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ENHANCING GROWTH CHARACTERISTICS AND ACCUMULATION POTENTIAL OF BEACH MORNING GLORY (*Ipomoea pes-caprae*) USING *Bacillus subtilis*

Naegil P. Acinas, Jr.*, Leo Gabriel V. Bautista, Wency Vience L. Tagsip and Jerry T. Cuadrado

Bayugan National Comprehensive High School – Science, Technology, Engineering and Mathematics, Bayugan City, 8502 CARAGA, Philippines

*Corresponding author: acinasken@gmail.com

ABSTRACT – This study was carried out to determine the effect of *Bacillus subtilis* to the physical growth characteristics of *Ipomoea pes-caprae* under different nickel concentrations (25, 50, 100 ppm). Plants were stem-cut and pre-planted for seven days, before subjecting to pot experimentation for a duration of three weeks using a completely randomized design with three replications. Results of the study revealed that plants grown in nickel-contaminated soil with *Bacillus subtilis* significantly increased its shoot length (48±2, 42.87±0.81, 39±1.73) and root length (36.53±1.53, 33.33±1.15, 26.17±0.76) compared to the plants grown in nickel-contaminated soil without *Bacillus subtilis;* shoot length (27±1.73, 25.67±0.58, 21±1) and root length (16.67±0.58, 15±3, 11.67±1.53). On the other hand, nickel concentration in the leaves of *I. pes-caprae* was higher in plants with *B. subtilis* (84ppm, 58ppm, 70ppm) compared to the plants without *B.* subtilis (48 ppm, 54 ppm, 82 ppm). *Bacillus subtilis* help *Ipomoea pes-caprae* to grow physically and become resistant even at 100 ppm of nickel concentration, thereby enhancing its accumulation potential to heavy metal. This work suggests that *Ipomoea pes-caprae* is a potential bioaccumulator and *Bacillus subtilis* is one of the most auspicious plant growth promoter rhizobacteria. Further investigation concerning the capability of *I. pes-caprae* as bioaccumulator using different heavy metals is highly recommended.

Keywords: Bioaccumulator, heavy metal contamination, phytoremediation, rhizobacteria

INTRODUCTION

Environmental contaminations are becoming a serious problem as a result of industrial and agricultural practices that contribute to the continuous deposition of pollutants into the environment (Wuana & Okieimen, 2011). One of these industrial activities is mining. Mining is the process of extracting useful minerals from the surface of the earth, including the seas (Hustrulid, 2017). Leaching of toxic constituents, such as arsenic, selenium, and metals, can occur even if acidic conditions are not present. Elevated levels of cyanide and nitrogen compounds (ammonia, nitrate, and nitrite) can also be found in waters at mine sites, from heap leaching and blasting (Norgate & Rankin, 2000). Heavy metals are among the contaminants released by mining operations in the environment. Migration of these contaminants into non-contaminated areas as dusts or leachates through the soil and spreading of heavy metals containing sewage sludge are some examples of events contributing towards the contamination of the ecosystem (Gaur & Adholeya, 2004). The threat that heavy metals pose is aggravated by their persistence in the environment and their tendency to bio-accumulate in the food chain (Nazir *et al.*, 2011).

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There are already various conventional remediation technologies that are used to clean heavy metal polluted environment such as soil incineration, excavation and landfill, soil flushing, solidification, and stabilization of electrokinetic systems. Though the said technologies are readily available, they are both costly and are heavy contributors towards the occurrence of secondary pollution.

Phytoremediation is an evolving field of science and technology to clean up polluted soil or water (Meagher, 2000). Phytoremediation is described as a natural process carried out by plants in cleaning up and stabilization of contaminated soils and ground water (Paz-Alberto & Sigua, 2012). Generally, phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root system, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body (Hinchman *et al*., 1995).

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and or indirectly. In last few decades a large array of bacteria including species of Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthobacter, Burkholderia, Bacillus and Serratia have reported enhancing plant growth (Glick *et al*., 1999).

This study addressed the need of such remediation technique by providing an alternative and environment friendly approach using plants to rehabilitate degraded areas. This study mainly focused on the investigation of the effect of *Bacillus subtilis* on the plant's physical growth and development. Specifically it aimed to determine and compare the growth characteristics of *Ipomoea pes-caprae* grown in nickel contaminated soil; and determine the level of nickel concentrations in *Ipomoea pes-caprae* with and without *Bacillus subtilis*.

MATERIALS AND METHODS

Sample Collection Sites

Soil samples were collected in Barangay Agata, Santiago, Agusan del Norte, Philippines and Plants sample in Aras-asan, Cagwait, Surigao del Sur, Philippines.

Plants and soil Collection and processing

The collected soil was freed from stones, pounded into fine particles, sieved and air-dried for 3 days in order to obtain a homogeneous soil mixture. The soil was then sterilized (121° C at 15 psi above atmospheric pressure for 30 minutes) using autoclave. Part of the soil was brought to the Department of Agriculture, Taguibo, Agusan del Norte, Philippines for physico-chemical analysis. The experimental plant was chosen based on the characteristics identified by Watanabe (1997) for phytoremediation: fast growing, has dense root system and has a high above ground biomass. In this regard, *I. pes-caprae* was chosen as the experimental plant as it satisfied the mentioned characteristics. *Ipomoea pes-caprae* plant were stem-cut at about 18 cm to obtain uniform length and washed with distilled water to remove unnecessary materials.

Pot Experimentation

A pot experiment was conducted to assess the physical growth characteristics of *I. pes-caprae*. Experiment was carried out on a completely randomized design with three replications (Heshmatpure & Rad, 2012). To ensure that the experimental plants were grown, it was pre-planted in the pots with a

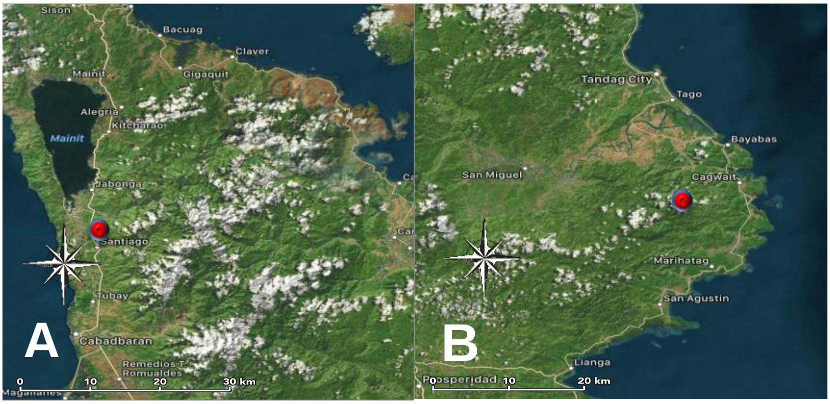

Figure 1. Map showing the collection site of **A.)** Soil Samples in Barangay Agata, Santiago, Agusan del Norte, Philippines and **B.)** Plant Samples from Aras-asan, Cagwait, Surigao del Sur, Philippines.

dimension of 21.2 cm x 18.2 cm x 13 cm for seven days before experimentation. The soil of the potted stem-cuts of *I. pes-caprae* was contaminated with 100 mL of 25, 50, and 100 ppm nickel concentrations prepared from $NiCl_2$. Separate pots of uncontaminated soil were used to serve as the control setup. On the other hand, inoculates of *B. subtilis* were added to the contaminated and non-contaminated soil samples. The plants were grown in a greenhouse and watered with 500 mL of water every two days and harvested after three weeks.

Evaluation of Physical Characteristics of I. pes-caprae

The plant shoots were measured weekly and root length was measured after three weeks of pot experiment using tape measure in cm. The measured shoot length and root length were then compared on each treatment.

Analysis of Nickel Concentration in Plants

After three weeks of growing in the pot experiments, leaves of *I. pes-caprae* were separated from the plant and carefully washed with tap water in order to remove any surface soil or dust deposits. After washing, plant samples were air dried at room temperature for one day, placed in zip plastic bags and brought to the Department of Agriculture, Butuan City, Philippines for the analysis of nickel concentration through Atomic Absorption Spectrophotometry.

Statistical Analysis of Data

Mean of plant shoot and root length as well as nickel concentrations were statistically analyzed. T-test was employed at 0.05 % level of confidence to test the differences of the said parameters.

RESULTS AND DISCUSSION

Plant Growth Characteristics

Shoot and root length of *I. pes-caprae* were measured to determine the impact of different nickel concentrations in the plant. Results of physical characteristics of *I. pes-caprae* are shown in Table 1.

Table 1. Physical growth characteristics of *I. pes-caprae.*

Physical Growth Characteristics	*Control*	Without *B. subtilis* in Different Nickel Concentrations			With *B. subtilis* in Different Nickel Concentrations		
		25	50	100	25	50	100
Plant Height (cm)	31.5 ± 1.32	27 ± 1.73	25.67 ± 0.58	21 ± 1	48 ± 2*	42.87 ± 0.81*	39 ± 1.73*
Root Length (cm)	20.67 ± 2.08	16.67 ± 0.58*	15 ± 3*	11.67 ± 1.53*	36.33 ± 1.53*	33.33 ± 1.15*	26.17 ± 0.76*

*Significantly different at P=0.05.

The results revealed that plants grown in nickel-contaminated soil with *Bacillus subtilis*, significantly increased its height compared to the plants without *B. subtilis*. This could be attributed to various direct and indirect mechanisms of PGPR that can affect plant growth such as increased solubility of mineral nutrients and nitrogen fixation making nutrients available for the plant and improving plant stress tolerance to drought, salinity, and metal toxicity through the production of phytohormones (Gupta *et al*., 2000).

Further, the results also revealed that *I. pes-caprae* with *B. subtilis* significantly increase its root length. The results were parallel to the study of Sengupta *et al.* (2015) where the seedling of Zea mays inoculated with single strains of *Bacillus subtilis* exhibited higher increases compared to the un-inoculated control. This is because the auxin produced by the rhizobacteria can positively influence the development of the root system, and then contributes to improve essential nutritive elements absorption for the plant growth (Ahemada & Kibret, 2014).

Effect of Bacillus subtilis in nickel accumulation potential of Ipomoea pes-caprae

Plants grown in different nickel concentration (25 ppm, 50 ppm, 100 ppm) with *Bacillus subtilis* have a concentration of 84 ppm, 58 ppm, and 70 ppm respectively, while plants without *Bacillus subtilis* have a concentration of 48 ppm, 54 ppm, and 82 ppm, as shown in Figure 2. According to Salt and Kramer (1999), plants can develop mechanisms by which they can effectively absorb metals from the soil solution and transport them to other parts within the plant. *Bacillus subtilis* helps the plant to become resistance to such elevated levels of heavy metals (Tak *et al.,* 2013). This ability to live and grow under in the presence of high metal concentration exists in many rhizospheric microorganisms.

PGPR have developed a range of mechanisms such as exclusion-metal ions that are kept away from target sites, extrusion-metals that are pushed out of the cell through chromosomal/plasmid mediated events, accommodation metals that form complexes with metal-binding proteins, and other cell components, Biotransformation, in which the toxic metal is reduced to less toxic forms, and methylation and demethylation (Kao *et al*., 2006; Umrania, 2006). One or more of the above-mentioned mechanisms allow the microbes to function metabolically in metal-contaminated sites/soils. Interest in exploiting these bacterial properties to remediate heavy-metal contaminated sites is growing, and early results from their application are promising (Lloyd & Lovley, 2001; Hallberg & Johnson, 2005).

Accumulation rate of plants greatly increase under *Bacillus subtilis*. Some microbial communities have the ability to sequester heavy metals, and therefore may be useful for bioremediating contaminated areas (Hallberg & Johnson, 2005; Umrania, 2006). When microbes are used to bioremediate a contaminated site, plant-associated bacteria can be potentially used to improve phytoextraction activities by altering the solubility, availability, and transport of heavy metals, and nutrients as well, by reducing soil pH and releasing chelators (Ma *et al*., 2011).

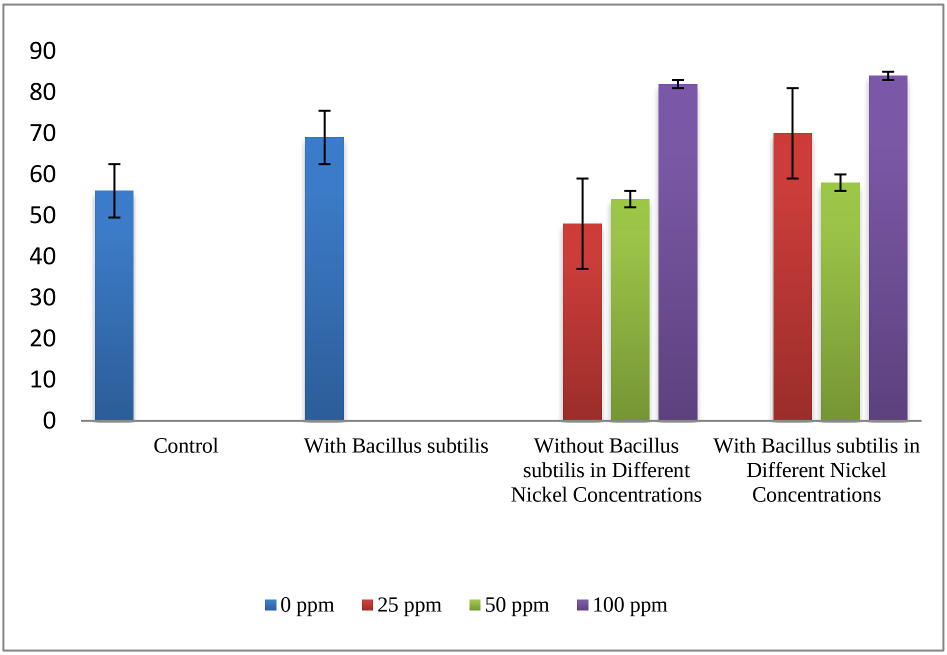

Figure 2. Concentration of Nickel in the leaf tissues of *I. pes-caprae* grown in a pot experiment with and without *Bacillus subtilis*.

Physicochemical properties of the Soil

Physicochemical properties of soil were also tested prior to the pot experimentation. Results of the test are shown in Table 2. The soil pH is a measure of the acidity and alkalinity in the soil. pH levels range from 0 to 14. The neutral measure of pH in the soil is 7. Below 7 is acidic and above 7 is alkaline. In this case, the measured soil pH is 6.55 which is slightly acidic, Texture is heavy, Organic matter is moderately low, Low P, Deficient K, Ca, and Mg, Moderately deficient Zinc and Sulfur.

Table 2. Physicochemical properties of the soil used in the pot experiments.

Soil pH	Texture	Organic Matter (%)	Available P (P_2O_5), ppm	Extractable K (K_2O), ppm	Ca (Qualitative Method)	Mg (Qualitative Method)	Zinc	Sulfur
6.55 (Slightly Acidic)	Heavy	2.5 (Moderately low)	2 Low	48 Moderately Deficient	Deficient	Deficient	Moderately Deficient	Moderately Deficient

Moreover, *I. pes-caprae* tolerated the soil conditions as depicted in the physicochemical analysis of the soil used in the pot experiments such as the marginal exchangeable K, deficient P and very low total N, which may limit plant growth. Furthermore, the high nickel accumulation of *I. pes-caprae*, its ability to tolerate nickel and other soil conditions which may limit plant growth and its invasiveness, high growth rate and dense root system showed possibility that the plant can be used in the phytostabilization of nickel in heavy metal contaminated site such as mining areas.

CONCLUSION AND RECOMMENDATIONS

Based on the growth parameters investigated in the plant *I. pes-caprae* grown in nickel-contaminated soil, the results revealed that the plant height and root length were significantly affected by the concentrations of nickel in the soil.

The *Bacillus subtilis* was used to test its effect on the nickel accumulation potential of *I. pes-caprae*. The inoculation of *Bacillus subtilis* on *I. pes-caprae* showed observable effects on the growth parameters evaluated. The height of *I. pes-caprae* under nickel-contaminated soil was significantly higher when *Bacillus subtilis* were introduced compared with the un-inoculated plants. Similarly, the inoculated *Bacillus subtilis* have significant effect on the root elongation of *I. pes-caprae* under nickel-contaminated soil. In addition, *I. pes-caprae* grown in nickel contaminated soil with *Bacillus subtilis* accumulated higher concentration of nickel compared to *I. pes-caprae* grown in nickel contaminated soil without *Bacillus subtilis*. These results could be attributed to the mechanism of PGPR that can affect plant growth, and its ability to sequester heavy metals, and improve phytoextraction activities of the plants.

Hence, *I. pes-caprae* could be a potential nickel hyper-accumulator plant, and when combined with *Bacillus subtilis* can be possibly used in the phytostabilization of nickel contaminated sites. Further investigation concerning the capability of *I. pes-caprae* as bio-accumulator using different heavy metals over a longer period is highly recommended.

STATEMENT OF AUTHORSHIP

N.P. Acinas, Jr., L.G.V. Bautista and W.V.L. Tagsip conducted the experiment, gathered the data and drafted the manuscript. J.T. Cuadrado performed the analysis and reviewed the paper. All authors discussed the results and commented on the manuscript.

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