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Phytoremediation of Pb and Hg by using *Scirpus mucronatus* with addition of bacterial inoculums

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Abstract Two heavy metal-resistant rhizobacteria bacteria (*Brevundimonas diminuta* SF-S1-5 and *Alcaligenes faecalis* SF-S1-60) were bioaugmented in sand and also spiked with 100 ppm Pb and 1 ppm Hg and the removal of these metals was monitored using plant, *Scirpus mucronatus*. The highest accumulation of Pb and Hg were obtained in the root of *S. mucronatus* inoculated with *A. faecalis* at day 42 and 28, respectively. Plant inoculated with *A. faecalis* also showed the highest bioaccumulation coefficient and bioconcentration factor values > 1 compared to plant inoculated with *B. diminuta* and control.

Keywords Bioconcentration · Bioaccumulation · Hyperaccumulator · Lead · Mercury · *Scirpus mucronatus*

Introduction

Oil sludge contains heavy metals such as mercury (Hg) and lead (Pb) and needs to be treated before it can be disposed of in the environment to reduce threats to soil and water resources, as well as human health [1]. Physical and chemical methods of treatment encounter some limitations like high cost, labor involved, alteration of soil properties and disturbance of soil native microflora [2]. Phytoremediation have been successfully used to remediate metal-contaminated sites [3, 4] especially with hyperaccumulator plants [5]. The efficiency of phytoremediation depends on the type of plant used, the type of metal present, the phytoremediation process that takes place, the presence of soil microorganism specifically the rhizosphere microorganisms and the medium properties [6]. The high uptake of metals by plants can harm the growth, symbiosis and consequently the yields of crops by the plants [7]. However, a rhizosphere microorganism known as plant growth promoting rhizobacteria can be used to overcome this situation as they are able to immobilize, mobilize or transform metals. The combination of both hyperaccumulator plants and effective rhizosphere bacteria in phytoremediation, will enhance the phytoremediation process [8]. The ability of plant to absorb metals from soils, transport and store them in the above-ground part can be assessed based on three factors: i.e. bioaccumulation coefficient (BAC), bioconcentration factor (BCF) and translocation factor (TF) [9] and for plants with the coefficient values greater than 1 indicates that the plant is a hyperaccumulating plant [10].

Scirpus mucronatus (syn. *Schoenoplectus mucronatus*) commonly known as roughseed bulrush or bog bulrush, is an aquatic plant, grass-like species in the family Cyperaceae (the sedges), growing in moist and wet terrestrial habitats and in shallow water, was used in this study. It has grass-like leaves, and clusters of small spikelets, often brown in colour. The *S. mucronatus* was isolated from the Lake Chini, Pahang, Malaysia and has been used as hyperaccumulator plant for phytoremediation of heavy metals [11] and diesel contaminants [12]. Two heavy metal-resistant rhizobacterial isolates *Brevundimonas diminuta* SF-S1-5 and *Alcaligenes faecalis* SF-S1-60, were used in this study as bacterial inoculums. Both bacteria

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have been screened for its resistance potential towards several heavy metals and the capability of producing plant growth promoting activities (PGPA) [13]. The objective of this study was to determine the effects of two heavy metal-resistant rhizobacterial inoculums (*Brevundimonas diminuta* SF-S1-5 and *Alcaligenes faecalis* SF-S1-60) on removal of Pb and Hg from soil by using *Scirpus mucronatus*.

Experimental procedures

Experimental Set up

Scirpus mucronatus plants were grown in plastic container (52 cm length × 30 cm width × 25 cm height) filled with 30 kg sieved sand (5 mm mesh). Pb and Hg stock solutions were prepared using $Pb(NO_3)_2$ and $HgCl_2$ (Merck) diluted with deionized distilled water (ddH_2O) from an ultrapure grade water instrument (type 7/15 fitted with ELGA Biofilter, Purelab, U.K.). Standard inoculums for each bacterial isolates were prepared as described by Hamzah et al. [14]. In the plastic container, 10 % (v/v) of standard bacterial inoculums, Pb and Hg solutions with final concentration of 100 and 1 mg kg^{-1} respectively were spiked in the treatment container. Since *S. mucronatus* grew in aquatic condition, the height of water in container was maintained at 10 cm above the sand.

Secondary generation of *S. mucronatus* was propagated for 2 months in the greenhouse and acclimatized in greenhouse condition at temperature ≈30 °C. Fifteen plants of the same age with height 15–20 cm were planted in each container. The uptake of Pb and Hg was estimated in plant root, shoot and soil at day 1, 7, 14, 28 and 42. For a control, the plant was grown without addition of bacterial inoculums. The soil moisture, pH, and temperature in each container were monitored, and physical observation of the plant growth was also done. Each experiment was done in duplicate.

Extraction of Pb and Hg from plant and soil samples

The metals from the plant were extracted using a wet digestion procedure according to Method 3050B, United States Environmental Protection Agency (USEPA) [15]. The metals from soil samples were leached out using ethylenediaminetetraacetic acid (EDTA) method [16] whereby the EDTA act as a chelator to examine enhanced metal mobilization and distribution of heavy metals in soil.

Blank extractions were also carried out for each set of analysis using the same reagents with plant or sand samples. All solution from plant extract and the supernatant liquid from sand extract were transferred into polyethylene container and at stored at 4 °C prior to analysis.

Analysis of heavy metal accumulation

The total concentrations of Pb and Hg in the samples were determined using inductively coupled plasma-mass spectrometry (ICP-MS) Elan 9000 (Perkin Elmer, US) and atomic absorption spectrometer (AAS) Analyst 800 (Perkin Elmer, US), respectively. Concentration means of Pb and Hg were calculated from three composite samples.

Bioconcentration Factor was calculated using the formula by Ghosh and Singh [17], TF using formula by Marchiol [18] and BAC according to formula by Yanqun [19].

$$\text{BCF} = \frac{\text{metal concentration in plant tissue}}{\text{initial concentration of metal in soil}}$$

$$\text{BTF} = \frac{\text{metal concentration in shoot}}{\text{metal concentration in roots}}$$

$$\text{BAC} = \frac{\text{metal concentration in shoot}}{\text{metal concentration in soil}}$$

Statistical analysis

One-way and two-way analysis of variance (ANOVA) were employed to assess the relationship between day, treatment, and interaction between day and treatment at level $p < 0.05$ level according to IBM SPSS.

Results and discussion

In this study, *S. mucronatus* plant accumulated Pb and Hg in its root and shoot. The accumulation of Pb and Hg in the root was always higher than in the shoot for the whole 42 days of experiment (Fig. 1). The result also showed that the absorption of Pb by *S. mucronatus* was higher than for Hg. The highest absorption of Pb in the root was obtained with addition of *A. faecalis* SF-S1-60 to the soil at day 42. The efficiency of Hg uptake in the root by *B. diminuta* and *A. faecalis* is the highest at day 28. A two-way ANOVA was conducted to examine the effect of treatment (*B. diminuta* and *A. faecalis*) and day on the uptake of Pb and Hg in the root. There is a statistically significant interaction between these two bacteria and day on Pb and Hg uptake ($p = 0.0$) (Table 1).

The absorption level of Pb in the shoot showed a significant difference ($p < 0.05$) between different days and treatment. For the plant with added *B. diminuta* the absorption of Pb increased from day 14 and was the highest at day 28, whereas, addition with *A. faecalis* showed a lower absorption in the shoot. The average absorption of

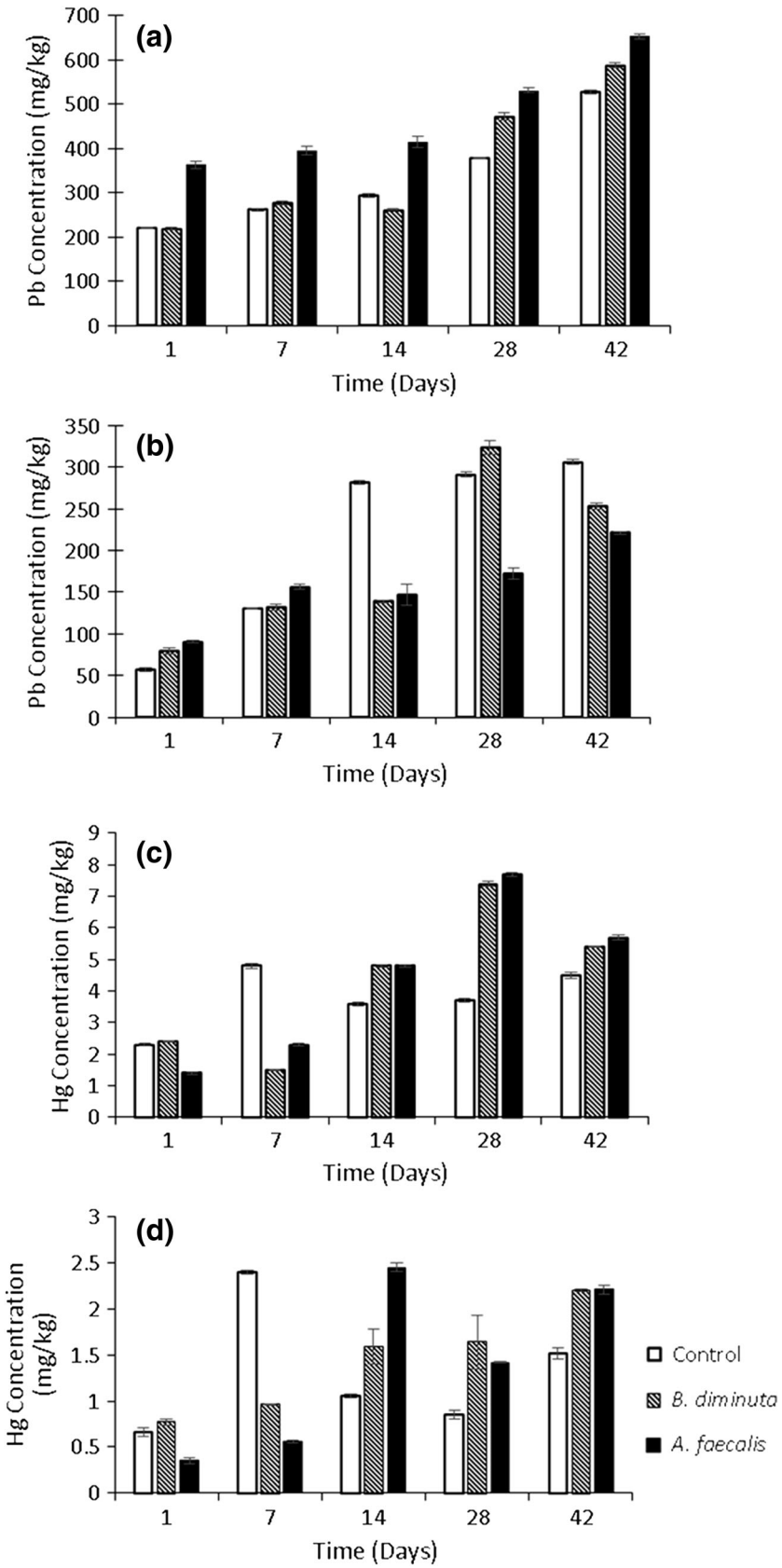

Fig. 1 The uptake of Pb and Hg by *S. mucronatus* with addition of different bacterial isolates: **a, c** by root, **b, d** by shoot

Table 1 Interaction between parts of plant on day and treatment (different microbes) in the absorption of Pb and Hg by *S. mucronatus*

Metal and parts of plant	Source	Degree of freedom (df)	F	Significance
Pb (shoot)	Day	4	715	0.0 S
	Treatment (T)	2	105	0.0 S
	Day*T	8	115	0.0 S
Pb (root)	Day	4	665	0.0 S
	Treatment (T)	2	1,367	0.0 S
	Day*T	8	20	0.0 S
Hg (shoot)	Day	4	88	0.0 S
	Treatment (T)	2	3	0.08 NS
	Day*T	8	49	0.0 S
Hg (root)	Day	4	665	0.0 S
	Treatment (T)	2	1,368	0.0 S
	Day*T	8	20	0.0 S

S signicance ($p < 0.05$), *NS* not significance ($p > 0.05$)

mercury among different treatments in the shoot was not significant (Table 1). However, there is a significant difference in the interaction between day and treatment in the Hg absorption.

Throughout the 42 days of experiment, the physical appearance of the plant grown in each container was observed. At day 14, some of the plants (added with heavy metals with/without addition of bacteria) became slightly dry and brownish compared to the plants grown without addition of heavy metals or bacteria. This result showed that the toxicity of Pb and Hg affected the plant growth since there is no addition of nutrient during this experiment. From day-28 onwards, it was recorded that the growth of plant with the addition of bacterial inoculums was better than for the plants without addition of bacteria in terms of shoot and root elongation (data not shown).

Brevundimonas diminuta SF-S1-5 and *A. faecalis* SF-S1-60 are plant growth promoting bacteria (PGPR) that have the capability of producing indole-3-acetic acid, ammonium, phosphate solubilization and nitrogen fixation. It has been found that *B. diminuta* SF-S1-5 does not produce siderophore but produces HCN, while *A. faecalis* SF-S1-60 does not produce HCN but produces siderophore [13]. According to Abou-Shanab [20] microbial populations can affect the heavy metals mobility and availability to the plant through releasing of chelating agents, acidification, phosphate solubilization, and redox changes. Therefore, the use of rhizobacteria in combination with plants is expected to provide a high efficiency for phytoremediation.

The BCF of metal was used to determine the quantity of heavy metals absorbed by a plant from soil. This index showed the ability of the plant to accumulate the particular metal with respect to its concentration in the soil. The higher the BCF value, the more suitable is the plant used for phytoextraction. If the BCF value > 2, it is regarded as a higher value [21]. Based on this criterion, the highest BCF value was for the absorption of Pb with the addition of both bacteria, followed by Hg (Table 2). The highest BCF value for translocation of Pb and Hg was obtained by the addition of *A. faecalis* at day 42. Metal translocation from the root to the shoot rate may depend on metal concentrations in the root [18, 22]. In this study the initial concentrations of Pb and Hg were 100 and 1 mg kg^{-1} ppm, respectively.

Table 2 The BAC, BCF and TF of plant *S. mucronatus* with the present of Pb, Hg and microbes

Day	Treatment	BAC Pb	BCF	TF	BAC Hg	BCF	TF
1	Control	1.17	3.45	0.25	0.78	2.51	0.28
	B. diminuta	1.35	4.34	0.36	1.80	5.43	0.32
	A. faecalis	1.46	5.50	0.24	0.36	1.35	0.24
7	Control	1.58	3.00	0.49	2.68	5.53	0.49
	B. diminuta	2.14	4.61	0.47	1.78	2.54	0.65
	A. faecalis	2.30	6.30	0.39	0.89	3.44	0.26
14	Control	3.60	3.76	0.96	0.64	2.02	0.29
	B. diminuta	2.70	5.23	0.59	0.97	2.91	0.33
	A. faecalis	2.33	6.20	0.38	2.25	4.87	0.51
28	Control	2.67	3.35	0.77	1.51	5.60	0.23
	B. diminuta	3.76	6.49	0.68	1.11	7.23	0.22
	A. faecalis	3.08	10.04	0.49	1.41	7.63	0.19
42	Control	2.83	5.62	0.57	3.07	8.38	0.33
	B. diminuta	4.01	9.58	0.43	3.69	9.10	0.41
	A. faecalis	4.68	14.80	0.33	5.55	14.33	0.39

Translocation factor ratio was used as an indicator of the ability of the plant to translocate metals from the roots to the plant aerial parts [18]. If the ratio >1, the plant is considered as a hyperaccumulator of heavy metals. Based on results in Table 2, *S. mucronatus* is not the hyperaccumulator plant since the TF ratio is less than 1. The standard for hyperaccumulator plant has not been defined scientifically but it is based on several opinions. They include four standard rules [23], among which the concentration of heavy metals in plant shoot should reach the hyperaccumulating level (e.g., Pb and Cu > 1,000 mg kg^{-1} and Zn > 10,000 mg kg^{-1}). The BAC value for *S. mucronatus* was >1 for Pb starting from day 1 and for Hg consistently increased after day 28. The bioaccumulation occurs when contaminants taken by the plant were not degraded rapidly, resulting in the accumulation in the plant. In this study, Pb concentration did not exceed >1,000 mg kg^{-1} in the shoot [24], nevertheless the ability of *S. mucronatus* to tolerate and accumulate heavy metals is useful for phytostabilization.

Our results showed that the BAC and BCF values increased when the plant was inoculated with bacterial inoculums compared to the control plant. This indicates that bacteria can help in increasing the efficiency of the phytoremediation process. The low TF value is referring to the lower concentration of heavy metals in the shoot part compared to the root. This is probably due to the passive heavy metal translocation from roots to the shoots. The bacteria are only capable of increasing the bioavailability of heavy metals in the soil for the plant uptake but the species of plant will determine the capacity for the metal-uptake by the roots and for its, translocation and storage in the shoots [10].

Many species of *Scirpus* plant, e.g., *S. mucoratus* have the capability as hyperaccumulator for diesel [12], *S. lacustris* for the chromium uptake [25], *S. grossus* for phytoremediation wastewater containing Pb [26] and *S. americanus* for removal of Pb, Cr and Mn [27]. All the previous studies found that the plants and shrubs can be used as hyperaccumulator plants for heavy metals such as Pb, Zn, Cd [19], Pb, Ni [22], Pb, Zn, Cu [23], Hg [28, 29] but none of them dealt with the effect of rhizobacteria addition to facilitate the metal uptake by the plants.

Conclusions

Phytoremediation of Pb and Hg in soil using the combination of plant *S. mucronatus* and bacterial inoculums was significant. The accumulation of both metals in plant was the highest with the addition of bacteria *A. faecalis* SF-S1-60 followed by *B. diminuta* SF-S1-5 compared to control plant. Based on BCF and BAC values, *S. mucronatus* is a hyperaccumulator plant. With the help of bacterial activity *A. faecalis* SF-S1-60 and *B. diminuta* SF-S1-5 60, *S. mucronatus* is suitable to be used for phytoremediation of Pb and Hg.

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