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INFLUENCE OF BACTERIA ON RAISING OF PLANT NUTRIENTS ABSORPTION FROM SOIL

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***Abstract*: Investigating various bacteria on elevating of plant nutrients absorption from soil can be useful for raising of plant's growing and phytoremediation. In this study, Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60) were examined for their ability to increase the plant nutrients absorption of Scirpus mucronatus in soil. Three sample treatments were utilized in this study. Two of the treatments enriched with bacteria 5 or bacteria 60, and the third treatment was growth without the addition of bacteria (control sample). After 1 and 42 days of enrichment, the Scirpus mucronatus was prepared, and the B, C, Ca, Cu, Fe, K, Mg, Mn, Mo, N, Ni, P, S, and Zn concentrations were determined using inductively coupled plasma mass spectrometry. To evaluate statistical differences of elemental concentration in our results, the one-way ANOVA test was used. Comparison of elemental concentrations in our samples demonstrated which there were statistically significant differences between concentration of all elements in bacterium and control samples except for Mn and Mo (for all elements, p>0.05). Moreover, our findings was proved the concentration of most of the elements in bacteria 5 and bacteria 60 were strongly correlated to the results in control sample using Pearson's correlation test (R>0.3). The results showed that bacterium increased the plant nutrients absorption from soil.**

***Keywords*: Plant nutrients, Scripus mucronatus, Brevundimonas diminuta and Alcaligenes faecalisin bacterium, ICP-MS**

I. INTRODUCTION

Plant nutrition is the study of the chemical elements and compounds that are necessary for plant growth, as well their external supply and internal metabolism. In 1972, E. Epstein [1] defined two criteria for an element to be essential for plant growth: (1) in its absence the plant is unable to complete a normal life cycle; (2) that the element is part of some essential plant constituent or metabolite. There are 14 essential plant nutrients. Carbon and oxygen are absorbed from the air, while other nutrients including water are obtained from the soil. Plants must obtain the following mineral nutrients from the growing media: (1) the primary macronutrients: nitrogen (N), phosphorus (P), potassium (K); (2) the three secondary macronutrients: calcium (Ca), sulphur (S), magnesium (Mg); (3) the micronutrients/ trace minerals: boron (B), chlorine (Cl), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), molybdenum (Mo), nickel (Ni) [1].

In this study, we utilized Scirpus mucronatus, a monocot weed in the Cyperaceae family. This plant is known by the general name of ricefield bulrush, as well as by its local name in Malaysia, 'rumput kerecut'. It is a perennial herb growing from a short, hard rhizome in shallow water and moist and wet terrestrial habitats. The triangular stems grow in thick clumps up to a meter tall with leaves that wrap around the base of the stem in sheaths, although they often do not have blades. This plant is very useful and appropriate on phytoremediation technique [2, 3].

Plant nutrition is a difficult subject to understand completely, partially because of the variation between different plants and even between different species or individuals of a given clone. An element present at a low level may cause deficiency symptoms, while the same element at a higher level may cause toxicity. Further, deficiency of one element may present as symptoms of toxicity from another element. An abundance of one nutrient may cause a deficiency of another nutrient. Also a lowered availability of a given nutrient, such as SO_2^{4-} can affect the uptake of another nutrient, such as NO_3^-. Also K^+ uptake can be influenced by the amount NH_4^+ available [1].

The aim of this study was to assess the effect of two bacterial inoculate, Brevundimonas diminuta (bacteria 5) and Alcaligenes faecalis (bacteria 60), on the increase the plant nutrients absorption of Scirpus mucronatus from soil, as well provide database of plant Scirpus mucronatus.

II. EXPERIMENTAL METHOD

The Scirpus mucronatus was propagated from seeds in a greenhouse at the Universiti Kebangsaan in Malaysia using garden soil in polyethylene crates (40 cm × 58 cm × 30 cm). After 5 weeks, bacteria 5 and bacteria 60 were added to the soil crates as well a crate with no bacteria (control). Samples of the Scirpus mucronatus were prepared 1 and 42 days after the addition of the bacteria to the soil. The samples were first rinsed in ultra-pure water (18.2 MΩ quality, model: LPTA/PB/7/1 - Maxima Ultra-Pure Water, ELGA company, Italy) to remove dust-borne contamination, after which each plant was cut crosswise from root to shoot into 5 sections of equal length before drying in an oven at 90 °C for 2 days. Subsequently, 200 mg samples were placed in heat-resistant tubes, and 5 mL 69% HNO3 and 3 mL H2O2 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 each sample by inductively coupled plasma mass spectroscopy (ICP-MS) [2]. 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 [4]. 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.

Statistical analyses were performed with IBM SPSS software version 22. A one-way ANOVA with the significance level set at 95% was employed to identify the factors influencing B, C, Ca, Cu, Fe, K, Mg, Mn, Mo, N, Ni, P, S, and Zn concentrations in our samples. P-values <0.05 were considered no statistically significant for this test. Pearson's correlation test was performed to examine the correlation between elemental concentrations in bacteria 5 and bacteria 60 versus those in control sample [5].

III. RESULTS AND DISCUSSION

Table 1 has presented mean value of elemental concentrations in our samples. As well, Figure 1 shows the ratio of B, C, Ca, Cu, Fe, Mg, Mn, Mo, N, Ni, P, S, and Zn concentration in Scirpus mucronatus enriched with bacteria 5 and bacteria 60 versus control sample. Our findings demonstrated plant nutrients absorption from soil was increased using bacteria 5 and bacteria 60 for most of the elements. Bacteria 5 increased plant nutrients absorption from soil for C, Ca, Cu, Mn, and Zn; on other hand, bacteria 60 was useful for raising of C, Ca, Cu, Mn, N, Ni, S, and Zn absorption from soil. Therefore, bacteria 60 is more effective than bacteria 5 in Scirpus mucronatus nutrients absorption from soil. Absorption of other elements in enriched samples by bacteria 5 and bacteria 60 were decreased than control sample. 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. Beckett and Davis [6] 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 [7]. It is clear that the presence of Zn in the growing environment reduced the Ni toxicity to the alfalfa plants. Yuji and Kazuo [8] reported that in rice plants, the presence of zinc reduced the toxicity of nickel.

To evaluate the statistical differences in the elemental concentrations of our samples, the one-way ANOVA test was used [5]. Present results demonstrated statistically significant differences were found for all elements (for all elements, $p>0.05$) except Mn and Mo with p-values 0.013 and 0.003 respectively (as presented in Table 1). Pearson's correlation test was used to find the correlation between trace element levels in bacteria 5 and bacteria 60 versus control sample. Table 1 shows that correlations between the elements found in bacteria 5 strongly correlate the results in control sample ($R>0.3$) for elements Ca, Cu, Fe, Mg, Mn, Mo, Ni, and Zn. As well, correlation of all elemental concentrations in bacteria 60 and control except for B concentration ($R=0.229$). Figures 2 and 3 exhibit regression between elemental concentrations in bacteria 5 and bacteria 60 versus control sample. 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. In the present study, we found a significant correlation between elemental concentrations in bacteria 5 and bacteria 60 versus control sample in plant Scirpus mucronatus (Figure 2 and Figure 3).

The plant nutrients absorption from soil varies from plant to plant as well as from species to species within a genus [9]. Therefore, the obtained results of this study may be useful as database on Scirpus mucronatus plant nutrients absorption from soil.

Element	One-way ANOVA test (p-value)	Mean value in control	Mean value in bacteria 5	Pearson's correlation coefficients	Mean value in bacteria 60	Pearson's correlation coefficients
B	0.110	9.03	7.15	R=0.216, P=0.549	8.6	R=0.229, P=0.525
C	0.424	6100	6373	R=0.273, P=0.446	6284	R=0.586, P=0.075
Ca	0.671	2630	2681	R=0.400, P=0.913	2664	R=0.550, P=0.099
Cu	0.282	5.39	5.48	R=0.670, P=0.034	7.54	R=0.791, P=0.006
Fe	0.653	2277	1455	R=0.986, P=0.000	1832	R=0.924, P=0.000
Mg	0.442	1078	884	R=0.524, P=0.120	981	R=0.755, P=0.012
Mn	0.013	629	770	R=0.802, P=0.005	892	R=0.905, P=0.005
Mo	0.003	1.54	1.36	R=0.422, P=0.224	0.94	R=0.898, P=0.000
N	0.734	127757978	64214939	R=0.243, P=0.449	130592714	R=0.566, P=0.088
Ni	0.386	4.05	3.98	R=0.779, P=0.006	6.03	R=0.846, P=0.002
P	0.610	687	424	R=0.342, P=0.334	572	R=0.737, P=0.015
S	0.122	56697	21422	R=0.031, P=0.932	73295	R=0.929, P=0.000
Zn	0.692	25.06	27.67	R=0.959, P=0.000	31.56	R=0.860, P=0.001

Table I. Comparison of elemental concentrations of plant nutrients in Scirpus mucronatus for the two treatments of bacteria 5 and bacteria 60 versus control sample

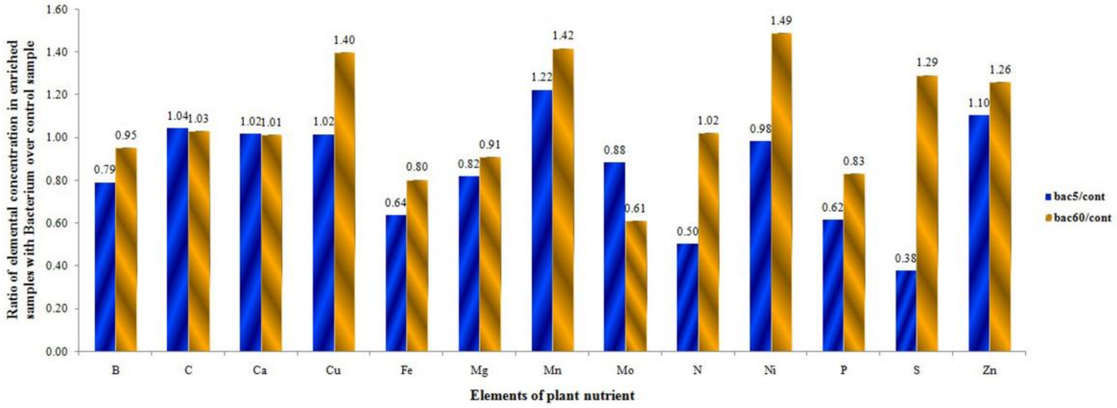

Fig. 1. Ration of elemental concentration in enriched samples with bacteria 5 and bacteria 60 versus those in control sample

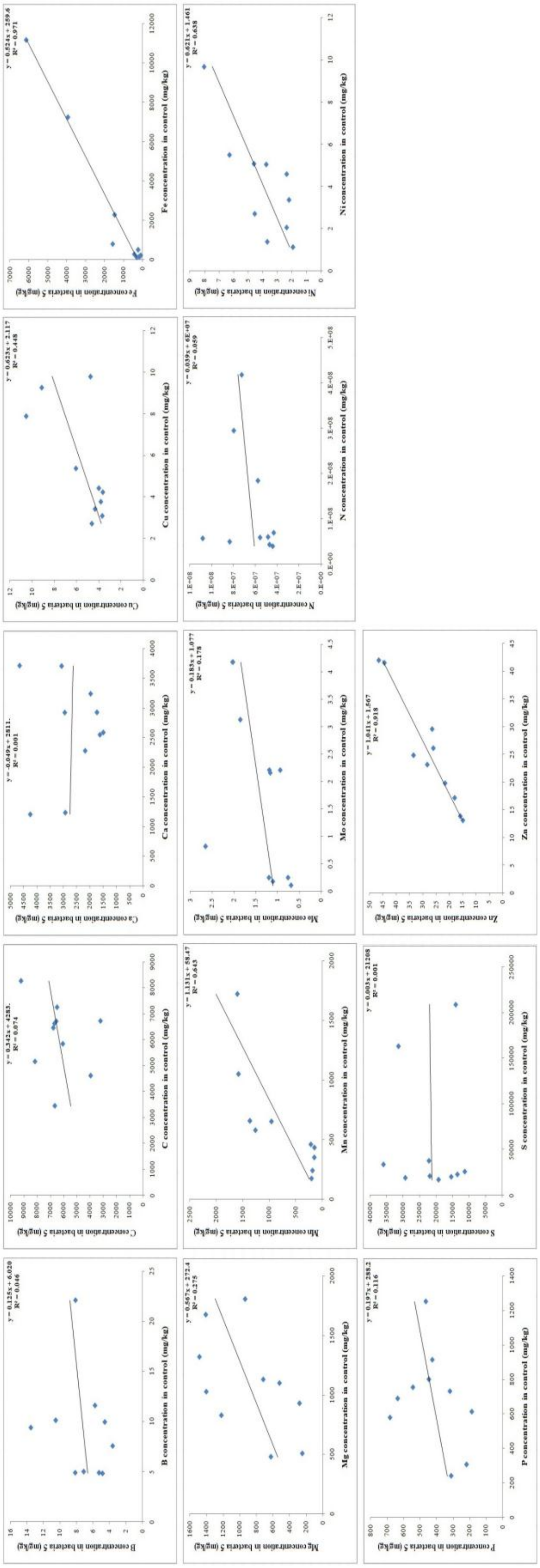

Fig. 2. Relationships between B, C, Ca, Cu, Fe, Mg, Mn, Mo, N, Ni, P, S, and Zn concentrations in bacteria 5 samples versus control sample.

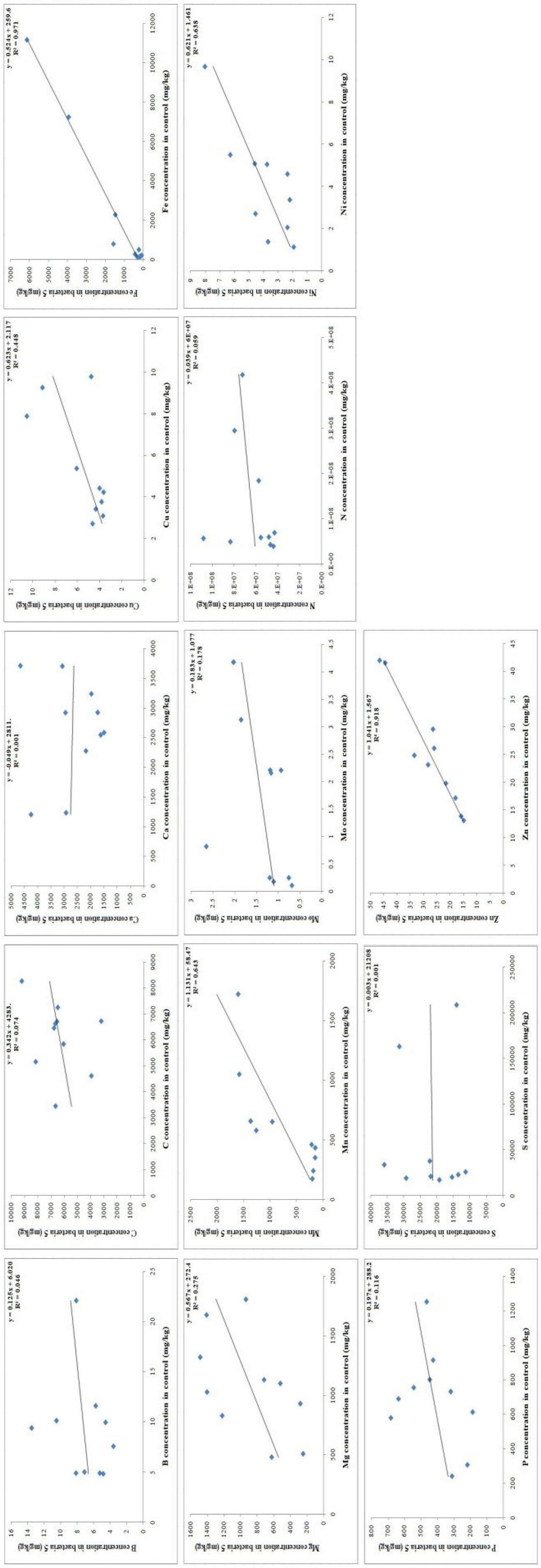

Fig. 2. Relationships between B, C, Ca, Cu, Fe, Mg, Mn, Mo, N, Ni, P, S, and Zn concentrations in bacteria 5 samples versus control sample.

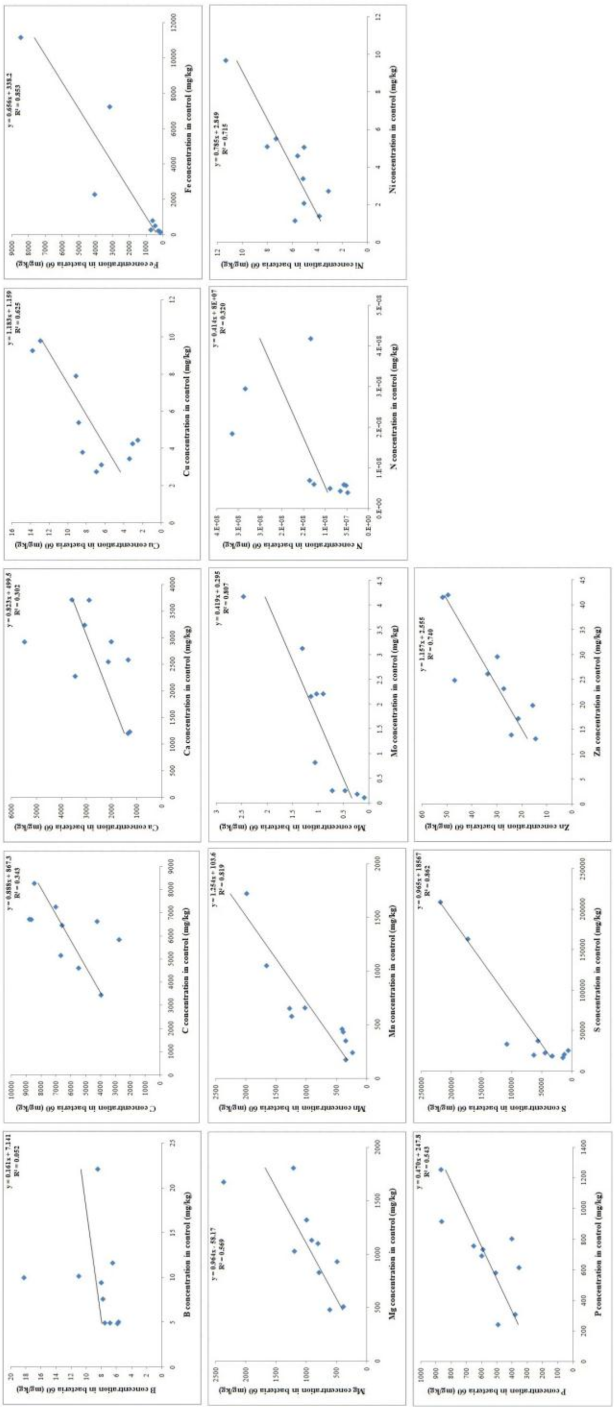

Fig. 3. Relationships between B, C, Ca, Cu, Fe, Mg, Mn, Mo, N, Ni, P, S, and Zn concentrations in bacteria 60 samples versus control sample.

IV. Conclusion

This study investigated the effectiveness of two bacterium, Brevundimonas diminuta and Alcaligenes faecalis, for Scirpus mucronatus nutrients absorption from soil. Bacteria 5 increased plant nutrients absorption from soil for C, Ca, Cu, Mn, and Zn; as well bacteria 60 was useful for raising of C, Ca, Cu, Mn, N, Ni, S, and Zn absorption from soil. Absorption of other elements in enriched samples by bacteria 5 and bacteria 60 were decreased than control sample. It may be due to interactive effect of elements in Scirpus mucronatus. One-way ANOVA test proved which there were statistically significant differences between most elemental concentrations in our findings ($p>0.05$). Moreover, strong correlations between elemental concentrations in bacteria 5 and bacteria 60 versus control sample in plant Scirpus mucronatus were found using Pearson's correlation test. Therefore, the obtained results of this study may be useful as database on Scirpus mucronatus plant nutrients absorption from soil.

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