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Effects of Heavy Metal-Tolerant Microorganisms on the Growth of "Narra" Seedlings

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Abstract: The effectiveness of heavy metal-tolerant microorganisms for supporting plant growth needs to be understood before it can be used as a soil bioremediation agent. The purpose of this study was to determine the effect of heavy metal tolerant microorganisms on the growth of "Narra" seedling (*Pterocarpus indicus* Wild). Three heavy metals-resistant (Pb, Cd, and Cu) rhizobacteria from a copper (Cu) mined-out site in Marinduque, Philippines showed plant growth promotion in vitro. A treatment combination of formula inoculant A (CuNFbM 4.1, MGR 333), B (CuNFbM 4.1, MGR 333, PbSM 2.1), and O (Uninoculated); compost (0%, 4%); and lime + inorganic fertilizer {without or with lime and inorganic fertilizer (LF0; LF1)} were applied to Narra seedlings planted on 445 mg/kg Cu-contaminated soil. Lime (2 mg/ha) and the recommended dose of soybean inorganic fertilizer were used as positive controls to evaluate the ability of inoculations and composts to promote the growth and used as positive controls to evaluate the ability of inoculants and composts to promote the growth and copper accumulation of narra in greenhouse experiments. All treatment combinations resulted in significant differences in plant height, leaf number, stem diameter, shoot and root dry weight, as well as, shoot, root Cu content, and plant Cu uptake of 13-week-old "Narra". Inoculated "Narra" could thrive better in mine-degraded soil containing 445 ppm Cu with 4% compost. Inoculant B demonstrated the best plant performance while *Pseudomonas synxantha* (PbSM 2.1) probably increases the plant's growth due to 1-aminocyclopropane-1-carboxylate (ACC) deaminase it produces. Accumulation of Cu was higher in the root compared other plant parts. More research is necessary to elucidate the mechanism of plant growth promotion and heavy metal re mediation by *P. synxantha*.

Keywords: heavy metal; tolerance; microorganism; growth; "Narra" seedling

1. Introduction

Mining activities in many countries have brought about environmental issues such as physical damage to soil, and pollution of terrestrial and aquatic environments that threaten human, animal, and plant life. Heavy metal (HM) pollution and land marginalization due to removal of the original soil along with its organic matter (OM) and associated nutrients, as well as the unstable condition of the land are also problems posed by mining.

These problems have raised great interest in coming up with environment-friendly and sustainable agriculture, since these are linked with food security. Hence, the restoration of these soils is essential to restore biodiversity and ecosystem integrity [1]. The use of plant growth-promoting rhizobacteria holds promise as a means of bringing back the ability of the soil to sustain plant growth.

Bioremediation is the process of removing contaminants from the environment, such as soil, or converting them into nontoxic forms using bacteria, fungi, and plants, in order to restore the soil to its pristine, pollution-free and productive environment. Increased beneficial soil microbes are important in nutrient recycling, degradation of pollutants and organic materials and maintenance of soil health. Improvement of soil health means a good amount of organic matter that help increase cation exchange capacity (CEC), good soil pore formation and water holding capacity (WHC) [2]. Phytoremediation efforts have been tried to remedy the situation of HM-contaminated soil [3–7]. Phytoremediation refers to the use of plants to absorb, accumulate, stabilize or volatilize HM contaminants from soil [3,8]. In phytoremediation, HM-resistant bacteria with growth-promoting properties contribute to plant fitness and its survival in harsh soil environments, such as drought and HM pollution, through nutrient and phytohormone supply, as well as biofilm formation and ACC deamination enzymes produce [9–11]. The synergic approach among microorganism-OM-plant in soil bioremediation is an effective strategy to rehabilitate mine-degraded soil and to improve the soil quality in terms of physical, chemical, and biologic characteristics [2,8].

Degraded soils from mines have been shown to exhibit high level of heavy metals, low pH, reduced supply of nutrients (i.e., nitrogen, phosphorus, potassium), in addition to lack of organic matter, malformed soil structure, and truncation of microbial communities. Under these conditions, plants used for phytoremediation often do not grow well and hardly survive [9].

Several studies reported that HM-resistant bacteria function as growth promoter and enhancer for a plant to accumulate HM [12,13], increasing the length of root and shoots, as well as increasing the fresh or dry weight plants [9,14]. Some copper-tolerant rhizosphere bacteria have been isolated from talahib (*Saccharum spontaneum*) growing in the rhizosphere of the Marinduque copper mine in the Philippines. These rhizobacteria displayed plant growth-promoting properties in vitro and were identified based on 16S rDNA sequence analysis as *Fulvimonas yonginensis* (CuNFb M4.1), *Rhizobium* sp. (MGR 333), and *Pseudomonas synxantha* (PbSM 2.1) [15,16].

"Narra" (Philippines) or Angsana (Indonesia) (*Pterocarpus indicus* Will) belongs to the family *Fabaceae* (Papilionoideae), is an endangered species native to Asian regions [16]. The natural distribution of this tree began from Burma to Southeast Asia up to the Philippines and the Pacific islands and it is widely cultivated in the tropics. "Narra", which is recommended as one of the trees in agroforestry provides shade to coffee and other crops and is adaptable in drought land areas [17]. This plant has ecological, industrial, and economic importance in Southeast countries due to its use in greening program, in medicine, in agroforestry, and as a source for natural dye, and furniture staff [18–27]. This plant has potential for phytoremediation of heavy metal compounds such as hexavalent chromium [28] or Cadmium [29].

This study was carried out because of the need to specifically test the synergistic effect of HM-resistant plant-promoting rhizosphere rhizobacteria, compost amendments on the growth of "Narra" trees in phytoremediation of copper-contaminated soils. Specifically, this research aimed to determine the effect of inoculation of the selected isolates on the growth of "Narra" by measuring different growth parameters such as plant height, midline length of stem, leaf number, as well as the dry weight of root and shoot part. In addition, the ability of narra to adsorb Cu on artificial copper-contaminated soil was tested by analyzing the presence of HM in the shoots, roots and soil before and after the pot experiment. The effect of inoculation on the pH and cation exchange capacity (CEC) of the soil before and after the experiment were also ascertained.

2. Materials and Methods

The research was conducted in a greenhouse at the Indonesian Agricultural Biotechnology and Genetic Resources Center in Bogor. The soil was collected from Kentrong, Banten Province, Indonesia. The soil composite at a depth of 0–20 cm was taken from ten points on an area of 1500 m^2 using scoops, labelled, and packed in airtight polypropylene woven sack bag and then transferred to the Soil Biology Laboratory of Indonesian Soil Research Institute, and stored at sample room (temperature at 21 °C, humidity at 50%, with 12 h:12 h (L:D)) until used for the next experiment. Prior to soil analysis and pot experiment, the soils were air-dried and passed through a 2 mm sieve before mixing. The homogenized soils were placed in airtight polypropylene woven sack bags stored in sample room.

The chemical characteristics of the soil before experiment were as follows: pH (H_2O) = 4.56; silty clay loam = 16% sand, 45% silt, 40% clay; total P_2O_5 = 14 mg·100 g^{-1}; available P = 2 ppm; soil organic carbon (SOC) = 2.13%; K_2O = 5 mg·100 g^{-1}; −1 CEC = 12.21 $cmol_{(+)}\cdot kg^{-1}$; Cu = 14 ppm; Cd = 1.25 ppm; and Pb = 3.35 ppm. The low pH and nutrient content reflected the situation of the mine-degraded soil from where the bacteria were taken. Kentrong soil was used as artificial soil in this study because in preliminary work, we found that the soil from Marinduque copper mined-outside area showed Cu concentrations which varied from 167 ppm to 776 ppm, due to homogenized soil samples containing copper ores in the form of fine granules. In order to provide measured and uniform soil-contaminating Cu, artificial soil was employed.

Three rhizobacteria used in this experiment, i.e., CuNFbM 4.1, MGR 333, and PbSM 2.1 are HM resistant and isolated from the Cu mining site in Marinduque. They demonstrated in vitro plant growth promoting properties such as biofilm formation (PbSM 2.1 and CuNFbM 4.1), N fixation, phosphate (P) solubilization and IAA production (all isolates), stress controller, ACC deaminase production (PbSM 2.1) [15,16]. The experiment used twelve treatments in a Randomized Complete Block Design with three replications and two subsamples. The treatments consisted of: (1). No need for lime inorganic fertilizer (LIF), compost and inoculation; (2). Without LIF and compost, use of inoculant consortium A (IA); (3). Without LIF and compost, with inoculant consortium B (IB); (4). Without LIF, with compost, and without inoculation (I0); (5). Without LIF, with compost and inoculant consortium A (IA); (6). Without LIF, with compost and inoculant consortium B (IB); (7) LIF, without compost and inoculation; (8). LIF, without compost and with inoculant consortium A (IA); (9). LIF, without compost and with inoculant consortium B (IB); (10). with LIF, compost, and without inoculation (I0); (11). With LIF, compost, and inoculant consortium A (IA); (12). With LIF, compost, and inoculant consortium B (IB).

The pot experiment consisted of 12 treatment combinations, 3 replicates, and 2 seedling sub samples, forming 72 experimental units. The experiment was carried out in a pot (3 × 3 × 13 cm) filled with 1 kg of air-dried soil. Soil preparation: the $CuCl_2$ solution was added to each kg of soil until the desired concentration was attained. Lime was applied two weeks before planting which coincided with the addition of the HM solution. The copper-treated soils were incubated for two weeks in order for the lime and copper to come to equilibrium. Fertilizers and compost were incorporated into the soil in the afternoon, a day before planting.

Seedling preparation: the seeds were soaked in water overnight and were uncoated. The uncoated seeds were placed in a container containing a mixture of sterile soil and compost (1:1) for germination. When the seedlings reach a height of about 6–10 cm, which as around 2–3 weeks, they were transplanted to the pots. Inoculant preparation and inoculation. The rhizobacteria were grown in nutrient broth (NB) up to 12 h or the log phase stage (10^9 cells/mL). Inoculant A consisted of MGR 333 and CuNFbM4.1, sterile dH_2O (1:1:1), whereas Inoculant B was a consortium of MGR 333, CuNFbM 4.1, and PbSM 2.1 (1:1:1). The total volume of both inoculants was 3 mL. Prior to mixing three bacterial cultures for each inoculant consortium, each broth culture was centrifuged (6000 rpm, 10 min) and the pellet was washed once with sterile dH_2O. To achieve the original volume of the broth, sterile dH_2O was added to the pellet and mixed with vortex.

The roots of the seedlings were soaked in the corresponding inoculants for 10 min, and then transplanted into soil holes in experimental pots. However, before adding soil to the pot, 10 mL of the inoculant was poured into the roots of the seedlings to further soak them. Afterwards, the roots of the seedling in the hole were covered with soil.

Planting and plant maintenance. Each inoculated seedling was transplanted immediately to a pot containing 1 kg of soil, one "Narra" seedling per pot. The potted experimental setups were placed in the greenhouse of ICABIOGRAD, Ministry of Agriculture, Indonesia. The plants were maintained for 13 weeks and were watered once every two days during the first four weeks and once a day during the last period by using tap water. About 100 mL of water was added, and no dripping was observed.

Growth parameters: the study assessed plant growth by measuring plant height, stem diameter, and counting the number of leaves, a day before the experiment was termited. After 13 weeks, the shoots were cut, and the plants were fully uprooted. Any remaining soil that adhered to the roots was removed and soil-detached the roots were cleaned with tap water. The shoot and root parts were separated and were placed individually in paper bags. All plant material were then placed in an oven at 70 °C for 3–5 days until a constant weight (dry weight) was attained.

Soil and plant tissue analysis. The soil, shoot, and root samples were analyzed for total Cu, soil pH, and CEC in the Soil Analytical Chemistry Laboratory of the Indonesian Soil Research Institute (ISRI). Soil parameters assay used in this study have been previously described in [30].

Statistical analysis. Data were analyzed by analyses of variance (ANOVA) using SPSS ver. 25 (www.ibm.com/legal/copytrade.shtml (accessed on 5 January 2022)). Differences among all parameters were tested using Duncan's Multiple Range Test (DMRT) if the ANOVA F showed a significant difference. The correlation between soil chemical characteristics and plant growth parameters, as well as the correlation test between Cu content in the soil and plant Cu concentration were evaluated.

3. Results

3.1. Soil Characteristics Used in the Greenhouse Experiment

The soil in the Cu mined-out site in Mogpog, Marinduque is characterized by low pH, low nutrient (i.e., N and K) contents, low OM, and high Cu contamination. As a consequence, there is a loss of biodiversity and biological productivity since most of the area is barren. Kentrong soil which was used in the greenhouse experiment (Table 1) reflected Marinduque soil from where the isolates were taken. In this experiment, the Kentrong soil was artificially contaminated with 445 ppm of Cu to reflect the situation of the Cu-contaminated soil in Marinduque.

Physico-chemical analyses of the Kentrong soil show that it has silty clay loam texture, acidic with pH 4.6, and high exchangeable aluminum content (4.01 $cmol_{(+)}$ kg^{-1}) which led to the high capability of soil in phosphorus fixation resulting inefficient phosphorus fertilization in the soil. Also, the high Al content level observed could be toxic to plant and microorganism growth. In acidic soil, such as Kentrong's soil (pH 4.6), ion H+ as exchangeable H^+ but could also attack the structure of mineral soil, releasing Al^{3+} in the soil which toxic to many organisms and has potency to fix P in soil [31].

Low CEC (12.21 $cmol_{(+)}$ kg^{-1}) and base saturation (31%), as well as high Al saturation value, indicated the inability of the soil to hold essential nutrients as shown by low to very low soil nutrient content, namely (N = 1.8%; P_2O_5 = 14 mg·100 g^{-1}; K_2O = 5 mg·100 g^{-1}; P available = 2 ppm). Liming and compost were needed to improve the fertility of this soil. Liming treatment was intended to neutralize exchangeable H^+ and Al^{3+}, and replacing the exchange sites with Ca, as well as toxicity Al, Mn, and Cu. Treatment of 4% compost was given as a pH-dependent CEC which would improve the ability of the soil in holding essential nutrients needed for plant growth, also as C for the growth and activity of the microorganisms. Fertilizer was included as a soil treatment to assess the efficacy of inoculation and compost application in improving soil productivity and plant growth. The effects of treatment to soil chemical characteristics are presented in Table 2.

Table 1. Chemical and Physical Characteristic of Ultisol Soil Sample of Kentrong, Banten Province, Indonesia (0–20 cm depth).

Soil Parameters	Unit	Value	Category
Textural Grade (pipet)			Silty Clay Loam
Sand	%	16	
Silt	%	45	
Clay	%	40	
pH (1:5, H_2O)		4.56	Acid
Organic matter			
C (Walkley and Black)	%	2.13	Medium
N (Kjeldahl)	%	0.18	Low
C/N	%	12	Medium
Extractant (HCl 25%)			
P_2O_5	mg·100 g^{-1}	14	Very low
K_2O	mg·100 g^{-1}	5	Low
P-Bray 1	ppm	2	Very low
Cation Exchangeable value (NH4-Acetate 1N, pH 7)			
Ca	$cmol_{(+)}\ kg^{-1}$	2.61	Low
Mg	$cmol_{(+)}\ kg^{-1}$	1.03	Low
K	$cmol_{(+)}\ kg^{-1}$	0.07	Very low
Na	$cmol_{(+)}\ kg^{-1}$	0.1	Low
CEC	$cmol_{(+)}\ kg^{-1}$	12.21	Low
Base saturation	%	31	Low
Exchangeable (KCl 1 M)			
Al^{3+}	$cmol_{(+)}\ kg^{-1}$	4.01	
H^+	$cmol_{(+)}\ kg^{-1}$	0.14	
Al saturation	%	32.84	High
Heavy Metal (ppm)			
Cu	ppm	14.00	
Cd	ppm	1.25	
Pb	ppm	3.35	

Table 2. Treatment Effects on Soil Chemical Characteristics and Soil Cu Content.

No	Treatments *	pH **	CEC (cmol(+)kg^{-1}) **	Soil Cu (mg·kg^{-1}) **
1	LF0 C0 I0	4.7 fg	14.53 ab	338.5 ab
2	LF0 C0 IA	4.5 g	14.48 ab	332.0 ab
3	LF0 C0 IB	4.6 fg	14.31 ab	334.5 ab
4	LF0 C4 I0	4.9 de	15.98 ab	347.5 ab
5	LF0 C4 IA	4.8 ef	11.87 b	396.5 a
6	LF0 C4 IB	4.8 ef	16.26 ab	366.0 ab
7	LF1 C0 I0	5.0 cd	14.78 ab	372.5 ab
8	LF1 C0 IA	5.1 cd	14.99 ab	402.0 a
9	LF1 C0 IB	5.2 c	15.77 ab	377.5 ab
10	LF1 C4 I0	5.5 b	18.54 a	294.5 b
11	LF1 C4 IA	5.8 a	17.10 ab	341.5 ab
12	LF1 C4 IB	5.6 b	17.06 ab	390.5 ab

* LF0 = without liming and inorganic fertilize, LF1 = with liming and inorganic fertilizer; C0 = 0% compost, C4 = 4% compost; I0 = No inoculation, IA = Inoculant consortium A, IB = Inoculant consortium B. ** Means in a column followed by the same letter are not significantly different at 5% level by DMRT.

3.2. Effect of Treatments on Plant Growth Parameters

There were significant differences between treatments on growth parameters, i.e., plant height, leaf number, stem diameter, shoots, and roots DW (dry weight) as presented in Table 3. The value of plant height, leaf number, stem diameter, shoots, and roots DW were about 10.45–35.25 cm, 4.67–14.67, 0.15–0.57 cm, 0.38–3.62 g, and 0.19–1.40 g, respectively. Treatments 12 (LF1C4IB) and 6 (LF0C4IB) showed the highest value of a seedling growth parameters, i.e., plant height, leaf number, stem diameter, shoots, and root DW (dry weight) in amount of 35.25 and 35 cm; 12.83 and 12.5 leaves; 0.5 and 0.47 cm; 3.62 and 3.08 g, and 1.27 and 1.38 g, respectively.

Table 3. Treatments Effects on Growth Parameters of "Narra" Seedlings.

No	Treatments *	Height (cm) **	Leaf Number **	Stem Diameter (cm) **	Shoot DW (g) **	Root DW (g) **
1	LF0 C0 I0	10.25 d	4. 67 b	0.18 c	0.38 d	0.21 c
2	LF0 C0 IA	10.50 cd	7.17 b	0.15 c	0.43 d	0.38 bc
3	LF0 C0 IB	10.58 cd	5.33 b	0.20 c	0.50 d	0.19 c
4	LF0 C4 I0	23.42 b	11.50 a	0.45 a	2.38 bc	1.10 ab
5	LF0 C4 IA	23.08 b	13.33 a	0.42 ab	2.00 bc	0.92 abc
6	LF0 C4 IB	35.00 a	12.50 a	0.47 a	3.08 ab	1.38 a
7	LF1 C0 I0	11.42 cd	5. 33 b	0.25 c	0.58 d	0.33 bc
8	LF1 C0IA	13.33 bcd	6.83 b	0.27 bc	0.58 d	0.35 bc
9	LF1 C0 IB	13.17 bcd	5.50 b	0.25 c	0.60 d	0.32 bc
10	LF1 C4 I0	18.42 bcd	11.50 a	0.42 ab	1.38 cd	0.63 abc
11	LF1 C4 IA	21.00 bc	14.67 a	0.57 a	2.37 bc	1.40 a
12	LF1 C4 IB	35.25 a	12.83 a	0.50 a	3.62 a	1.27 a

* LF0 = without liming and inorganic fertilizer, LF1 = with liming and inorganic fertilizer; C0 = 0% compost, C4 = 4% compost; I0 = No inoculation, IA = Inoculant consortium A, IB = Inoculant consortium B, DW = dry weight. ** Means in a column followed the same letter are not significantly different at 5% level by DMRT.

3.3. Cu Accumulation in Narra Plants

There were significant differences among all treatments on shoot and root Cu content, as well as plant Cu uptake (Table 4). Cu accumulation data from all treatments ranged from 35.0 to 95.0 mg/kg, 114 to 155 mg/kg, and 61 to 320 µg/plant DW, respectively.

Table 4. Treatment Effects on Cu Accumulation in "Narra" Seedlings.

No	Treatments *	Shoot Cu (ppm) **	Root Cu (ppm) **	Seedling Cu Uptake (µg/plant DW) **
1	LF0 C0 I0	95.00 a	148.50 a	67.36 d
2	LF0 C0 IA	75.50 b	114.00 b	76.11 cd
3	LF0 C0 IB	46.50 ef	155.00 a	52.88 d
4	LF0 C4 I0	39.50 g	143.00 a	250.89 ab
5	LF0 C4 IA	50.00 d	124.50 b	214.04 abc
6	LF0 C4 IB	47.00 e	127.50 b	320.95 a
7	LF1 C0 I0	56.50 c	123.50 b	74.13 cd
8	LF1 C0 IA	57.50 c	119.00 b	75.23 cd
9	LF1 C0 IB	36.50 h	127.00 b	61.98 d
10	LF1 C4 I0	45.50 f	124.00 b	136.97 bcd
11	LF1 C4 IA	36.00 hi	126.50 b	262.48 ab
12	LF1 C4 IB	35.00 hi	119.50 b	277.24 ab

* LF0 = without liming and inorganic fertilizer, LF1 = with liming and inorganic fertilizer; C0 = 0% compost, LF1 = 4% compost; I0 = No inoculation, IA = Inoculant consortium A, IB = Inoculant consortium B. 260, DW = dry weight, ** means in a column followed by the same letter are not significantly different at 5% level by DMRT.

3.4. Plant Performance

Growth performance of "Narra" is presented in Figure 1, better growth performance was showed by the plants which received treatment of 4% compost without or with inoculation and treatment combination of LF1 + 4% without or with inoculation compared to other treatments (LF0 with or without inoculation and LF1 with or without inoculation.

LF0C0I0 LF0C0IA LF0C0IB LF0C4I0 LF0C4IA LF0C4IB LF1C0I0 LF1C0IA LF1C0IB LF1C4I0 LF1C4IA LF1C4IB

Figure 1. Plant performance of 13 week old "Narra" seedlings planted in soil 445 ppm Cu with combination treat ments of inoculant consortia (IO, IA, IB), without or with lime fertilizers (LF0 or LF1), and composts (C0%, C4%).

3.5. Cu Bioaccumulation and Translocation

To better understand the effects of soil characteristics with plant growth and plant Cu accumulation, a correlation test was conducted. The analysis results are presented in Table 5; soil pH had significant positive correlation with CEC (r = 0.374), and seedling growth parameter, i.e., leaf number (r = 0.436), stem diameter 0.583), shoots (r = 410) and roots dry weight (r = 0.364). Significant negative correlation between soil pH and shoot Cu content (r = −0.593), as well as between Cu plant uptake and shoot Cu content (r = −0.441 were observed.

Table 5. Pearson correlation coefficient values (R) among Soil Properties, as well as Seedling Growth against Soil Properties and Cu Accumulation.

Parameters	Seedling Cu Uptake (µg/plant)	pH	CEC	Soil Cu (mg/kg)
Height (cm)	0.896 **	-	-	-
Leaf Number	0.665 **	0.436 **	-	-
Stem Diameter (cm)	0.865 **	0.583 **	-	-
Shoot DW (g)	0.925 **	0.410 *	-	-
Root DW (g)	0.971 **	0.364 *	-	-
Shoot Cu (ppm)	−0.441 **	−0.593 **	-	-
Root Cu (ppm)	-	-	-	-
Seedling Cu uptake(µg/plant)	1	-	-	-
pH	-	1	0.374 *	-
CEC	-	0.374 *	1	-
Soil Cu (mg/kg)	-	-	-	1

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

4.1. Soil Characteristics Used in the Greenhouse Experiment

The effects of treatment on the chemical characteristics of the soil and soil Cu content are presented in Table 2. All treatments resulted to significant differences in soil pH, CEC, and Cu content. The treatment LF or compost per se, as well as, the combination of LF1 and 4% compost were able to increase soil pH about 4.8 up to 5.8. The treatment which affected the increase in soil pH from highest to lowest were as follows: treatment combination of LF1 and 4% compost, a single treatment of LF1, and a single treatment of 4% compost. In the first two treatments, inoculation of formula A or B resulted in a higher increase in soil pH compared to those without inoculation.

The effect of the treatment on the CEC value revealed that combination treatment of LF1 and 4% compost, as well as single treatment of LF1 or compost, gave a relatively higher CEC value than those of without LF (LF0) or 0% compost. The CEC value of all treatments after the experiment ranged from 11.87 to 18.54 $cmol_{(+)}$ kg^{-1}. The pH-dependent negative charge of clay and organic colloid was a contributor of pH-dependent soil CEC and would have high negative charge density by deprotonation of carboxyl and phenolic functional groups, which would occur at pH more than 5.5. The negative charge of soil and organic colloid is warehouse storage of essential cation elements for plant growth.

The medium of organic C content (2.13%) in Kentrong soil did not appear to be sufficient to effect higher soil CEC value due to acidic soil pH (from 4.5 to 4.7). There was a weak positive correlation between pH and CEC (r = 0.374) which means increasing soil pH would increase soil CEC (Table 1). Soil and soil organic matter have a variable charge that is pH-dependent so that they are important in cation exchange capacity. As pH increases, the degree of negative charge increases due to the deprotonation or dissociation of H^+ from functional groups. The major acidic functional groups are carboxyls, quinones that may dissociate as readily as carboxyl groups, phenolic OH groups, and enols. Since carboxyl and phenolic groups can deprotonate at pH's common in many soils, they are major contributors to the negative charge of soils. It has been estimated that up to 55% of the CEC from SOM is due to carboxyl groups while about 30% of the CEC of SOM up to pH 7 is due to the quinone, phenolic, and enolic groups [32]. Aside from hydrogen and aluminum ion toxicity in acid soil, the toxicity of Mn and added Cu became a constraint to plant and microorganism growth.

Liming and compost application were needed to improve the fertility of this soil. In agricultural practice, liming is carried out for reducing soil acidity. Lime in soil hydrolyzes to release basic conjugate such as carbonate ($CO_3{}^{2-}$), hydroxide (OH^-), and silicate ($SiO_2{}^-$) and Ca^{2+}/and Mg^{2+}. The basic conjugate could react with H^+ to form weak acid such as water while Ca^{2+}/and Mg^{2+} replace H^+ and Al^{3+} on exchange site of colloidal complex of clay or humus [33]. Liming effects to soil fertility, namely to reduce soil H^+ concentration and soil acidity; to increase the availability of nutrients, particularly Ca, Mg, K, P, and Mo; liming reduces the toxicity of Al, Fe and Mn; to stimulate the activities of the heterotrophic soil organism or those responsible for the decomposition of organic matter with subsequent mineralization of nitrogen; to enhance the symbiotic nitrogen fixation of rhizobium in legume; and to stimulate soil granulation [33–37].

Efficiency of liming to soil pH increase is depending on type and amount of lime, soil properties (pH, SOM, CEC, and clay), management pattern, and crop types [33–37]. He et al., 2021 [38] in their experiment found that when compared to their individual higher soil background values, the addition of limes had a significantly larger impact on soil pH when there was a low background value of soil pH, soil organic matter (SOM), CEC, and clay. This suggests that liming to raise soil pH may be more effective when there is a low background value of soil pH, SOM, CEC, and clay. The soil pH buffering capacity may play a role in how other soil characteristics, excluding the soil background pH, affect the soil pH. The ability of soil pH to maintain a generally constant level after the introduction of alkaline or acidic substances varies depending on the type of soil. The capacity of soils to buffer pH is produced by the precipitation/dissolution and deprotonation/protonation of minerals with varying charges and SOM. The addition of limes to soils with high levels of SOM, CEC, and clay will have a less impact on the soil pH than that would be seen with soils with lower levels of those three components [38].

In this study, liming increased the soil pH so that it could increase soil CEC. However, the increase in pH and CEC were produced by combination treatment of LF1 + 4% compost in ranges 5.5 to 5.8 and 17.06 to 18.54, respectively. The value of pH increase included in pH of 5.5–6.5, which most plants grow well at this pH range, caused liming treatment on acidic soil while the value of CEC was lower than the lowest soil CEC value according to Buni [35] (19.18 $cmol_{(+)}$ kg^{-1}), and the highest (33.34 $cmol_{(+)}$ kg^{-1}).

Compost as a single treatment or as a combination treatment with LF1 was able to improve soil pH and soil CE. The commercial compost which was used in this study was made from a blend of natural and nontoxic sieved grass clippings, palm fronds, green coconut husks, and dried animal manure which have completely decayed following natural decomposition in the forest and it has a pH of 6.3, C/N ratio 22, OM content 19%. The increase in soil pH by compost addition as stated by [39] is mainly due to the addition of basic cations, ammonification, and production of NH_3 during decomposition of the added compost. Additionally, in soils modified with compost, adsorption of H+ ions, the establishment of reducing conditions as a result of increased microbial activity, and the displacement of hydroxyls from sesquioxide surface by organic anion can all contribute to pH increase. It is similar to the report of Sulok et al. [40] that compost has a liming effect due to its high content of calcium, magnesium, sodium, and potassium, and when organic matter decomposes these base cations are released.

The mobility and availability of heavy metals in the soil are commonly low, especially when the soil is high in pH, clay and organic matter [41–43]. Copper heavy metal has a strong affinity for organic matter; especially for dissolved organic matter which is a more important determinant of Cu solubility and bioavailability than pH [43]. As stated by [42], OM buffers the concentrations of cations in the soil solution due to its high cation exchange capacity, and its incorporation into soils can reduce concentrations of HMs in the soil solution, thereby preventing their uptake by roots and their leaching into groundwater. After the experiment, the soil total Cu content was about 294.5–402.0 $mg \cdot kg^{-1}$ where treatment 5 (LF0C4IA) and 8 (LF1C0IA) showed significantly higher soil Cu concentration at the end of the experiment, i.e., 396.5 and 402.0 $mg \cdot kg^{-1}$, respectively. Before the experiment, all soils were created to have final total Cu content of about 445 mg/kg. However, total soil Cu content from composite soil samples before treatment was about 378 $mg \cdot kg^{-1}$. The change in soil total Cu content before and after the experiment could not be discussed since this factor was not measured in the individual soil pot unit before the experiment. Overall, soil Cu concentration up to the end of the experiment relatively the same values. Soil total Cu contents in each pot unit showed as expected values, throughout the experiment of all soils were in a situation of Cu contamination. According to Liu et al. [44] the regulatory concentrations of Cu in agricultural soils ($mg \cdot kg^{-1}$) of different countries are as follows: $\leq$100, Australia; $\leq$63, Canada; $\leq$50 (pH < 6.5), $\leq$100 (pH $\geq$ 6.5, China, Mainland); $\leq$200, China, Taiwan; $\leq$150, European union; <125 in paddy soil Japan; $\leq$270, USA.

4.2. Effect of Treatments on Plant Growth Parameters

The treatment combination of LF1 + 4% compost, as well as compost treatment alone was able to increase plant height, leaf number, stem diameter, shoots, and roots DW. Of both treatments, inoculant consortium A application was able to enhance leaf number, stem diameter, and roots DW (14.67, 0.57 cm, and 1.40 g) while inoculant consortium B was able to enhance plant height and shoot DW (5.25 cm and 3.63 g).

The application of liming and in organic fertilizer (LF1) could not improve "Narra" growth. The seedling performance of this treatment (treatments 7, 8, 9) were not different from the seedlings which did not receive lime and inorganic fertilizer (treatment 1, 2, 3). The addition of 4% compost or combination of LF1 and 4% compost could improve seedling growth which showed better performance compared to those treated without LF and compost (treatments 1, 2, 3) or LF1 only (treatments 7, 8, 9). In the former treatment, the inoculated seedling had better performance than the uninoculated treatment. It seemed that the inoculant has not yet optimally expressed the seedling growth promotion traits on "Narra" planted in Kentrong soil which had low soil pH and only 2.13% organic carbon content. Inoculant requires lime and additional organic matter in the soil to affect the growth of "Narra" seedlings (Figure 1).

The effects of liming to seedling growth as described previously due to its effects to the increase in soil pH and soil CEC. These results were confirmed as in a significant positive correlation between soil pH and growth parameters such as leaf number (0.436), stem diameter (0.583), shoot DW (0.410), and root DW (0.364) (Table 4).

An increase in soil pH would increase "Narra" tree growth through increasing availability of soil nutrients (N, Ca, Mg, P, K, S, Mo), decreasing solubility or toxicity of heavy metal (A^{3+}, Cu^{2+}, Mn^{2+}, Fe^{2+}), and improving the suitable environment for inoculated rhizobacteria to grow and express plant growth promotion on "Narra" seedlings. Moreover, as has been stated previously, the increase pH would promote decomposition activity of organic material which is nutrient such as nitrogen was released and available for plant uptake.

This corroborates the research result of [45] who reported that poultry litter addition to soil can enhance soil pH, CEC, and exchangeable cation, except K. The significant increase in soil pH following the application of poultry litter results from the reduction in exchangeable aluminum of organic colloids. Similarly with another research result those soils with a high CEC are more produce high availability and minimal leaching of K, Ca, Mg, Na and other cations [39].

4.3. Cu Accumulation in Narra Plants

The highest Shoot Cu content was obtained from treatment 1, i.e., without application of liming, inorganic fertilizer, and inoculation (LF0C0I0) while the highest root Cu content was exhibited by treatment 3 (LF0C0IB), i.e., 155.0 g which was similar to those of treatment 1 (LF0 C0 I0) and 4 (LF0 C4 I0) in the amount of 148.5 and 143.0 mg/kg, respectively. Significant negative correlation between soil pH and Shoot Cu content ($r = -0.593$) (Table 4) occurred which means an increase in soil pH would decrease shoot Cu content. Plant Cu uptake had a significant negative correlation with shoot Cu content ($r = -0.441$) (Table 4). An increase in plant Cu uptake decreased shoot Cu content. According to [40], reduced Cu contents in plants with the presence of increased supply of some nutrients are often related to secondary effects such as Cu dilution because of enhanced growth rates of the plant where its growth rate is faster than Cu uptake.

4.4. Plant Performance

The organic carbon in Kentrong soil has decomposed as a material similar to hummus in organic soil, which plays a role in the process of nutrient exchange; the formation of aggregates between organic substances and mineral particles, and the immobilization of toxic compounds. The additional 4% compost in this study was as a source of labile organic matter, the organic compound which could be used for rhizobacterial growth and functional activity. After lime addition, soil pH increased close to neutral pH which was suitable for bacterial growth with added organic matter as nutrient sources, eventually, they could express their plant promotion characteristics on "Narra" plant growth.

Inoculant B was able to contribute to better performance of seedling growth on soil with 4% compost or combination LF1 + 4% compost. ACC deaminase attributed to *Pseudomonas synxantha* (PbSM 2.1) was a factor for survival of the "Narra" seedlings on the degraded Kentrong soil. ACC deaminase is an enzyme which could breakdown ACC to ketoglutaric acid and ammonium so that it could not be used as precursor of ethylene formation in plant. Excessive ethylene is usually produced in plant growing in soil with abiotic or biotic stress where excessive presence of this compound could cause senescence impact to the plant. Therefore, ACC deaminase facilitates plant growth by IAA and other plant growth promotion compounds of the inoculant in abiotic stress situation as described by [10].

In this study, factors which were expected as constraints for seedling growth, i.e., Cu and Al heavy metal. However, kinetics and isotherm of the absorption of these metal ions onto Kentrong soil was not evaluated so that it could not determine whether delayed seedling growth of treatment 1, 2, and 3 (Figure 1) due to Cu or Al, and or due to other factors which were associated with soil acidity. Similarly, the available Cu and Al in Kentrong soil was not measured so that it could not predict the toxicity of the Cu and Al in Kentrong soil toward the growth of the seedling.

4.5. Cu Bioaccumulation and Translocation

Metal which are taken up by plants do not accumulate quickly in the environment, but accumulate in plants, resulting in heavy metal accumulation. Plant metal extraction, also known as phytoextraction, necessitates the transfer of heavy metals to an easy-to-harvest region of biomass, particularly the upper part [6,43,46]. There is significant positive correlation among growth variable, i.e., plant height, number of leaves, stem diameter, shoot dry weight, and root dry weight with plant Cu uptake of about r = 0.896, 0.665, 0.865, 0.925, and 0.971, respectively. However, plant Cu uptake was not affected by soil Cu concentration soil pH, and CEC as shown in Table 5. Soil pH indicated positive correlation with plant growth parameter, namely number of leaves (r = 0.436), stem diameter (r = 0.583), shoot dry weight (r = 0.410), and root dry weight (r = 0.364). Interestingly, shoot Cu content indicated negative correlation with plant Cu uptake (r = −0.441) and soil pH (r = −0.593). Increasing the shoot Cu content due to a decrease in soil pH would cause a decrease in plant Cu uptake and (Table 5). Plant biomass when subjected to a safe and controlled procedure will result in the release of metals contained therein. Tolerance plants limit metal transmission from soil to roots and from roots to shoots, resulting in tiny metal accumulations in their biomass. Hyperaccumulation plants, on the other hand, actively take up and transmit metals to aboveground biomass. Plants with lower Cu concentrations in their roots than in the soil (low bioaccumulation factor/BCF) and shoot parts with lower Cu concentrations than in their roots (low translocation factor/TF) are good candidates for phytoextraction [43,46].

The TF and BCF values of "Narra" plants grown in 445 ppm Cu-polluted soil were less than one in this study. The "Narra" plants were not acceptable for phytoextraction of Cu in the metal contaminated soil, even with the addition of lime + inorganic fertilizer, 4% compost, and/or inoculation. From Table 5, we can see that the decrease in pH caused an increase in the shoot Cu content but the Cu uptake of plants decreased. This shows that the tolerance mechanism of plants was root-based. Shoot parts of the plant shows limitation in Cu accumulation which is associated with its tolerance mechanism. Similarly, in 9 month old tropical plants Khaya ivorensis and Cedrela fissile, it was reported that these plants showed BCF and TF factor values of <1 and most of the Cu was compartmentalized by the root [6]. However, this conclusion may be premature; more research based on data analysis from mature Narra trees is needed. Furthermore, it is projected that mature trees would accumulate more copper. More or less from this study it was known that "Narra" will be able to grow and become a Phyto stabilizer in Cu-contaminated soil with the help of fertilizer or 4% compost combined with inoculation. According to [47], "Narra" is a suitable plant for phytoremediation in terms of Phyto stabilization, in which heavy metals are locked or sequestered over time and limited to a single location. "Narra" is a semi deciduous plant and is suitable for phyto stabilization of ex-mining land. Fallen leaves with less Cu content would improve soil fertility through increase in soil organic carbon.

Several report about effects inoculation of PGPR on halophyte plant growth and metal bioaccumulation have been provided. Metal-resistant PGPR (*Pantoea agglomerants* RSO6 and RSO7, and *Bacillus Aryabhata* RSO25) contribute to alleviate metal stress on wheat plant. After inoculation, the oxidative stress index (OSI) reduced by between 50% and 75%, phenylalanine ammonium lyase (PAL), which is involved in secondary metabolism and/or lignin synthesis, plays a significant role in managing metal stress in this halophyte when it is inoculated with the proper PGPR. Metal tolerant (1400 μg mL^{-1} Cu, 1000 μg mL^{-1} Cd, and (1000 μg mL^{-1} Cr) bacterium isolated from chilli rhizosphere (*Pseudomonas aeruginosa*), had multiple plant growth promoting biomolecules in the presence and absence of metals. Strain CPSB1 solubilized P at 400 μg mL^{-1} of Cd, Cr and Cu. The strain was positive for indole-3-acetic acid (IAA), siderophores, hydrogen cyanide (HCN), ammonia (NH_3) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase when grown with/without metals. The phytotoxic effects on wheat increased with increasing Cd, Cr and Cu rates. The *P. aeruginosa* CPSB1 inoculated wheat in contrast had better growth and yields under Cu, Cd and Cr stress. The root dry biomass of inoculated plants was enhanced by 44, 28 and 48% at 2007 mg Cu kg^{-1}, 36 mg Cd kg^{-1} and 204 mg Cr kg^{-1}, respectively. The bioinoculant enhanced number of spikes, grain and straw yields by 25, 17 and 12%, respectively. *Pseudomonas aeruginosa* CPSB1 significantly declined the levels of catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD), proline and malondialdehyde (MDA), and reduced metal uptake by wheat. Single inoculation of PGPR (*Bacillus cereus* and *Pseudomonas moraviensis*) decreased 50% of Co, Ni, Cr and Mn concentrations in the rhizosphere soil. Co-inoculation of PGPR and biofertilizer treatment further augmented the decreases by 15% in Co, Ni, Cr and Mn over single inoculation except Pb and Co where decreases were 40% and 77%, respectively. The maximum decrease in biological concentration factor (BCF) was observed for Cd, Co, Cr, and Mn. *P. moraviensis* inoculation decreases the biological accumulation coefficient (BAC) as well as translocation factor (TF) for Cd, Cr, Cu Mn, and Ni. The PGPR inoculation minimized the deleterious effects of heavy metals, and the addition of carriers further assisted the PGPR [48].

5. Conclusions

Inoculation of the contribution of plant growth promoting rhizobacteria showed significant effects on seedling growth when incorporated with lime, inorganic fertilizer, and compost. Future observations on the improvement of efficiency in organic matter use should be considered in phytoremediation work. Based on the results of this study, "Narra" could grow in Cu-contaminated soil containing 445 ppm Cu when compost at 4% level is added, indicating that the tree could take up the HM thereby reducing Cu in the soil. Moreover, for phytoremediation of Cu contaminated soil using "Narra", the following rhizobacteria bacteria CuNFBM 4.1, MGR and PbSM 2.1 in combination with compost is recommended.

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