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Screening of Rhizobacteria from *Scirpus mucronatus* in Pb and Hg Contaminated Soil

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Abstract

In present work, effect of two types of rhizobacteria was investigated on *Scirpus mucronatus* plant grown in contaminated soil spiked with 1 ppm Hg and 100 ppm Pb using phytoremediation technique. The concentrations of Hg and Pb in root and shoot of *Scirpus mucronatus* plant were determined on 1th, 7th, 14th, 28th and 42th days after enrichment by bacteria using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). To evaluate statistical differences in the Hg and Pb concentrations, the one-way ANOVA test was used. There were statistically significant differences between Hg and Pb concentrations between samples of control, bacteria 5 and bacteria 60. The results were demonstrated that these two bacterial inoculums namely *Brevundimonas diminuta* and *Alcaligenes faecalis* were known as plant-grown promoting rhizobacteria and they can increase the heavy metal uptake from soil contaminated.

Keywords: ICP-MS; Lead and mercury; Rhizobacteria; *Scirpus mucronatus*; Phytoremediation

Introduction

Heavy metals are among the most common contaminants in the environment. Of those, two of the most common in the environment are lead (Pb) and mercury (Hg) which are sourced from such industries as paint and battery manufacture and lead smelting. The Pb and Hg are also known to act as an accumulated toxin. Inorganic Pb and Hg is inhibitor enzyme which are harmful to the nervous system. If these metals enter the environment in an ionic form, their most toxic form, they can enter the human body through drinking water, food or air [1].

Since the traditional treatments for contaminated soil through excavation, soil vapor exposure, and bio-ventilation are expensive, treatment using phytoremediation, which is cost-effective and environmentally friendly has been used widely. Phytoremediation uses plants to degrade, stabilize, and remove contaminant from the soil [2]. Several types are used in phytoremediation, such as grasses, legumes, and aquatic plants. Phytoremediation has also been used to treat contamination with organic compounds, such as petroleum-derived contaminants. The mechanism for removal of petroleum is through rhizodegradation, i.e. degradation in the rhizosphere (the zone of influence of the root system). Rhizodegradation is the breakdown of an organic contaminant in soil through microbial activity that is presence in the root zone [3-7]. The *Scirpus mucronatus* is a monocot weed in the Cyperaceae family. This plant is known by the common name of ricefield bulrush. It grows in moist and wet terrestrial habitat, and in shallow water. It is a perennial herb growing from a short and hard rhizome. The erect, three-angled stems grow in dense clumps and reach a meter tall. The leaves take the form of sheaths wrapped around the base of stem, but they generally do not have blades [8]. This plant also is planted in Tasik Chini Wetland, Pahang, Malaysia.

Phytoremediation is not a very well-known process. Some of the mechanisms involved in accumulation and detoxification of the metals are still unidentified. The role of bacteria in the improvement of the phytoremediation technique seems to be very important. Understanding the mechanisms of plant growth promotion is important when deciding what type of bacteria to use with a plant in a given situation [3-6]. The aim of this study was to assess the effect of two bacterial inoculums, *Brevundimonas diminuta* (bacteria 5) and *Alcaligenes faecalis* (bacteria 60), on the phytoremediation of mercury and lead contaminated soil planted with *Scirpus mucronatus*.

Experimental

Scirpus mucronatus was propagated from seeds in a greenhouse in University Kebangsaan Malaysia using soil garden in polyethylene crates (40 cm - 58 cm - 30 cm dimensions). The crates were watered with deionized water, to avoid further intake of nutrients or other elements. After 5 weeks, Hg and Pb solutions and bacterial inoculums namely *Brevundimonas diminuta* (bacteria 5) and *Alcaligenes faecalis* (bacteria 60) were added into crates. After 1th, 7th, 14th, 28th and 42th days of growth the plants were harvested; rinsed by Milli-Q water, and separated to root and shoot. The samples were dried in oven at 90°C for 2 days. About 200 mg of plant samples were accurately weighed into a heat-resistant digestion vessel, 5 mL 69% HNO3 and 3 mL H2O2 were added to the vessel and waited for about 20 min before the vessel is closed. Decomposition of the samples was carried out in a microwave for 55 min with 1400 W energy. The extract was transferred into a volumetric flask and made up to 100 mL with double distilled water. Blank experiments were carried out in the same way. Digested samples were analyzed for multi-element analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique [3,8,9]. The advantage of ICP-MS is the multi-element capacity in combination with superior precision and sensitivity. In addition, the sample consumption can be as low as 100 µl/min if using a micro-nebulizer, allowing the analysis of multiple elements even in small sample volume [10].

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Statistical analyses were performed with IBM SPSS software version 22.0. A one-way ANOVA with the significance level set at 95% was employed to identify the factors influencing Hg and Pb concentrations in our samples. The p-value <0.05 is considered no statistically significant for all tests [11].

Results and Discussion

The shoot and root of plants were collected on 1th, 7th, 14th, 28th and 42th days after addition of heavy metal (Hg and Pb) solution and bacterial inoculums. The Figures 1 and 2 illustrate Pb concentration

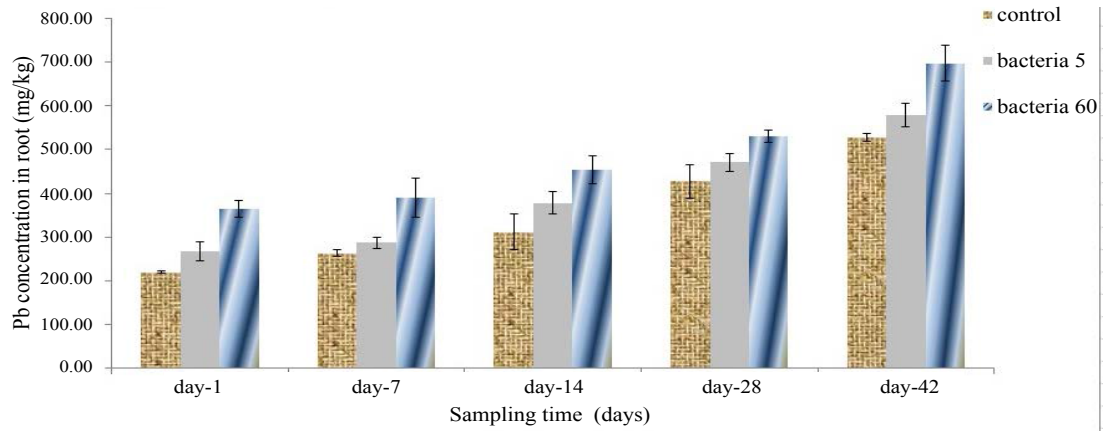

Figure 1: Pb concentration in root of *scirpus mucronatus* plant contaminated by 100 ppm Pb versus days.

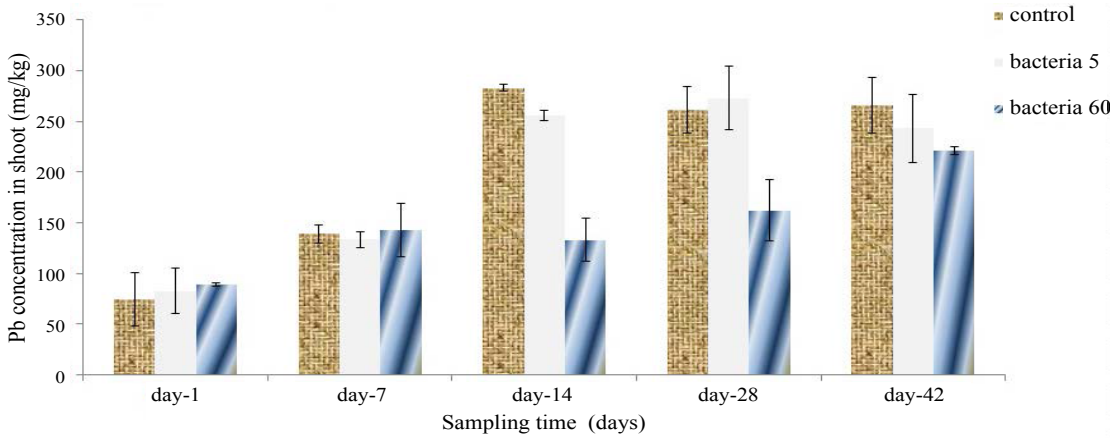

Figure 2: Pb concentration in shoot of *Scirpus mucronatus* plant contaminated by 100 ppm Pb versus days.

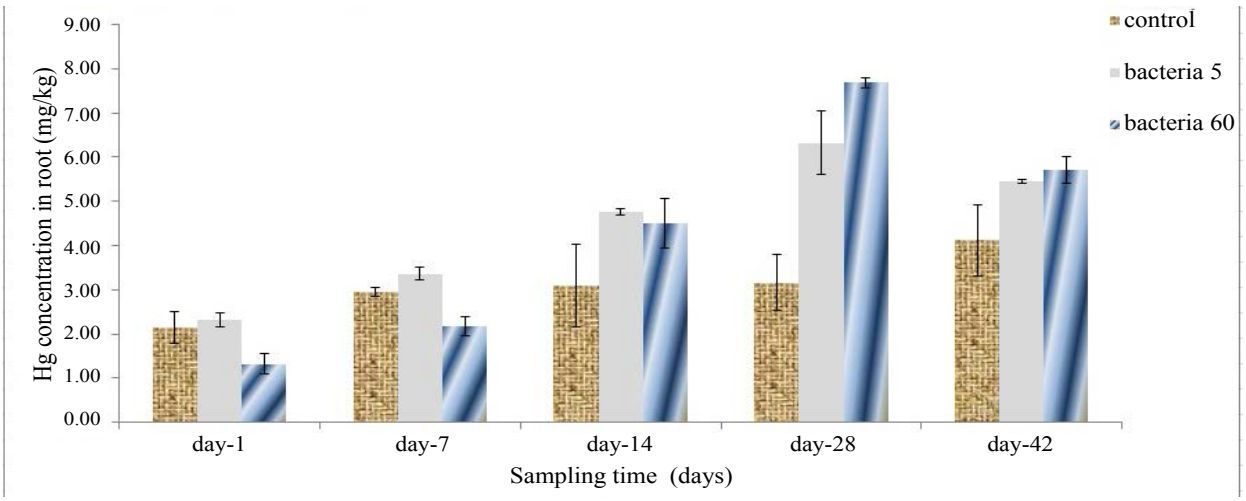

Figure 3: Hg concentration in root of *Scirpus mucronatus* plant contaminated by 1 ppm Hg versus days.

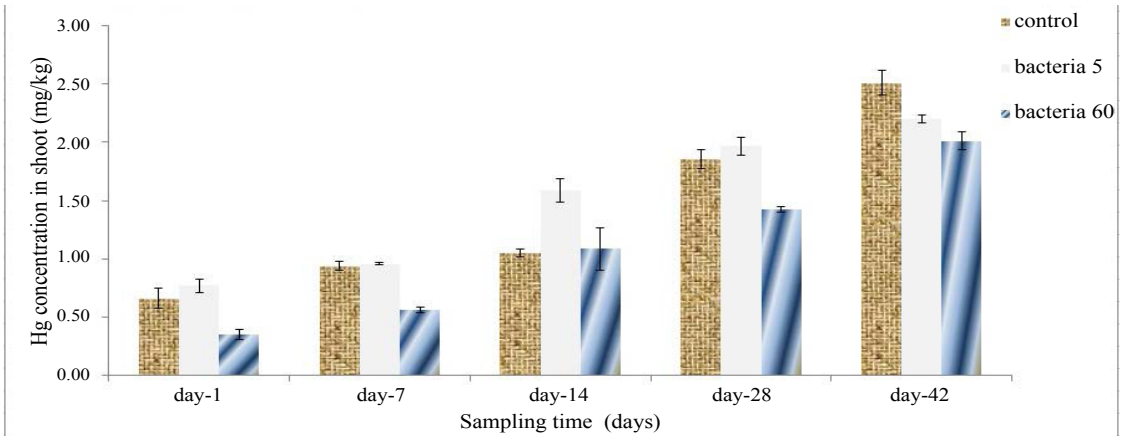

Figure 4: Hg concentration in shoot of *Scirpus mucronatus* plant contaminated by 1 ppm Hg versus days.

in root and shoot for 42 days, respectively. The Pb concentration in root and shoot has increased from 1th day to 42th day. As it shown in Figure 2, Pb concentration in bacteria 5 and bacteria 60 samples were lower than those in control sample. The Figures 3 and 4 show Hg concentration in root and shoot, respectively. Hg concentration in root of bacteria 60 sample was lower than those in control sample on 1th and 7th days. However it was increased after 7th day. On the other hand, Hg concentration in shoot of control sample was higher than those in bacteria 60 sample on all 42 days. It may be caused by internal detoxification mechanism of *Scirpus mucronatus*. Generally, to protect themselves from metal poisoning, plants must have developed a mechanism by which the heavy metal entering the cytosol of the cell, is either immediately excluded or complexed and inactivated, thus preventing the metal from inactivating catalytically active or structural proteins, presumably by adapting mechanism that may also be involved in the general homeostasis of essential mineral ions, and tolerate them. Such plants are resistant to certain metal ions suggesting their potential use for cleaning of contaminated soil [12]. Furthermore, the chemical properties of a small number of pollutant trace elements, mainly mercury, allow the use of the technology of phytovolatilization. Instead of accumulating inside the plant, the trace element is enzymatically transformed into a less toxic, volatile compound and is subsequently released into the atmosphere [13]. Additionally, it may be due to interactive effect of elements in *Scirpus mucronatus*. Overall, the interaction at root level among some transition elements could be antagonistic, synergistic or multiplicative. In this study, result showed that Pb and Hg concentrations in roots were more than in shoots, which the results were consistent with other studies. Baldantoni et al. [14] have reported that roots of Phragmitesaustralis contained more Pb than its leaves. Firitioff and Greger [15] also found that the highest accumulation of Pb by Potamogetonnatans was in the roots. A study of Pb uptake and accumulation in Brassica juncea [16] also found that it accumulated Pb primarily and largely in its root tissues and transported and concentrated it in its hypocotyls and shoots with much smaller concentrations. A study on uptake of cadmium, lead and zinc by Siam weed [17] found that roots accumulated higher concentration of metals than its other parts. Our results have indicated that the activities of the bacteria 5 and bacteria 60 in the soil have a significant effect on increasing the mobility of Hg and Pb in the rhizosphere of the plants from soils.

Since the data are nonparametric, one-way ANOVA test was used to evaluate statistical differences in the elemental concentration between bacteria 5, bacteria 60 and control samples. Comparison of Hg and Pb concentrations in all samples were proved in which there were statistically significant differences between three sample treatments (control, bacteria 5 and bacteria 60) with p-values of 0.642, and 0.196 for Hg and Pb concentrations, respectively ($p>0.05$).

Although phytoremediation has received great attention in the last decade, and a considerable number of reports exist suggesting that it should become the technology of choice for the cleanup of various types of environmental contamination, this technology still does not work sufficiently. Nevertheless, it is calculated to account for about 10-15% of the environmental remediation market. On the other hand, to realize the potential of this technology, it is necessary for plants to grow as large as possible in the presence of various environmental contaminants. One way to achieve this goal is to utilize plant growth promoting bacteria to facilitate the growth of the plants used for phytoremediation [5,8,9]. Furthermore, tolerance and accumulation of metal vary from plant to plant as well as from species to species within a genus [18]. Therefore, the obtained results of this study are useful as database on properties of phytoremediation of *Scirpus mucronatus* plant.

Conclusion

The effect of two bacterial inoculums namely *Brevundimonas diminuta* and *Alcaligenes faecalis* were investigated in Hg and Pb contaminated soil using phytoremediation method. The *Scirpus mucronatus* plant was utilized in present work. The results were shown a good ability to transport Hg and Pb from roots to shoots and cleanup the contaminated soil. Hg and Pb content in shoot of bacteria 60 sample was lower than those in control sample in which it may be caused by internal detoxification mechanism of *Scirpus mucronatus*; the chemical properties of their elements that allow the use of the phytovolatilization technology; interactive effect of elements in *Scirpus mucronatus* plant as well. One-way ANOVA test proved wherein there were statistically significant differences between three sample treatments (control, bacteria 5 and bacteria 60) with p-values of 0.642, and 0.196 for Hg and Pb concentrations, respectively. That is economical and necessary that any country finds the local plant to mobilize the metals to decrease the environmental contaminations. Aside from, development and understanding the effects of various bacteria in plant growth promotion and metal uptake is necessary in phytoremediation method. The *Scirpus mucronatus* plant as local phytoremediation plant is found adequate in

Malaysia. Consequently, obtained results of this study are valuable as database on phytoremediation properties.

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