoot Part

To measure the capability of *H. cordata* to take in Pb, the shoot part and root part of *H. cordata* were collected separately to measure the internal concentration of Pb. Different concentrations of Pb (500, 5000 and 10000 mg kg^{-1}) were applied to *H. cordata* without the inoculation of *Bacillus*. It was shown that in both Fig. 2 and Fig. 3, the internal concentration of Pb in both the root part and shoot part increases as the age goes by. In Fig. 2, the internal concentrations of Pb in the shoot part are 34 and 132 mg kg^{-1}, respectively under the concentration of 500 and 5000 mg kg^{-1} at the age of 30 days. When Pb concentration increases in soil arranges from 500 to 5000 mg kg^{-1}, the internal concentration of Pb in the shoot part increases from 58.8 to 392 mg kg^{-1} as well. However there is no clear difference of Pb concentration between plants with 500 and 5000 mg kg^{-1} of Pb in the soil. When the concentration increases from 500 to 5000 and 10000 mg kg^{-1}, the internal root Pb concentration increases from 181 to 910 mg kg^{-1} and 1510 mg kg^{-1} separately at the age of 30 days (Fig. 3). Additionally, as the surrounding concentration of Pb increases from 500 to 5000 mg kg^{-1} and 10000 mg kg^{-1}, the internal Pb concentration of root increases from 218 to 1210 mg kg^{-1} and 2330 mg kg^{-1} respectively in Fig. 3. Taken together with the hindered growth of *H. cordata*, it suggests that the uptake of Pb is harmful for *H. cordata.* Moreover, the capability of taking in Pb in the shoot part of *H. cordata* is saturated with 5000 mg kg^{-1}. Thirdly, the capability of absorbing Pb is much higher in the root part than in the shoot part. When at highest Pb concentration of 10000 mg kg^{-1}, the root Pb content is more than 4 times of the shoot content. The higher accumulation of pb in the root is consistent with the former research with general higher concentrations of heavy metals in the root in the phytoremediation species (Bang *et al*., 2015).

Inoculation of *Bacillus* Facilitates Intake of Pb

To explore the effect of *Bacillus* on the capability of *H. cordata* to absorb the Pb, the internal concentration of Pb was measured in the shoot part and root part of *H. cordata* separately after inoculation of *Bacillus.*

As shown in Fig. 4, the internal concentration of Pb in the shoot part reaches a maximal value of around 100 mg kg^{-1} with *Bacillus* more than the value of 50 mg kg^{-1} without *Bacillus* (Fig. 4), when the external concentration of Pb is

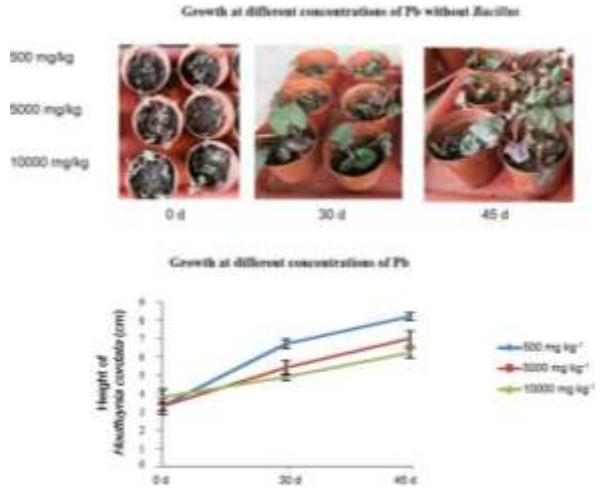

Fig. 1: Height of *H. cordata* was measured different days later after being transferred to different concentrations of Pb (500 mg kg^{-1}, 5000 mg kg^{-1} and 10000 mg kg^{-1}). The soil was mixed with Pb acetate to a required Pb concentration in advance then transplanted with ten-day seedlings of *H. cordata* from the field. Values represent the means and standard deviations were obtained from six individual plants as biological replicates

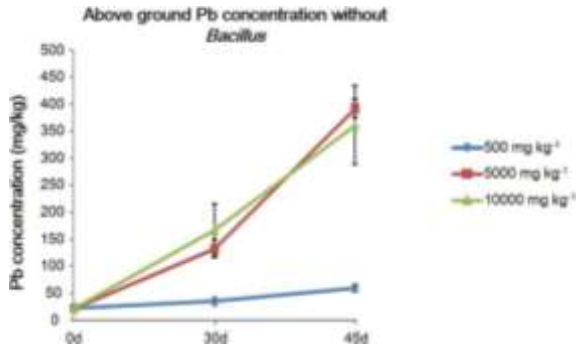

Fig. 2: The internal concentration of Pb in the above ground part of *H. cordata* was measured different days later at different concentrations of Pb (500 mg kg^{-1}, 5000 mg kg^{-1} and 10000 mg kg^{-1}). The soil was mixed with Pb acetate at a required Pb concentration in advance then planted with small seedling of *H. cordata*. The soil was without *Bacillus* additionally. Values represent the means and standard deviations were obtained from six individual plants as biological replicates

500 mg kg^{-1}. Similarly at the external concentration of 5000 mg kg^{-1}, the internal concentration of Pb with *Bacillus* riches 300 mg kg^{-1}, higher than 100 mg kg^{-1} without *Bacillus* at 30th day. At the external concentration of 10000 mg kg^{-1}, the internal concentration of Pb with *Bacillus* riches a maximal value of around 800 mg kg^{-1} (Fig. 4), obviously higher then around the maximal value of 400 mg kg^{-1} (Fig. 2) without *Bacillus*. When measuring Pb in the root part of *H. cordata*, the internal concentration of Pb with

Table 2: The ratio of concentration of Pb in *H. cordata* with *Bacillus* to without *Bacillus* in the shoot part and root part of *H. cordata* separately

ratio (+)/(-) *Bacillus*	500 mg kg^{-1}	5000 mg kg^{-1}	10000 mg kg^{-1}
30d (+)/30d (-)_shoot	1.34	2.45	3.75
45d (+)/45d (-)_shoot	1.21	1.00	2.23
30d (+)/30d (-)_root	1.19	1.24	1.14
45d (+)/45d (-)_root	1.35	1.40	1.03

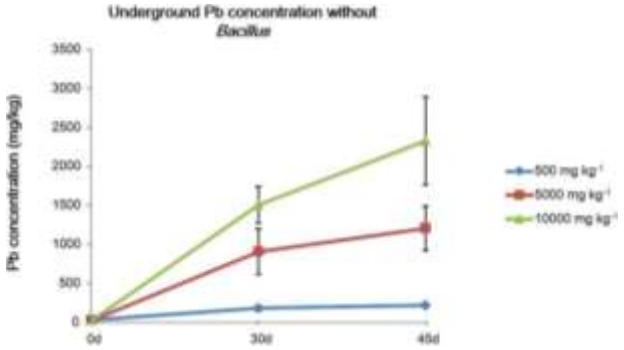

Fig. 3: The internal concentration of Pb in the underground part of *H. cordata* was measured different days later at different concentrations of Pb (500 mg kg^{-1}, 5000 mg kg^{-1} and 10000 mg kg^{-1}). The soil was mixed with Pb acetate at a required Pb concentration in advance then planted with small seedling of *H. cordata*. The soil was without *Bacillus* additionally. Values represent the means and standard deviations were obtained from six individual plants as biological replicates

the inoculation of *Bacillus* is higher than the case without *Bacillus* both 30 days and 45 days later after the transplanting at the external concentration of 5000 mg kg^{-1} (Fig. 3 and Fig. 5). At the external concentration of both 500 and 10000 mg kg^{-1} Pb, though the internal concentration of the shoot part is still higher with than without *Bacillus*, the degree of increasing is not as obvious as the root part. As shown in Table 2, the increase of Pb in the shoot part is much more than the increase of Pb in the root part. Overall, the inoculation with *Bacillus* increases the capability of *H. cordata* to absorb the Pb.

Inoculation of *Bacillus* Facilitate the Transportation of Pb from the Root to the Shoot

From the above results by us, we have shown that the inoculation of *Bacillus* differs in facilitating the Pb uptake in the shoot part and root part of *H. cordata*. To explore the influence of *Bacillus* on the transportation of Pb, the ratio of the internal Pb concentration of the shoot part to the root part was calculated. The initial ratio is 0.75 at the starting point of 0 day. After growing in Pb, the ratio decreases, suggesting that the root mainly absorbs Pb (Table 3). Interestingly all the ratios with the inoculation of *Bacillus*

Table 3: The ratio of concentration of Pb in the shoot to the root of *Houttuynia cordata*

above/below	500 mg kg^{-1}	5000 mg kg^{-1}	10000 mg kg^{-1}
0d (-)	0.75	0.75	0.75
0d (+)	0.75	0.75	0.75
30d (-)	0.19	0.15	0.11
30d (+)	0.21	0.29	0.36
45d (-)	0.27	0.32	0.15
45d (+)	0.24	0.23	0.34

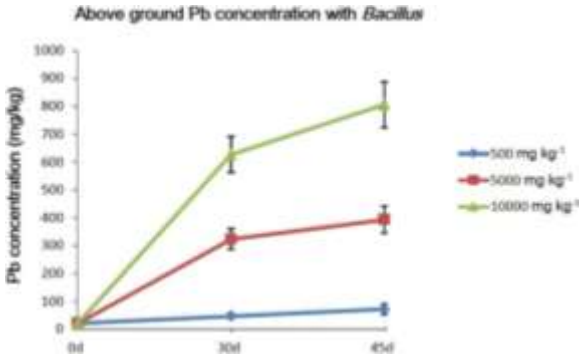

Fig. 4: The internal concentration of Pb in the shoot part of *H. cordata* was measured different days later at different concentrations of Pb (500 mg kg^{-1}, 5000 mg kg^{-1} and 10000 mg kg^{-1}). The soil was mixed with Pb acetate at a required Pb concentration in advance then planted with small seedling of *H. cordata*. The soil was inoculated with *Bacillus* 14 days before the 30th day and 45th day. Values represent the means and standard deviations were obtained from six individual plants as biological replicates

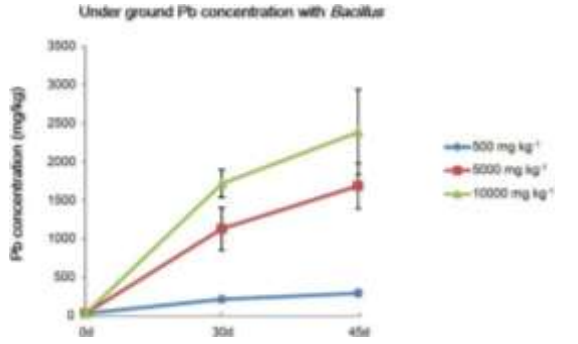

Fig. 5: The internal concentration of Pb in the root part of *H. cordata* was measured different days later at different concentrations of Pb (500 mg kg^{-1}, 5000 mg kg^{-1} and 10000 mg kg^{-1}). The soil was mixed with Pb acetate at a required Pb concentration in advance then planted with small seedling of *H. cordata*. The soil was inoculated with *Bacillus* 14 days before the 30th day and 45th day. Values represent the means and standard deviations were obtained from six individual plants as biological replicates

grown in different external concentration of Pb were more than the ratios without *Bacillus* at the 30th growing day. However, the situations differ at 45th growing day. Both ratios of 500 and 5000 mg kg^{-1}Pb decrease with *Bacillus* compared with the cases without *Bacillus*. At the external concentration of 10000 mg kg^{-1}Pb, the ratio is 0.34 more than two times of the ratio without *Bacillus*. The difference might be due to the reason that the ability of transporting Pb to the shoot part reaches highest after growing 45 days at the concentration of 500 and 5000 mg kg^{-1} and could not be further enhanced by *Bacillus*. The ability can be still elevated by *Bacillus* after growing 45 days in a higher concentration of 10000 mg kg^{-1}. Therefore, the inoculation of *Bacillus* can facilitate the transportation of Pb from the root to the shoot, especially at the high concentration of Pb.

Discussion

Phytoremediation is evaluated by the ability of plants to tolerate the heavy metal and transfer the heavy metal to the above ground part. Many measurements including bimolecular tools were taken to achieve this (Barabasz *et al*., 2010). The study of phyto extraction of Pb is quite limited due to the lack of plant, which can hyper accumulates Pb. In this study, we have shown that *H. cordata* can grow under increasing concentrations of Pb (500, 5000 and 10000 mg kg^{-1}), indicating that *H. cordata* can tolerate high concentration of Pb, though the growth of *H. cordata* was hindered by Pb (Fig. 1). This is consistent with the former research that *H. cordata* was found to grow in the mining area consisting of high concentrations of heavy metals including Pb (Nguyen *et al*., 2011). The amount of Pb accumulation in planta is very limited. In addition, the transfer of Pb from the root to shoot is usually very low. It was shown that the transfer coefficient (shoot/root ratio of Pb concentration) with a value arranging from 0.04–0.10 in the hyper accumulator higher than the non-hyper accumulators. Furthermore, the transfer coefficient of Pb was 0.15 higher in the accumulating ecotype of *S. alfredii* higher than 0.03 in the non-accumulating ecotype at a Pb concentration of 50 µmol L^{-1} (Luo *et al*., 2017). When grown at an equivalent Pb concentration of 10000 mg kg^{-1}, the transfer coefficient is 0.11 and 0.15 at the age of 30 days and 45 days respectively (Table 2) without the inoculation of *Bacillus*. When at the Pb concentration of 500 and 5000 mg kg^{-1}, the transfer coefficient is even higher with values of 0.19 at the age of 30 days and 0.27 at the age of 45 days and with values of 0.15 at the age of 30 days and 0.32 at the age of 45 days. This indicates that *H. cordata* belongs to the hyper accumulating species of Pb and therefore has the potential of treating Pb by phytoremediation.

However compared with transfer coefficients of other heavy metals in hyper accumulating species, the transfer coefficient of Pb is still very low. Thus, the phytoremediation is limited by the low transfer efficiency to shoot and leaves. Many strategies were taken to improve the transfer coefficient. Many soil modifiers including the chemical compounds and micro beings are added to

improve the ability of phytoremediation. In the present study, *Bacillus*, the most common bacteria in soil was added to explore the change of the transfer coefficient. When measuring the root and shoot concentration of Pb in *H. cordata* before and after adding *Bacillus*, it was shown that the inoculation of *Bacillus* obviously promote the absorption of Pb by both root and shoot (Fig. 2, 3, 4 and 5). It has been shown in Table 1, the increase of shoot absorption by adding *Bacillus* is much more than the increase of root absorption by adding *Bacillus.* Surprisingly, the transfer efficiencies were increased by adding *Bacillus* to soil with different concentrations of Pb. As shown in Table 3, the transfer efficiency increased from 0.19 to 0.21 at 500 mg kg^{-1} of Pb, 0.15 to 0.29 at 5000 mg kg^{-1} of Pb and 0.11 to 0.36 at 10000 mg kg^{-1} at the age of 30 days after adding *Bacillus*. At the age of 45 days, the transfer efficient increased from 0.15 to 0.34 at the concentration of 10000 mg kg^{-1} after adding *Bacillus*. When comparing the transfer efficient at different plant age, it increases as the age goes at all three concentrations (500, 5000 and 10000 mg kg^{-1} Pb). However at lower concentration of 500 and 5000 mg kg^{-1}, there is no increase at all after adding *Bacillus* at the age of 45 days. Probably the transfer efficient cannot be further increased anymore by *Bacillus* at the age of 45 days. The above results clearly showed that *Bacillus* could improve Pb absorption by *H. cordata* and the transportation of Pb from the root to shoot, facilitating treating Pb contamination. When treating Pb contamination, the above ground part then can be collected to remove Pb from the soil.

Microorganisms play an essential role in cycling the metals and nutrients, thus improving the fitness of plants and the tolerance of the plants. *Bacillus* was reported to promote the growth of *Vinca rosea* and phytoextraction of nickel (Waheed Ullah Khan *et al.*, 2017). However, in our case, as shown in Table 1, no clear difference of the height of *H. cordata* suggests that *Bacillus* does not improve Pbphytoextraction by better growth of *H. cordata*. Another possibility might be that *Bacillus* can facilitate the bioavailability of the heavy metals. For instance, Rhizobacteria produces metabolites to mobilize Nickel to improve the uptake of plants (Becerra-Castro *et al*., 2013). *Bacillus* could produce some metabolites to interact with Pb and thus it can be more easily taken up by *H. cordata*. Another reason might be due to the improved detoxification of *Bacillus*, since excess metals cause injures by oxidative stress. In plants, there are many antioxidant enzymes such as glutathione peroxidase, APX, POD, CAT and SOD and metabolites including proline and flavonoids which can degrade ROS. Pb treatment can upregulate the activity of ascorbate peroxidase and glutathione reductase in Duckweed. Moreover, Pb toxicity triggers the antioxidant responses in *Luffa cylindrical* Seedlings (Nan *et al*., 2010). Plants can activate their antioxidant system to alleviate the stress from Pb. The inoculation of *Bacillus* might strengthen the antioxidant system to detoxify Pb to further improve the capability of tolerating and further absorbing Pb. Therefore in future, the antioxidant metabolites and antioxidant enzymes can be measured before and after inoculation of *Bacillus*.

Conclusion

The current research indicated that: (1) *H. cordata* hyper-accumulates Pb; (2) The main part of accumulating Pb is the root part of *H. cordata*; (3) The inoculation of *Bacillus* facilitates capability of taking in Pb in both root and shoot and the transportation of Pb from the root to the shoot. Therefore, the inoculation of *H. cordata* with *Bacillus* might be used for phytoremediation of Pb contaminated soil. The present study brings a better prospect for understanding plant-microbe crosstalk under Pb stress. In future, it would be interesting to explore the phytoremediation effect of Pb on an outdoors field scale. Moreover, identification of potential genes involved in regulation of plant tolerance to Pb stress might be helpful for developing new Pb treating cultivars.

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