IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 6 Ver. III (Jun. 2014), PP 16-24
www.iosrjournals.org

Characterization of Toxic Elements on Phytoremediation of Scirpusmucronatus Enriched with Bacteria using ICP-MS Technique

A. Yavar[a], S. B. Sarmani[b], A. Hamzah[c], K. S. Khoo[a]
[a]*School of Applied Physics, Faculty of Science and Technology, The National University of Malaysia (UKM), 43600 Bangi, Selangor, Malaysia*
[b]*School of Chemical Sciences & Food Technology, Faculty of Science and Technology, The National University of Malaysia (UKM), 43600 Bangi, Selangor, Malaysia*
[c]*School of Biosciences and Biotechnology, Faculty of Science and Technology, The National University of Malaysia (UKM), 43600 Bangi, Selangor, Malaysia*

Abstract: *Investigating various bacteriato promotea plant's phytoremediation capacityis essential for environmental health. In this study, Brevundimonasdiminuta(bacteria 5) and Alcaligenesfaecalis(bacteria 60) were examined for their ability to increasethe phytoremediation capacity of Scirpusmucronatusin soil contaminated with100 ppmlead. After 1 and 42 days of contamination with the lead and inoculated with the bacteria,the Scirpusmucronatuswas cut crosswise into fivesections of the same size from root to shoot, and theelemental concentrations in each piece were determined using inductively coupled plasma mass spectrometry.To evaluate statistical differences in the elemental concentration between our findings, one-way ANOVA test was used. Comparison of elemental concentrations in all samples were demonstrated which there were statistically significant differences between all elemental concentrations except Ba, Mn and Mo with p-values of 0.000, 0.013, and 0.003 respectively (for other elements, p>0.05). Moreover, Pearson's correlation test was proved the elements values found in bacteria 5 and bacteria 60 were strongly correlated to the results in control sample (R>0.3). The results showedthatbacteriumincreased the heavy metal uptake from the lead-contaminated soil.*

Keywords: *Heavy metals, Phytoremediation, Scripusmucronatus, Lead contamination, Brevundimonasdiminuta and Alcaligenesfaecalisin bacterium, ICP-MS*

I. Introduction

ICP-MS is an analytical method utilized for elemental determinations that has the benefit of being able to analyse for multiple elements simultaneously and to do so with high precision and sensitivity[1, 2].

Heavy metals are the most widespreadcontaminants in the environment,and one of the most common of these is lead, with industries like paint and battery manufacturers and lead smeltingplants being the main sources of lead production. In addition, it has beenrecognizedthat lead operates as an accumulated toxin, and inorganic lead acts asan inhibitor enzyme that is harmful to the nervous system. The most toxic form is ionic lead,whichwhen it spreads to the environment, can be absorbed by the body through drinking water, food, or the air[3, 4].The general spread of toxic elements in the environment poses a serious problem for human health, and the U.S. Environmental Protection Agency has designated silver (Ag), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), and zinc(Zn) as potentially toxic elements[5].

Phytoremediation is a procedure that uses plants to decrease and/or remove contaminants from the environment. It is a better option as a recovery technique than other methods because it is cost effective and environmentally friendly. Globally, soil contamination is avital environmental problem affecting human health, and remediation technologies are necessary to resolve the problem of lead-contaminated soil[3, 6]. Hyper-accumulator plants have the ability to take up large amounts of toxic metals in contaminated soils. Adam and Duncan [7]have shown that grasses are good hyper-accumulator plants for contaminated-soil remediation due to their fibrous root systems that have an extensive surface area for microbial colonization. The fibrous root system forms a continuous dense rhizosphere, which provides the ideal conditions for phytoremediation.

In this study, we utilizedScirpusmucronatus, a monocot weed in the Cyperaceae family. This plant is known by the general name of ricefield bulrush. It is a perennial herb growing from a short, hard rhizome inshallow water and moist and wet terrestrial habitats. The triangular stems grow in thick clumps up to a meter tall with leavesthat wrap around the base of the stem in sheaths, although they often do not have blades[8, 9].

Toobtainthefull potential of a phytoremediationtechnique, it is essential that the plant grow as large as possible in order to take up thevarious environmental contaminants. One way to achieve this is to employ plant

growth-promoting bacteria to assist the growth of the phytoremediation plant [8, 10, 11]. The aim of this study wasto assess the effect of two bacterium,Brevundimonasdiminuta (bacteria 5) and Alcaligenesfaecalis (bacteria 60), on thephytoremediation of lead-contaminated soil planted with Scirpusmucronatus.

II. Experimental Procedures

TheScirpusmucronatuswas propagated from seeds in a greenhouse at theUniversitiKebangsaan in Malaysia using garden soil in polyethylene crates (40 cm × 58 cm × 30 cm).After 5 weeks, a lead solution (100 ppm)was added to the crates with no bacteria (control), inoculants of bacteria 5,or bacteria 60. Samples of theScirpusmucronatuswere prepared 1 and 42 days afterthe addition of the lead solution and the bacteria to the soil. The samples were first rinsed inultra pure water (18.2 MΩ quality, model: LPTA/PB/7/1 - Maxima Ultra Pure Water, ELGA company, Italy) to remove dust-borne contamination, after whicheach plant was cut crosswise from root to shootinto 5 sectionsof equal length before drying in an oven at90 °C for 2 days. Subsequently, 200 mg samples were placedin heat-resistant tubes, and 5 mL 69% HNO_3 and 3 mL H_2O_2 were added; the samples were then heated in a microwave (Milestone Start D Microwave Digestion System, VAC-1000) with 300 (W) energy, 20-120 (°C) temperature, for 55 min. Microwave digestion was used instead of classical methods because of its shorter time, less acid consumption, and ability to retain volatile compounds in the solutions. The residues were filtered by using a 0.45 µm Whitman filter paper. Finally, the digested samples were diluted to 50 mL with ultra pure water, and a multi-element analysis was conducted on eachsampleby inductively coupled plasma mass spectroscopy(ICP-MS). This study used the PerkinElmer Elan 9000 ICP-MS (USA). After calibrating the instrument by using standard solution derived from commercial materials, the system was optimized based on the recommendation of the manufacturer. The analytical conditions for the analysis of heavy metals with the use of ICP-MS are described in Table 1.

Table 1. Operating parameters for the Inductively Coupled Plasma Mass Spectrometry

Parameters	Condition
RF Generator	40MHz
RF Power	1000 W
Spray Chamber	Ryton Scott
Nebulizer	Cross Flow
Plasma gas flow	15.0 L/min
Auxiliary gas flow	1.0 L/min
Nebulizer gas flow	0.60 L/min
Sampler & skimmer cone	Nickel
Sweeps/Reading	20
Reading/Replicates	3

Statistical analyses were performed with IBM SPSS version 22. A one-way ANOVA with the significance level set at 95% was employed to identify the factors influencing Ag, As, Ba, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, and Zn concentrations in our samples. P-value<0.05 is considered no statistically significant for all tests. Pearson's correlation test was performed to examine the correlation between elemental concentrations in bacteria 5 and bacteria 60 versus those in control sample [12].

III. Results and discussion

Three sample treatmentswere utilized in this study. Two of the treatments enriched the lead-contaminated soil with bacteria 5or bacteria 60, and thethirdtreatment was growth without the addition of bacteria (control). Fig. 1 to Fig. 13 show the concentrations of the toxic elementsAg, As, Ba, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, and Zn, respectively, ineach of the 5 plant sections (root to shoot)of Scirpusmucronatusfor the 3 treatments. The control samples were used to assess the effects of bacteria 5 and bacteria 60 on the phytoremediation capacity of theScirpusmucronatus. As well, Table 2 presents mean and range of elemental concentrations in all treatments. The concentrations of theall elements inthe bacteria 5,bacteria 60,and control samples were higher after 42 days than afterthe first day, especially in the shoots (sections 4 and 5), except Ag, Cr, Hg and Ni. It may be caused by internal detoxification mechanism of Scirpusmucronatus. 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 [13]. Further, the chemical properties of a

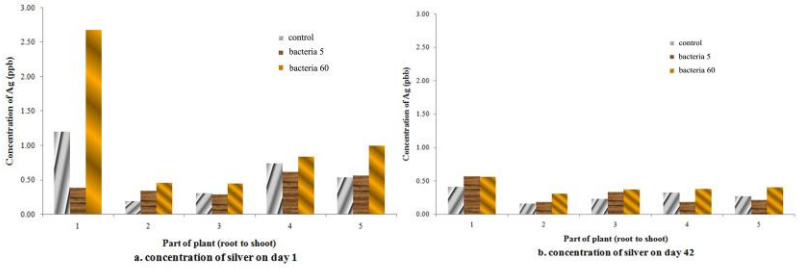

Fig. 1 Concentration of silver (Ag) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

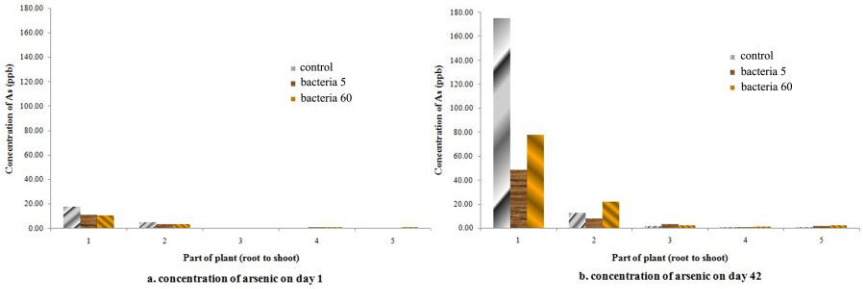

Fig. 2 Concentration of arsenic (As) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

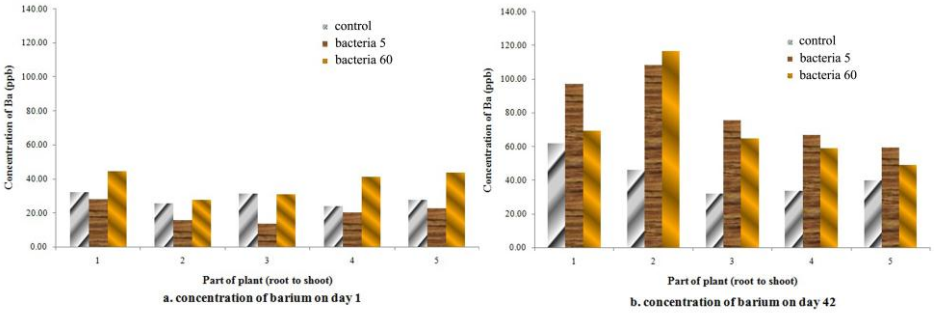

Fig. 3 Concentration of barium (Ba) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

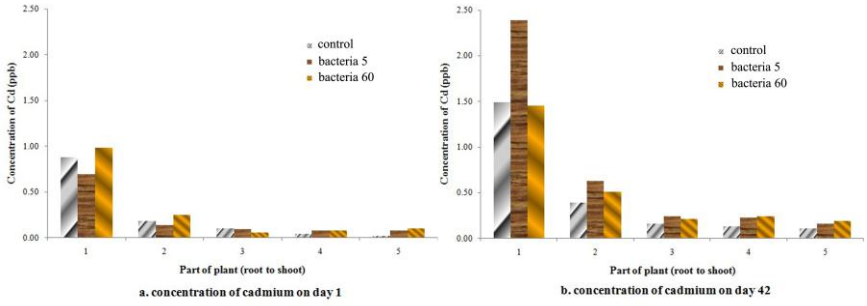

Fig. 4 Concentration of cadmium (Cd) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

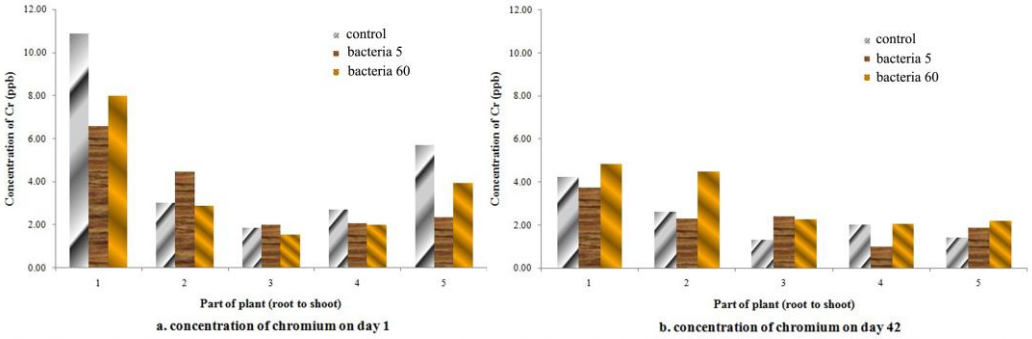

Fig. 5 Concentration of chromium (Cr) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

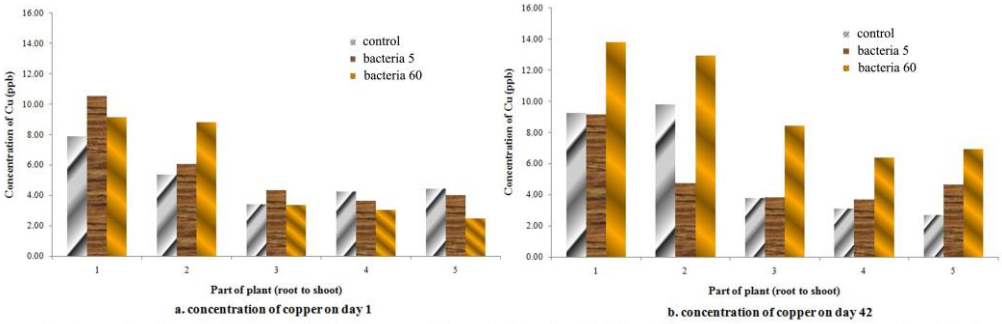

Fig. 6 Concentration of copper (Cu) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

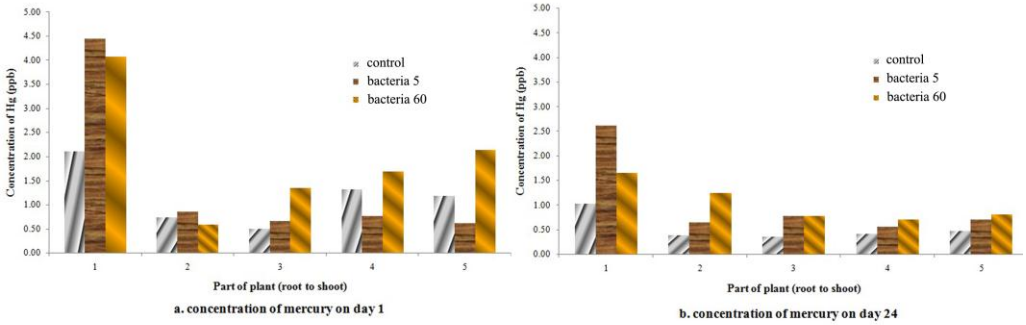

Fig. 7 Concentration of mercury (Hg) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

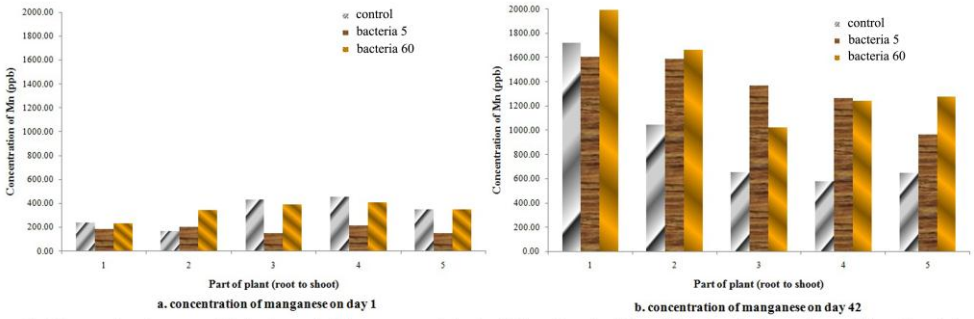

Fig. 8 Concentration of manganese (Mn) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

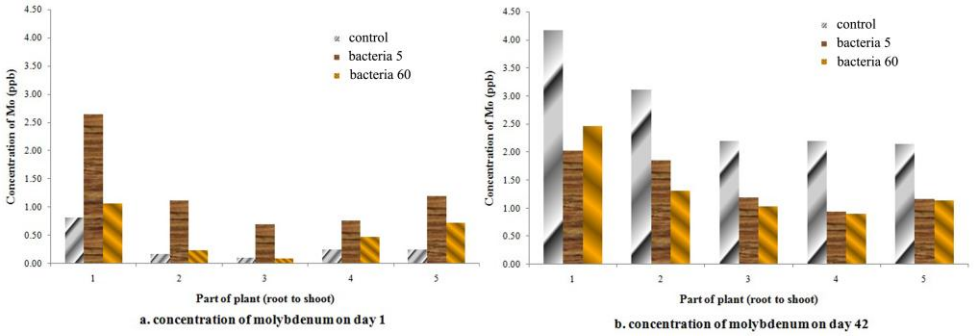

Fig. 9 Concentration of molybdenum (Mo) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

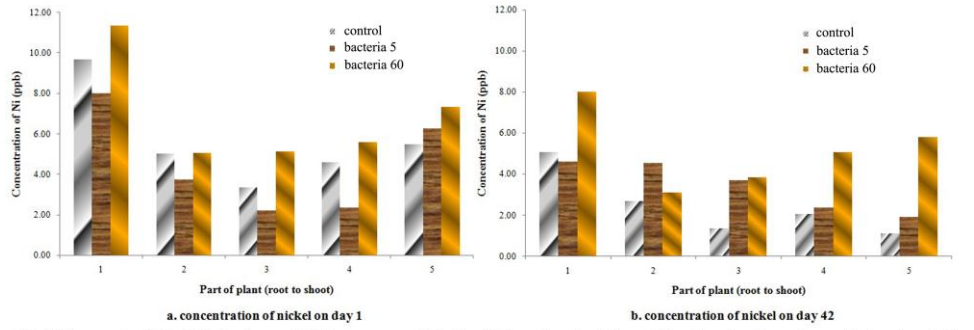

Fig. 10 Concentration of nickel (Ni) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

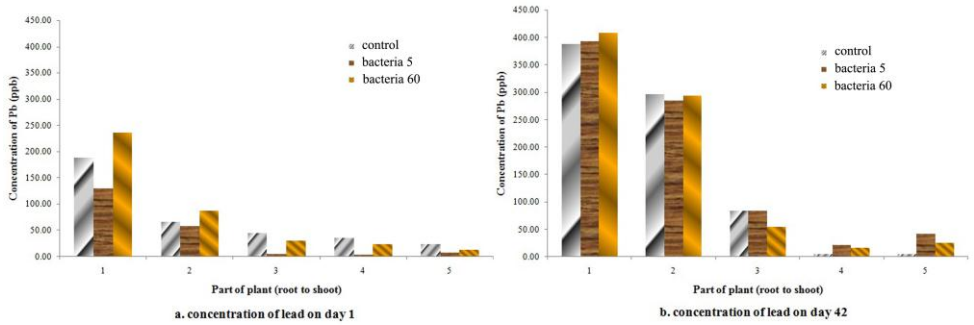

Fig. 11 Concentration of lead (Pb) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

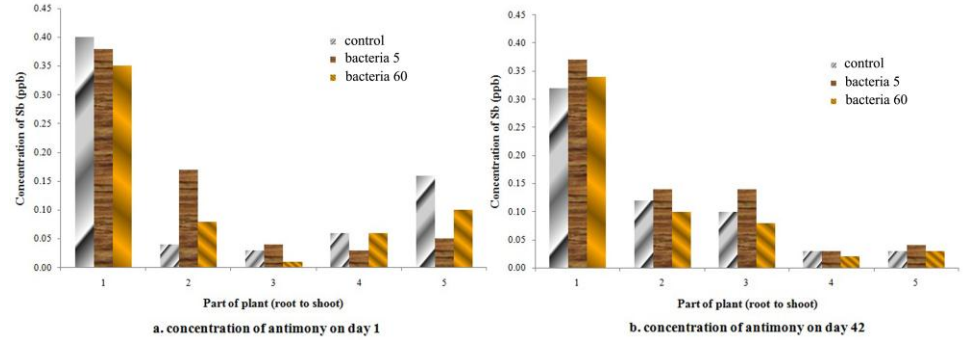

Fig. 12 Concentration of antimony (Sb) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

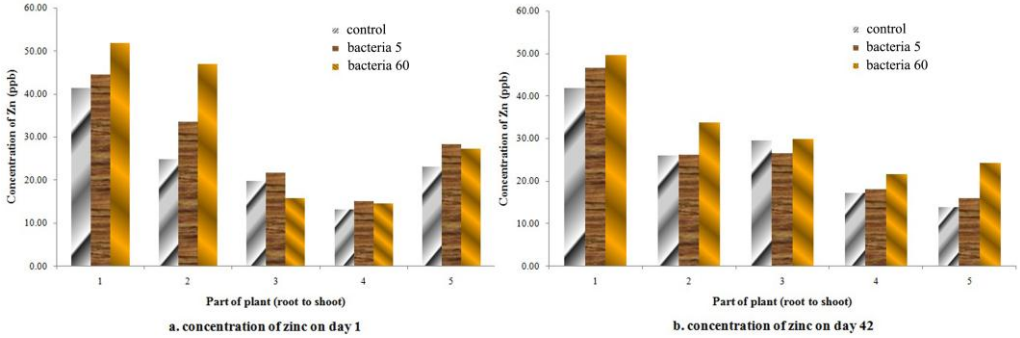

Fig. 13 Concentration of zinc (Zn) the plant sample (Scirpus mucronatus) after 1 and 42 days of growth with bacterial inoculums from Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) in lead-contaminated soil, and a control sample (Control) without the addition of bacteria. One and forty-two days after inoculation, the plant was harvested and cut into 5 sections of identical length from root (1) to shoot (5) and the concentrations of the elements were determined by inductively coupled plasma mass spectrometry.

Table 2. Comparison of concentrations of the toxic elements in Scirpusmucronatus for the two treatments of bacteria 5 and bacteria 60 versus control sample using one-way ANOVA test

Elements	One-way ANOVA (p-value)	Control		Bacteria 5		Bacteria 60	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Ag	0.504	0.44±0.32	0.16–1.20	0.37±0.17	0.18–0.62	0.75±0.72	0.31–2.68
As	0.575	21.51±54.28	0.32–175	7.75±14.71	0.40–48.37	12.17±24.03	0.45–77.82
Ba	0.000	35.45±11.44	22.83-62.08	50.77±35.34	13.64-108	54.69±25.62	27.79-116
Cd	0.972	0.35±0.47	0.02–1.49	0.47±0.71	0.08–2.39	0.41±0.46	0.06–1.45
Cr	0.571	3.58±2.89	1.33–10.88	2.89±1.63	0.99–6.60	3.42±1.97	1.56–7.99
Cu	0.282	5.39±2.62	2.72–9.78	5.48±2.44	3.66–10.56	7.54±3.91	2.49–13.79
Hg	0.296	0.85±0.57	0.35–2.11	1.26±1.27	0.55–4.45	1.50±1.03	0.58–4.07
Mn	0.013	628±457	170-1719	769±644	148-1606	892±633	234-1991
Mo	0.003	1.54±1.43	0.10–4.17	1.36±0.62	0.69–2.65	0.94±0.67	0.09–2.47
Ni	0.386	4.05±2.54	1.12–9.67	3.98±1.97	1.93–8.02	6.03±2.36	3.11–11.34
Pb	0.914	113±133	4.52-388	103±133	4.08-393	119±141	13.36-409
Sb	0.832	0.13±0.13	0.03–0.40	0.14±0.13	0.03–0.38	0.12±0.12	0.01–0.35
Zn	0.692	25.06±10.19	13.09-41.86	27.67±11.07	15.02-46.60	31.56±13.71	14.60-51.80

SD: standard deviation

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 [14]. Moreover, it may be due to interactive effect of elements in Scirpusmucronatus. Overall, the interaction at root level among some transition elements could be antagonistic, synergistic or multiplicative. Beckett and Davis [15] found that in barely, the toxic effect of Cu and Zn were antagonistic when the tissue concentration of these heavy metals surpassed a critical concentration. On the other hand, in vegetables grown in pots, high concentration of Zn in the soil solution was synergistic for Cd uptake. Ni combined with Cd, Mn, and Zn, presented a multiplicative effect in the reduction of wheat root growth. It is clear that the presence of Zn in the growing environment reduced the Ni toxicity to the alfalfa plants [16].

Since the data are nonparametric, one-way ANOVA test was utilized to evaluate statistical differences in the elemental concentration between bacteria 5, bacteria 60 and control samples. Comparison of elemental concentrations in all samples were demonstrated which there were statistically significant differences between all elemental concentrations except Ba, Mn and Mo with p-values of 0.000, 0.013, and 0.003 respectively (for other elements, $p>0.05$) as can be seen in Table 2. Pearson's correlation test was performed to find the correlation between trace element levels in bacteria 5 and bacteria 60 versus control sample [12].Fig. 14 and Fig. 15 exhibit correlations between the elemental concentrations found in bacteria 5 and bacteria 60 strongly correlate to results of control sample ($R>0.3$). To the best of our knowledge, there have been no studies describing the relationship between elemental concentrations in bacteria 5 and bacteria 60 versus control sample yet. In the present study, we found a significant positive correlation between elemental concentrations in bacteria 5 and bacteria 60 versus control sample in plant Scirpusmucronatus.

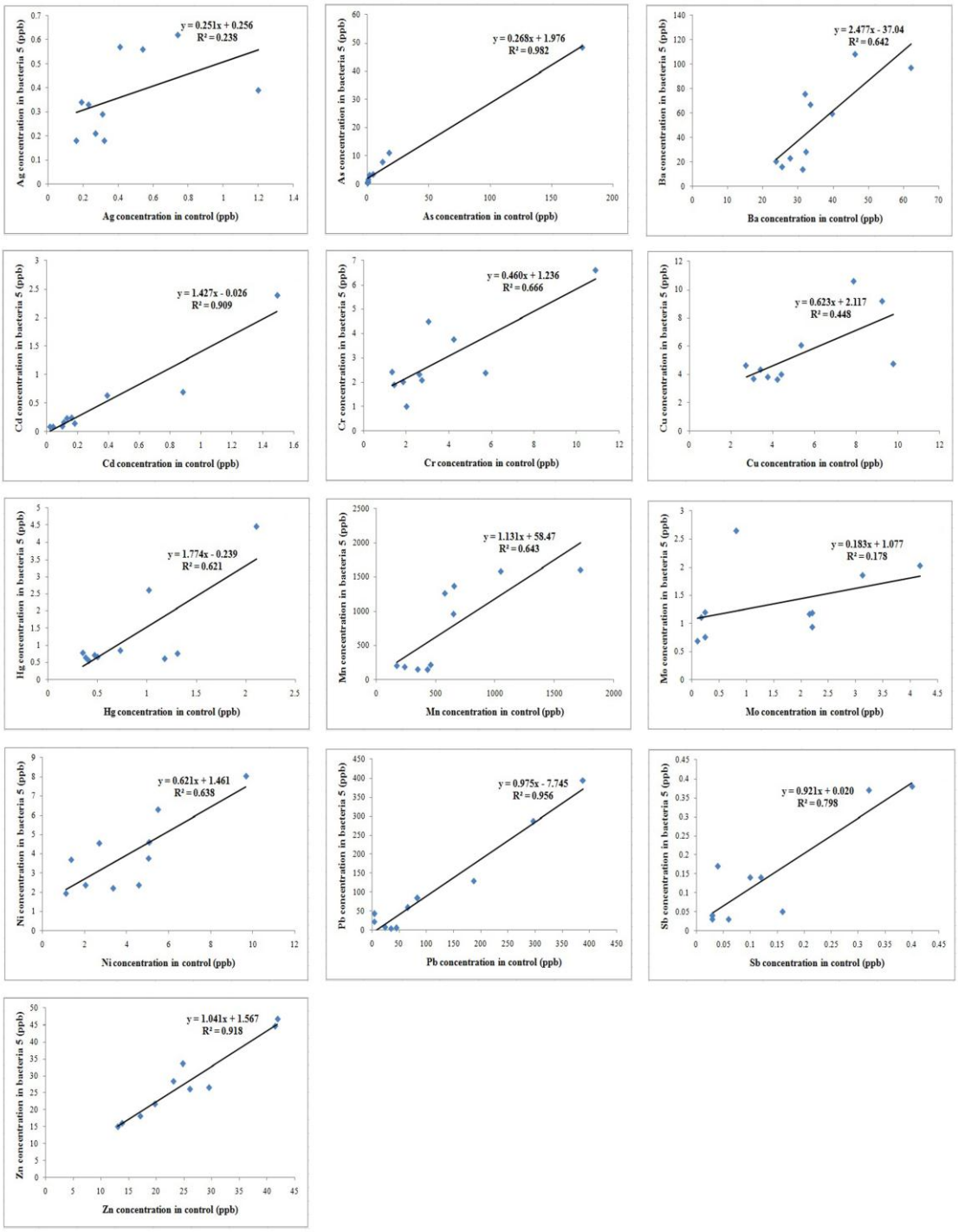

Fig 14. Relationship between Ag, As, Ba, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, and Zn concentrations in bacteria 5 sample versus control sample. R is Pearson's correlation cofficient.

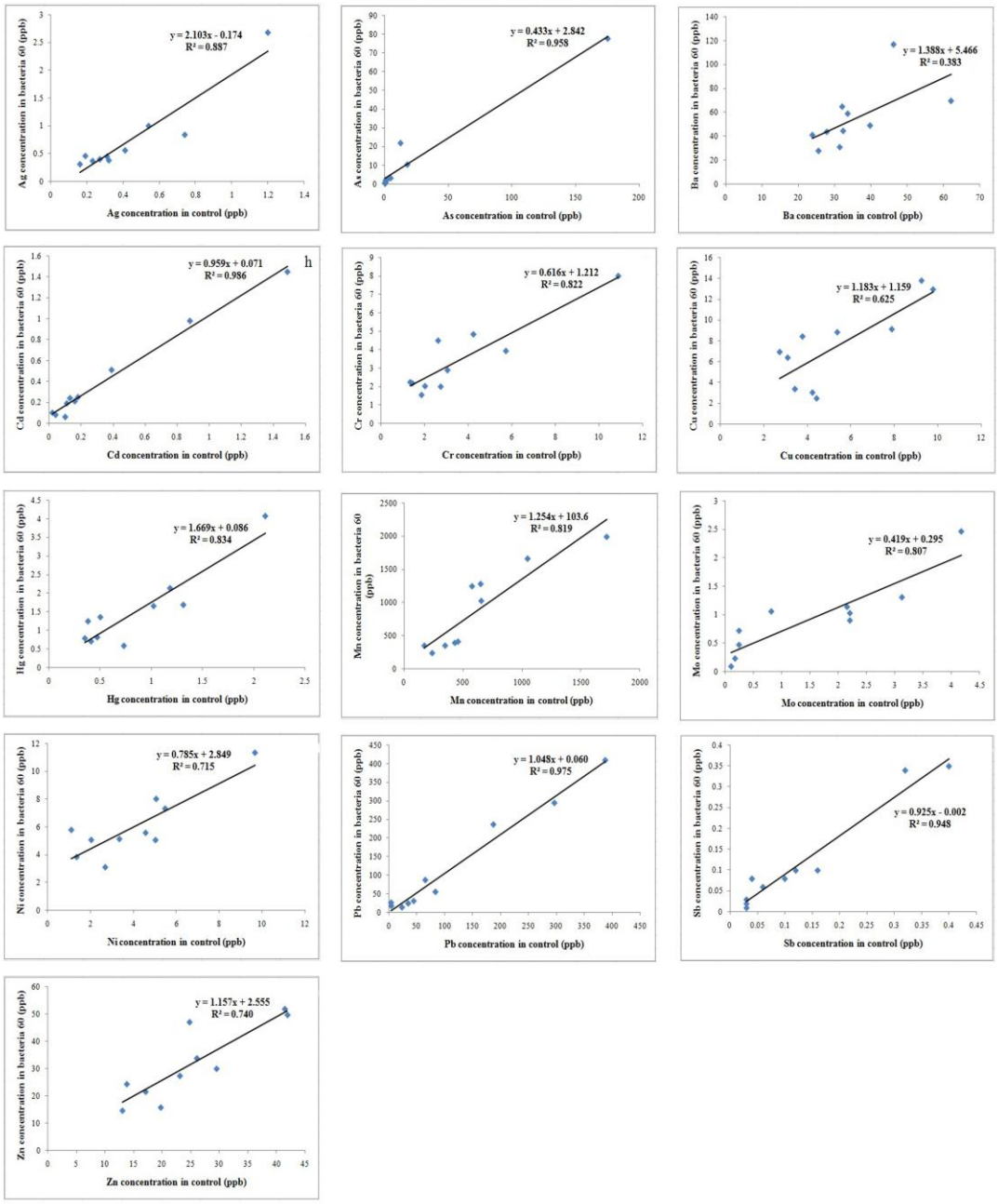

Fig 15. Relationship between Ag, As, Ba, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, and Zn concentrations in bacteria 60 sample versus control sample. R is Pearson's correlation confficient.

The tolerance and accumulation of metal vary from plant to plant as well as from species to species within a genus [17]. Therefore, the obtained results of this study may be useful as baseline on propertiesof phytoremediation of plant Scirpusmucronatus.

IV. Conclusion

This study investigated the effectiveness of two bacterium,Brevundimonasdiminuta and Alcaligenesfaecalis, for promoting heavy metal uptakeby the plant Scirpusmucronatus growing in lead-contaminatedsoil. The results showedthat thetoxic elements (Ag, As, Ba, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, and Zn)were transportedfrom thesoil to the plant and that the concentrations of the elements in the plant tissues were higher in the samples inoculated with bacteria than in the un-inoculated control.Theconcentrations of the elements for all of the treatments were also higher after 42 days than after 1 day, particularlyin the shoots, except Ag, Cr, Hg and Ni.It may be caused by internal detoxification mechanism of Scirpusmucronatus, and the chemical properties of their elements that allow the use of the technology of phytovolatilization, interactive

effect of elements in Scirpusmucronatusas well.One-way ANOVA test proved which there were statistically significant differences between all elemental concentrations except Ba, Mn and Mo with p-values of 0.000, 0.013, and 0.003, respectively. Moreover, strong correlations between elemental concentrations in bacteria 5 and bacteria 60 versus control sample in plant Scirpusmucronatus were found using Pearson's correlation test. The obtained results of this study may be useful as database on properties phytoremediation of Scirpusmucronatus.As a result,utilizing of these two bacteriumin this local plant (Scirpusmucronatus) in Malaysia could be a useful and economical means of decreasing the toxic elements in the environment.

Acknowledgments

We would like to express our special gratitude and thanks to United Nations University-International Institute for Global Health for supporting us by project funding DPP-2013-021.

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