A CONSORTIUM OF PLANT GROWTH-PROMOTING RHIZOBACTERIA STRAINS SYNERGISTICALLY ASSISTS JUJUNCAO (*PENNISETUM GIGANTEUM)* TO REMEDIATE CADMIUM CONTAMINATED SOILS

YANKEY, R.[1] – KARANJA, J. K.[2] – OKAL, E. J.[1] – OMOOR, I. N. A.[1] – LIN, H.[1] – BODJREMOU, D. M.[3] – LI, J.[1] – LIN, D. M.[1] – CAO, X. M.[1] – LIN, Z. X.[1*]

[1]*China National Engineering Research Center of Juncao Technology, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China*

[2]*Center for Plant Water-Use and Nutrition Regulation, Joint International Research Laboratory of Water and Nutrient in Crops, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China*

[3]*Institute of Horticultural Biotechnology, College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China*

[*]*Corresponding author*
e-mail: lzxjuncao@163.com

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Abstract. Plant growth-promoting bacteria (PGPB) have received much attention in recent years due to their ability to interact with plants and remediate contaminated soil. This research aimed to assess the potential synergistic effect of three rhizobacterium strains; *Enterobacter cloacae* RCB980 (A3), *Klebsiella pneumonia* kpa (A4), and *Klebsiella sp* XT-2 (A7) in the remediation of cadmium (Cd) contaminated soils using *Pennisetum giganteum* plant. *P. giganteum* seedlings were transplanted into pots with seven different concentrations of Cd (0, 25, 50, 75, 100, 150, and 200 mg/kg), and the rhizosphere treated with combinations of bacteria A3, A4, and A7, for 60 days. Plant height, shoot and root biomass, chlorophyll content, bioaccumulation (BAF) and translocation factors (TF) were then determined. Root and shoot BAF for plants inoculated with bacteria strains at soil Cd concentrations of 25 and 50 mg/kg were all above 1.0 whereas TF values were greater than 1.0 only at 25 mg/kg Cd concentration. The study revealed that the application of double and triple strain consortium of bacteria significantly enhanced plant growth parameters and phytoremediation as compared to single strain. These results suggested that the strains had the synergistic potential to be utilized in enhancing *P. giganteum* growth and phytoremediation of Cd stressed soils.
Keywords: *phytoremediation, heavy metals, chlorophyll, bacteria, pollution*

Introduction

In recent years, contamination of the environment by heavy metals has increased sharply as a result of increased industrialization and excessive population growth. This continuous release of heavy metal pollutants into the environment has become so alarming that, the past decade recorded yearly global figures of 22,000, 783,000, 939,000, and 1,350,000 metric tons for cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) respectively (Singh et al., 2003). This poses major environmental and human health problems worldwide. It has already been established that high concentrations of heavy metals in the soil affect the growth of plants and reduce agricultural productivity (Edelstein and Ben-Hur, 2018). The response of plants to heavy metals in soils differs. When most plants accumulate heavy metals into their tissues, cellular activities are

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negatively affected leading to retarded growth (Hall, 2002; Bücker-Neto et al., 2017). The common heavy metal pollutants are Cd, Pb, Cu, Hg, and Zn (He and Yang, 2007), therefore complete removal of such metals is the only way to effectively treat them since they cannot be easily broken down into harmless products (Wu et al., 2018).

Although several methods, with varying degrees of success and cost, are employed to address heavy metal pollution, the use of green plants in association with plant-growth promoting rhizobacteria (PGPR) has been generally accepted to be highly productive (Saxena et al., 2019). The use of grasses is most encouraged as they grow faster in low nutrient soils and usually have dense root and shoot biomass. Microorganisms play several roles in soil-water-plant-pollution relationships. Their importance in the heavy metal phytoremediation process cannot be over-emphasized, as their ability to endure metal toxicity, and change metal species into less toxic and soluble forms for plant uptake while promoting plant growth leading to enhanced biomass even under stressed environments.

Many studies have shown that bacterial strains of *Enterobacter and Klebsiella* species exhibit physiological and genetic characteristics which significantly enhanced growth of various plants (McKenzie-Reynolds, 2018; Liu et al., 2018; Dhungana and Itoh, 2019). *P. giganteum* also known as Jujuncao in Chinese is a tall herbaceous grass that is hardy and grows quickly under poor soil conditions while producing high biomass. The grass is widely cultivated and used for ecological remediation, animal feeding, and for production of edible and medicinal mushrooms. Although heavy metal accumulation capacities of certain plants such as *Brassica juncea*, *Helianthus annuus*, and *Zea mays* have been extensively studied, their large scale use for phytoremediation is limited due to their low biomass (Cui et al., 2004; Turgut et al., 2004; Szabó and Fodor, 2006). Despite high biomass trees such as *Salix sp* and *Populus sp* being demonstrated by Liphadzi et al. (2003) and Vervaeke et al. (2003) to potentially be ideal for phytoextraction, such trees generally take a longer period of time to grow and are therefore not good candidates for phytoremediation. Grasses, however, have generally become a good fit for this purpose and Jujuncao in particular, has been identified as potential good phytoremediators because of its wide growth adaptability, fast growth, and extensive roots and shoot biomass (Hayat et al., 2020). It would thus be interesting to explore the synergistic plant growth promotion association between these PGPR and Jujuncao for phytoremediation.

The success of bacteria assisted phytoremediation differs depending on the levels of tolerance of the plant and bacteria to the heavy metal in question. For these concerns, the selection of plant and bacteria species for phytoremediation of heavy metals depends mainly on the tolerance capacity of the bacteria and plant to the heavy metal and the plants' biomass production capabilities (Rezania et al., 2016). Moreover, it has been established that multiple contaminations of the same soil by different heavy metals are common and because different bacteria employ different mechanisms for remediation activities, some authors stress that bioremediation of heavy metals would be more successful if a cocktail of bacterial strains is utilized rather than using a single strain culture (Kang et al., 2016; Varjani et al., 2020). Previous studies have reported the ability of *Klebsiella sp* and *Enterobacter sp* to tolerate high Cd concentrations and to promote plant growth in heavy metal contaminated soils (Pramanik et al., 2018; Chuanboon et al., 2019). Therefore, this research aims to study the synergistic effect of these strains in the remediation of Cd contaminated soils using *P. giganteum* plants.

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Materials and Methods

Isolation of rhizobacteria strains

Following a random harvesting of 20 *P. giganteum* plants from the Lianjiang abandoned copper mines, Lianjiang, Fujian province, China and their subsequent movement to the laboratory in zip lock bags, bacteria was isolated from the composite pool of rhizospheric soil attached to the roots of the plants using Davis Minigioli (DM) agar medium according to the procedure of Sarkar et al. (2018). High Cd tolerance was taken as preliminary screening criteria during the isolation of the PGPR using the plate technique screening method according to the procedure of Rajesh et al. (2014). Subsequently, three PGPR strains of the Klebsiella and Enterobacter species were selected, identified as *Enterobacter cloacae* RCB980, *Klebsiella pneumonia* kpa, and *Klebsiella sp* XT-2 and were subsequently tagged as A3, A4 and A7, respectively, for the study of their synergistic growth and phytoremediation potentials on *P. giganteum.* The BLAST sequences were submitted to NCBI and assigned Genbank accession numbers MT103318, MT103319, and MT103320, respectively.

Soil preparation and pot experiment

The experimental soil was collected from Minhou, Fuzhou city, Fujian province, China. The basic physicochemical properties of the soil were pH 6.5, total organic carbon 16.3 g/kg, total nitrogen 1.2 g/kg, cation exchange capacity (CEC) 10.5 cmol/kg, and total Cd 0.95 mg/kg. The methods for determining the basic physicochemical properties of soils followed Tang et al. (1999). Soil total Cd was determined by ICP-MS (Agilent 7500a, USA) after digestion with HNO_3-$HClO_4$-HF. Cuttings of *P. giganteum* were prepared and nursed at the Juncao experimental field and were grown in mini pots for one month before being transferred to the experimental pots (40 (diameter) x 40 (height) cm) containing 9 kg of non-sterile field soil. The experiment was carried out in a greenhouse with a temperature range of 25 to 32°C and 50 to 80% relative humidity, while soil water content was maintained at 60% of water holding capacity.

The experiment employed a completely randomized design that had seven treatments of the three bacteria strains and their combinations; (A3, A4, A7, A3&A4, A3&A7, A4&A7, A3&A4&A7); applied on *P. giganteum* in pots under seven concentrations of Cd (0, 25, 50, 75, 100, 150 and 200 mg/kg) with three replicates each. Different concentrations of the Cd were prepared in distilled water, added to the soil in the pots, and mixed thoroughly. The control without Cd was mock-treated with the same amount of distilled water. The Cd contaminated soils and the controls were subsequently kept for 2 weeks for stabilization purpose before planting. *P. giganteum* plants of average height (75 cm) were then transplanted into the plastic pots with three replicates per treatment, resulting in a total of 168 pots. The three bacteria strains were cultured at 28°C for 48 h on Luria-Bertani (LB) agar medium. A single colony from a freshly streaked plate was selected, inoculated into LB broth, and incubated at 28°C for 48 h on a gyro-rotatory shaker at 200 rpm. For single strain treatment, 15 ml of each bacterium culture containing 10^6 CFU ml^{-1} was inoculated at the rhizosphere of plants in the pots while for the double strains, and triple strains treatments, 7.5 ml and 5.0 ml of each bacterium media were used respectively (Gamez et al., 2019). For bacteria treatment controls (CK), we added 15 ml of sterilized LB solution to the plant rhizosphere. The experimental design had two different controls, whereby the first group of CK consisted

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of plants with no bacterial inoculation in the soil while the second group of CK consisted of plants grown on soil with zero concentration of Cd.

Determination of chlorophyll and Cd contents

Plant heights were measured every 7 days for 60 days. After 60 days of growth, chlorophyll contents were determined by a SPAD-502 Plus chlorophyll content analyzer (Zhejiang Top Cloud-Agri Technology Co. Ltd, China) by measuring three locations along the third flag leaf of each plant. The plants were removed from the pots and the roots washed adequately with deionized water to remove any soil adhering to the root surface. The shoots were cut from the root and fresh weights of the shoot and root were recorded. The root and shoot samples were then dried at 70°C in an oven for six days in order to obtain their dry weights. The samples were then ground, sieved, and 0.5 g of each sample digested with a mixture of HCl/HNO_3 (3:1, v/v). The concentrations of Cd in the digests were then determined using atomic absorption spectroscopy (AAS). To quantify Cd, 0.5 g of each sample was placed in a 100 ml conical flask, 10 ml of a 1:2 mixture of Perchloric and Nitric acid was added to each conical flask, and then left overnight. Glass funnels were placed on each flask ensuring that funnel stem did not touch the liquid in the flask. The flasks were then placed on the digestor and temperature gradually increased until the contents of the flask fully digested. The volume of the digested material was increased to 50 ml with deionized water, then Cd content determined using AAS. Soils from pots of each treatment were thoroughly mixed and Cd content was determined in the soil samples following the digestion procedure by Sabienë et al. (2004).

Quantification of the efficiency of phytoremediation

Bioaccumulation factor (BAF) and Translocation factor (TF) were respectively determined as indicated below (González-Mendoza et al., 2007; Padmavathiamma and Li, 2007).

$$BAF = \frac{The\ metal\ concentration\ in\ plant\ tissue\ (mg/kg)}{The\ metal\ concentration\ in\ soil\ (mg/kg)} \quad \text{(Eq.1)}$$

$$TF = \frac{The\ metal\ concentration\ in\ shoots\ (mg/kg)}{The\ metal\ concentration\ in\ the\ roots\ (mg/kg)} \quad \text{(Eq.2)}$$

Statistical analyses

All the data were expressed as mean with standard errors of three replicates. Two-way ANOVA (Bacteria x Cd) was used to determine the statistical differences across and within different treatments by using SPSS Version 20.0. Least Significant Difference (LSD) and Duncan Multiple Range Test were determined at $P \leq 0.05$. Diagrams were prepared using Excel 2010.

Results

Increasing levels of Cd exposure is detrimental to plant growths

Plants grown on soils with medium to high Cd concentrations exhibited stunted growth with yellow leaves whereas the growth and height were better in the control.

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Plants inoculated with triple (A3A4A7) and double (A4A7) exhibited significantly higher plant heights (approx. 138 cm) than all other bacteria treatments. Again, the influence of the double A3A7 and A3A4 applications were significantly better than single bacteria inoculations. Heights of plants with no bacteria inoculation (CK) were lowest (102.52 cm) and differed significantly from all other treatments (*Table 1*). Furthermore, the results show a strong effect of the Cd stress on plant heights. Plant height significantly differed under the various Cd treatments with 0 mg/kg concentration the highest (194.15 cm) and 200 mg/kg the lowest at 85.54 cm.

Table 1. *Height of P. giganteum grown under different bacteria strains and their cocktails, and in soil amended with different concentration Cd (mg/kg soil)*

Treatments	**Plant height (cm)**							
Conc. (mg/kg)	**0**	**25**	**50**	**75**	**100**	**150**	**200**	**Mean (Bacteria)**
CK	156.8 ± 3.4^{f}	$116.3 \pm 6.3^{j\text{-}m}$	$103.3 \pm 4.8^{o\text{-}u}$	$98.8 \pm 1.8^{s\text{-}y}$	89.0 ± 6.5^{yza}	80.0 ± 3.2^{zab}	$73.3 \pm 2.4^{\text{-}b}$	102.52^{e}
A3	179.0 ± 1.7^{e}	125.3 ± 2.6^{hi}	$109.5 \pm 1.7^{k\text{-}p}$	$103.1 \pm 2.0^{m\text{-}s}$	$96.6 \pm 1.7^{p\text{-}v}$	$88.3 \pm 1.3^{u\text{-}z}$	79.6 ± 0.8^{za}	116.10^{cd}
A4	181.0 ± 2.1^{cd}	132.0 ± 2.3^{fg}	$113.3 \pm 2.9^{j\text{-}l}$	$106.0 \pm 3.2^{n\text{-}s}$	$101.3 \pm 6.3^{q\text{-}w}$	92.6 ± 1.4^{za}	80.0 ± 1.4^{zab}	119.00^{c}
A7	192.3 ± 5.0^{e}	147.0 ± 1.4^{ij}	$118.3 \pm 9.2^{l\text{-}r}$	$106.6 \pm 3.6^{o\text{-}u}$	$102.6 \pm 6.4^{s\text{-}z}$	$93.3 \pm 1.3^{v\text{-}z}$	87.3 ± 2.0^{zab}	112.62^{d}
A3A4	200.3 ± 2.9^{ab}	180.0 ± 1.8^{e}	121.6 ± 4.3^{gh}	$111.6 \pm 2.8^{k\text{-}p}$	$104.6 \pm 2.6^{n\text{-}t}$	$93.6 \pm 2.7^{t\text{-}z}$	$89.3 \pm 5.0^{x\text{-}z}$	133.38^{b}
A3A7	210.3 ± 4.3^{bc}	181.5 ± 1.7^{e}	140.0 ± 4.0^{jk}	$111.8 \pm 3.5^{k\text{-}n}$	$109.1 \pm 2.6^{k\text{-}o}$	$94.6 \pm 2.0^{u\text{-}z}$	90.6 ± 1.2^{yza}	130.38^{b}
A4A7	215.0 ± 1.5^{a}	184.0 ± 3.5^{de}	148.6 ± 2.3^{f}	$114.5 \pm 6.5^{k\text{-}p}$	$111.3 \pm 1.7^{l\text{-}q}$	$94.0 \pm 6.3^{r\text{-}x}$	$92.0 \pm 4.7^{w\text{-}z}$	137.86^{a}
A3A4A7	218.0 ± 3.2^{a}	188.6 ± 2.1^{de}	150.3 ± 3.2^{fg}	$117.6 \pm 2.6^{j\text{-}l}$	$113.1 \pm 5.0^{l\text{-}r}$	$100.6 \pm 5.2^{t\text{-}z}$	$92.0 \pm 3.4^{w\text{-}z}$	138.45^{a}
Mean (Cd)	194.15^{a}	156.85^{b}	125.44^{c}	108.79^{d}	103.50^{e}	92.25^{f}	85.54^{g}	

Values in each column represent the mean ± standard error of three replicates. Those marked with different superscripts in each of the column show significant difference at $P \leq 0.05$ as analyzed by LSD. The mean Cd analysis is within the row

The effects of the interactions of bacteria and Cd indicate that heights of plants inoculated with combinations of bacteria cultures were generally significantly higher than those under single strain inoculations. Height of plants with no bacteria inoculation (CK) always significantly differed from those under the influence of some bacteria showing the strong positive effect of PGPB on plant growth. Among all other applied combinations of bacteria and Cd with respect to plant height of Jujuncao, the highest was recorded at the influence of the triple A3A4A7 inoculation (218.0 cm) at 0 mg/kg Cd and the lowest at CK (73.3 cm) at 200 mg/kg Cd (*Table 1*).

PGPB enhance growth of P. giganteum even in the presence Cd

PGPB and their cocktails significantly improve growth (plant weight and biomass) of plants grown in soils with no Cd compared to those with Cd concentrations. The triple (A3A4A7) strain application showed a significantly higher shoot fresh weight (109.25 g) than all other treatments. The influences of the double strain inoculations were generally better and significantly different from the single strain inoculations. The CK recorded the lowest weight (48.05 g) and was significantly different from all other treatments. Each Cd treatment significantly differed from the other in terms of shoot fresh weight with 0 mg/kg Cd concentration recording the heaviest (128.01 g) and 200 mg/kg recording the least weight (60.39 g) (*Table 2*).

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Table 2. *Shoot fresh weights of P. giganteum grown on soil amended with different concentrations of Cd (mg/kg soil) under the application of different bacteria strains and their cocktails*

Treatments	**Shoot fresh weight (g)**							
Conc. (mg/kg)	**0**	**25**	**50**	**75**	**100**	**150**	**200**	**Mean (Bacteria)**
CK	$91.1 \pm 2.1^{p-s}$	73.3 ± 1.7^{x}	51.6 ± 1.4^{-a}	44.5 ± 2.3^{-b}	31.9 ± 1.0^{-c}	24.6 ± 2.3^{-d}	19.1 ± 0.7^{-e}	48.05^{f}
A3	115.3 ± 4.0^{3f}	$96.9 \pm 2.3^{l-n}$	82.6 ± 2.9^{vw}	69.3 ± 0.6^{xy}	62.2 ± 1.2^{z}	50.3 ± 1.2^{-a}	40.1 ± 1.5^{-b}	73.98^{e}
A4	123.5 ± 2.1^{de}	$103.1 \pm 2.2^{h-j}$	$84.0 \pm 1.2^{u-w}$	71.1 ± 1.2^{xy}	63.3 ± 1.3^{yz}	50.5 ± 2.1^{-a}	43.3 ± 1.2^{-b}	77.67^{d}
A7	121.0 ± 1.7^{e}	$99.9 \pm 1.1^{i-l}$	$86.6 \pm 1.4^{r-v}$	68.4 ± 1.6^{xy}	66.3 ± 1.8^{z}	54.4 ± 2.0^{-a}	42.8 ± 1.2^{-b}	75.92^{d}
A3A4	140.1 ± 0.8^{b}	180.0 ± 1.8^{cd}	104.3 ± 1.0^{hi}	$98.5 \pm 1.4^{j-n}$	$94.6 \pm 3.4^{m-p}$	$87.9 \pm 1.5^{r-u}$	$85.1 \pm 3.6^{t-w}$	105.57^{bc}
A3A7	142.3 ± 0.8^{ab}	128.3 ± 1.3^{c}	$102.4 \pm 2.0^{h-k}$	$95.5 \pm 0.4^{l-p}$	$93.7 \pm 0.9^{n-q}$	$88.5 \pm 1.1^{r-u}$	81.5 ± 1.1^{w}	104.76^{c}
A4A7	144.0 ± 0.6^{ab}	129.8 ± 1.1^{c}	106.9 ± 1.3^{gh}	$99.5 \pm 0.9^{i-m}$	$96.4 \pm 1.4^{l-o}$	$89.2 \pm 0.6^{q-t}$	$84.8 \pm 2.3^{t-w}$	107.25^{b}
A3A4A7	146.7 ± 1.2^{a}	127.7 ± 1.6^{cd}	111.8 ± 2.1^{fg}	$103.2 \pm 0.8^{h-j}$	$97.5 \pm 1.2^{k-n}$	$91.4 \pm 1.2^{o-r}$	$86.4 \pm 1.3^{s-w}$	109.25^{a}
Mean (Cd)	128.01^{a}	111.08^{b}	91.30^{c}	81.25^{d}	75.74^{e}	66.87^{f}	60.39^{g}	

Values in each column represent the mean ± standard error of three replicates. Those marked with different superscripts in each of the column show significant difference at $P \leq 0.05$ as analyzed by LSD. The mean Cd analysis is within the row

The best influence of the strains on root fresh weights were recorded at the double inoculations A4A7 (12.73 g), A3A4 (12.46 g) and A3A7 (12.44 g) which were significantly better than all other treatments. Generally, the single strain inoculations significantly differed from the cocktail inoculations. The CK recorded the lowest weight and differed significantly from all others. The effects of the Cd treatments on root fresh weights were significantly different from each other (*Table 3*).

Table 3. *Root fresh weights of P. giganteum grown on soil amended with different concentrations of Cd (mg/kg soil) under the application of different bacteria strains and their cocktails*

Treatments	**Root fresh weight (g)**							
Conc. (mg/kg)	**0**	**25**	**50**	**75**	**100**	**150**	**200**	**Mean (Bacteria)**
CK	$13.5 \pm 1.0^{f-i}$	$9.4 \pm 0.5^{q-t}$	$8.4 \pm 0.3^{u-z}$	$7.0 \pm 0.1^{-b-f}$	$6.8 \pm 0.4^{-b-f}$	6.1 ± 0.2^{-fg}	5.6 ± 0.5^{-g}	8.13^{e}
A3	14.4 ± 0.5^{ef}	$13.0 \pm 0.1^{h-j}$	$12.0 \pm 0.3^{k-m}$	$10.0 \pm 0.5^{p-s}$	$8.7 \pm 0.2^{t-y}$	$7.3 \pm 0.4^{-a-d}$	$6.4 \pm 0.1^{-d-g}$	10.26^{d}
A4	14.5 ± 0.4^{e}	$13.8 \pm 0.2^{e-h}$	$12.5 \pm 0.3^{i-k}$	$10.4 \pm 0.3^{o-q}$	$9.6 \pm 0.3^{q-t}$	$7.5 \pm 0.3^{z-c}$	$6.6 \pm 0.2^{-c-f}$	10.71^{c}
A7	$14.2 \pm 0.6^{e-g}$	$13.3 \pm 0.3^{g-i}$	$11.9 \pm 0.1^{k-n}$	$10.1 \pm 0.2^{p-r}$	$8.7 \pm 0.1^{t-x}$	$7.1 \pm 0.1^{-a-e}$	$6.3 \pm 0.1^{-e-g}$	10.22^{d}
A3A4	$17.3 \pm 0.6^{a-c}$	$17.2 \pm 0.4^{a-c}$	$13.1 \pm 0.5^{h-j}$	$11.8 \pm 0.2^{k-m}$	$10.9 \pm 0.4^{n-p}$	$9.1 \pm 0.3^{r-v}$	$7.7 \pm 0.5^{y-b}$	12.46^{ab}
A3A7	$17.0 \pm 0.3^{a-c}$	16.5 ± 0.5^{cd}	$13.1 \pm 0.4^{h-j}$	$12.2 \pm 0.3^{j-l}$	$11.2 \pm 0.3^{m-o}$	$9.0 \pm 0.1^{s-w}$	$8.0 \pm 0.3^{x-a}$	12.44^{ab}
A4A7	17.8 ± 1.1^{a}	$16.6 \pm 0.4^{b-d}$	$13.3 \pm 0.4^{g-i}$	$12.2 \pm 0.2^{j-m}$	$11.5 \pm 0.2^{l-n}$	$9.4 \pm 0.2^{q-u}$	$8.4 \pm 0.3^{v-z}$	12.73^{a}
A3A4A7	17.5 ± 0.7^{ab}	15.9 ± 0.4^{d}	$13.7 \pm 0.3^{e-h}$	$11.6 \pm 0.2^{k-m}$	$10.4 \pm 0.1^{o-q}$	$9.2 \pm 0.3^{r-v}$	$8.0 \pm 0.2^{w-a}$	12.31^{b}
Mean (Cd)	15.74^{a}	14.46^{b}	12.25^{c}	10.67^{d}	9.74^{e}	8.10^{f}	7.16^{g}	

The values in each column represent the mean ± standard error of three replicates. Those marked with different superscripts in each of the column show significant difference at $P \leq 0.05$ as analyzed by LSD. The mean Cd analysis is within the row

The effects of the bacteria on shoot dry weights indicated that combinations A3A4A7 and A4A7 showed significantly higher weights (approx. 36 g) than all other bacterial treatments. Here too, the single strain inoculations significantly differed from

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the cocktail inoculations. The CK recorded the least weight (18.70 g) and significantly differed from all other recordings. The effects of the Cd stress were significantly different from each other (*Table 4*). These observations were generally similar for the root dry weights as presented in *Table 5*.

Table 4. *Shoot dry weights of P. giganteum grown on soil amended with different concentrations of Cd (mg/kg soil) under the application of different bacteria strains and their cocktails*

Treatments	**Shoot dry weight (g)**							
Conc. (mg/kg)	**0**	**25**	**50**	**75**	**100**	**150**	**200**	**Mean (Bacteria)**
CK	32.3 ± 0.8^{mn}	21.5 ± 0.4^{-b}	19.0 ± 0.2^{-c}	16.6 ± 0.4^{-d}	6.8 ± 0.4^{-d}	13.5 ± 0.2^{-e}	12.3 ± 0.3^{-e}	18.70^{f}
A3	40.1 ± 1.3^{g}	34.2 ± 0.8^{l}	$30.1 \pm 0.9^{o-q}$	$27.4 \pm 0.7^{s-u}$	$8.7 \pm 0.2^{u-w}$	$24.6 \pm 0.2^{x-z}$	23.7 ± 0.3^{ya}	29.48^{e}
A4	43.8 ± 0.7^{cd}	39.3 ± 0.4^{gh}	34.0 ± 0.6^{l}	$28.8 \pm 0.2^{q-s}$	$9.6 \pm 0.3^{v-x}$	23.2 ± 0.2^{za}	$24.7 \pm 0.3^{w-z}$	31.33^{c}
A7	40.6 ± 0.6^{fg}	39.5 ± 1.2^{gh}	$33.7 \pm 0.6l^{m}$	$27.4 \pm 0.6^{s-u}$	$8.7 \pm 0.3^{v-x}$	$24.9 \pm 0.2^{w-y}$	23.0 ± 0.5^{-ab}	30.69^{d}
A3A4	46.6 ± 0.7^{b}	42.5 ± 0.8^{de}	37.1 ± 0.6^{ij}	33.6 ± 0.3^{lm}	10.9 ± 0.4^{no}	$28.7 \pm 0.4^{q-s}$	$27.8 \pm 0.3^{r-u}$	35.41^{b}
A3A7	46.9 ± 0.7^{ab}	42.2 ± 0.9^{ef}	36.5 ± 0.3^{jk}	33.8 ± 0.9^{lm}	$11.2 \pm 0.3^{n-p}$	$27.9 \pm 0.3^{r-t}$	$26.6 \pm 0.3^{t-v}$	35.00^{b}
A4A7	48.0 ± 0.3^{ab}	44.4 ± 0.5^{c}	37.3 ± 0.6^{ij}	34.5 ± 0.5^{l}	$11.5 \pm 0.2^{n-p}$	29.0 ± 0.2^{qr}	$27.8 \pm 0.3^{r-u}$	36.02^{a}
A3A4A7	48.3 ± 0.6^{a}	44.4 ± 0.6^{c}	38.5 ± 0.5^{hi}	35.1 ± 0.3^{kl}	10.4 ± 0.1^{no}	29.7 ± 0.3^{pq}	$27.9 \pm 0.5^{r-t}$	36.46^{a}
Mean (Cd)	43.34^{a}	38.49^{b}	33.29^{c}	29.66^{d}	27.23^{e}	25.21^{f}	24.23^{g}	

The values in each column represent the mean ± standard error of three replicates. Those marked with different superscripts in each of the column show significant difference at $P \leq 0.05$ as analyzed by LSD. The mean Cd analysis is within the row

Table 5. *Root dry weights of P. giganteum grown on soil amended with different concentrations of Cd (mg/kg soil) under the application of different bacteria strains and their cocktails*

Treatments	**Root dry weight (g)**							
Conc. (mg/kg)	**0**	**25**	**50**	**75**	**100**	**150**	**200**	**Mean (Bacteria)**
CK	$6.8 \pm 0.2^{j-n}$	$6.2 \pm 0.1^{q-t}$	5.8 ± 0.1^{tu}	5.7 ± 0.1^{uv}	$5.4 \pm 0.1^{w-z}$	4.8 ± 0.2^{-ab}	4.5 ± 0.2^{-b}	5.63^{e}
A3	$7.3 \pm 0.1^{f-h}$	$7.0 \pm 0.1^{i-m}$	$6.4 \pm 0.1^{p-s}$	5.9 ± 0.1^{tu}	$5.6 \pm 0.1^{u-w}$	$5.1 \pm 0.1^{y-a}$	5.1 ± 0.2^{za}	6.05^{d}
A4	$7.5 \pm 0.1^{d-f}$	$7.0 \pm 0.1^{i-l}$	$6.6 \pm 0.1^{n-p}$	6.1 ± 0.1^{st}	$5.7 \pm 0.1^{u-w}$	$5.5 \pm 0.1^{v-y}$	$5.2 \pm 0.1^{x-a}$	6.22^{c}
A7	$7.3 \pm 0.1^{f-i}$	$6.9 \pm 0.1^{j-n}$	$6.6 \pm 0.1^{n-p}$	$6.2 \pm 0.1^{q-t}$	$5.5 \pm 0.1^{v-x}$	5.1 ± 0.1^{za}	5.0 ± 0.1^{-a}	6.07^{d}
A3A4	8.1 ± 0.1^{bc}	$7.5 \pm 0.1^{d-f}$	$7.1 \pm 0.1^{h-k}$	$6.8 \pm 0.1^{k-o}$	$6.6 \pm 0.1^{m-p}$	$6.5 \pm 0.1^{o-r}$	6.1 ± 0.2^{st}	6.95^{b}
A3A7	8.2 ± 0.3^{ab}	$7.4 \pm 0.1^{e-h}$	$7.0 \pm 0.1^{h-k}$	$6.9 \pm 0.1^{i-l}$	$6.5 \pm 0.1^{n-q}$	$6.1 \pm 0.1^{r-t}$	5.9 ± 0.1^{tu}	6.89^{b}
A4A7	8.5 ± 0.2^{a}	7.7 ± 0.1^{de}	$7.3 \pm 0.1^{f-i}$	$7.0 \pm 0.1^{h-k}$	$6.6 \pm 0.1^{l-p}$	$6.4 \pm 0.1^{p-s}$	$6.2 \pm 0.1^{q-t}$	7.10^{a}
A3A4A7	8.5 ± 0.1^{a}	7.8 ± 0.2^{cd}	$7.4 \pm 0.1^{d-g}$	$7.1 \pm 0.1^{g-j}$	$6.9 \pm 0.1^{i-m}$	$6.5 \pm 0.1^{o-r}$	$6.2 \pm 0.2^{q-t}$	7.21^{a}
Mean (Cd)	7.80^{a}	7.17^{b}	6.78^{c}	6.48^{d}	6.10^{e}	5.75^{f}	5.52^{g}	

Values in each column represent the mean ± standard error of three replicates. Those marked with different superscripts in each of the column show significant difference at $P \leq 0.05$ as analyzed by LSD. The mean Cd analysis is within the row

The interaction effects of bacteria and Cd on weights of shoots and roots of Jujuncao generally indicate that combinations of bacteria culture recorded significantly higher values than single strains inoculations which were also always significantly higher than the CK at the various Cd stress levels. As concentrations of metals increased from 25 mg/kg onwards to 200 mg/kg, the influence of the bacteria on growths reduced

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progressively suggesting that a threshold exists beyond which the bacteria may not alleviate toxicity of the metals in plants. Similarly, as Cd concentration increased towards 200 mg/kg, the effect of the various bacteria treatments on plant growth dramatically decreased. The negative effect of the metal on plants grown in pots without PGPB was so severe that the yellowing of leaves and drying of leaf tips were profound at 200 mg/kg Cd concentration.

Plants inoculated with PGPB accumulated more Cd in their tissues

Cadmium concentrations in the different plant parts grown in the contaminated soil are presented in *Tables 6 and 7*. The effects of the PGPB on shoot Cd indicate that there was no significant difference between either the triple or double consortia applications. However, significant difference exists between cocktail of strains inoculation and single inoculation. The CK recorded the lowest shoot Cd of 28.18 mg/kg which was significantly different from all the bacteria treatments. Cd accumulation in shoots had an interesting pattern; for all Cd treatments, the Cd contents progressively increased up to 50 mg/kg Cd, after which it had an inverse relation with the Cd concentrations in the soil. The maximum cadmium uptake in shoot, 55.1 mg/kg, was recorded in plants inoculated with A3A4A7 at 50 mg/kg Cd concentration. The quantities of Cd in the shoot of plants treated with 0 to 50 mg/kg Cd was in the range of 0.01 to 55.1 mg/kg whereas treatments of 75 to 200 mg/kg Cd were in the range of 25.3 mg/kg (at 200 mg/kg Cd with no bacteria) to 46.0 mg/kg (at 75 mg/kg Cd with A3A4A7 bacteria inoculation). The pattern for shoot Cd content in terms of the PGPB influence was: cocktails > single > no bacteria (*Table 6*).

Table 6. *Concentrations of Cd in shoots of P. giganteum grown in different concentrations of Cd contaminated soil, under the application of different bacteria strains and their cocktails*

Treatments	**Shoots Cd (mg/kg)**							
Conc. (mg/kg)	**0**	**25**	**50**	**75**	**100**	**150**	**200**	**Mean (Bacteria)**
CK	0.01 ± 0.0^{x}	26.1 ± 1.6^{vw}	$43.1 \pm 1.2^{f-k}$	$36.7 \pm 1.8^{o-r}$	$36.7 \pm 1.3^{o-r}$	29.1 ± 1.8^{uv}	25.3 ± 1.0^{w}	28.18^{c}
A3	0.01 ± 0.0^{x}	$39.2 \pm 1.4^{m-p}$	48.7 ± 1.4^{cd}	$40.3 \pm 1.0^{k-n}$	$40.5 \pm 1.0^{k-n}$	$39.7 \pm 0.9^{l-o}$	31.1 ± 0.8^{tu}	34.26^{b}
A4	0.02 ± 0.0^{x}	$39.1 \pm 1.6^{m-q}$	52.3 ± 1.0^{ab}	$40.6 \pm 0.6^{k-n}$	$41.7 \pm 1.0^{h-m}$	$40.7 \pm 1.3^{j-n}$	32.0 ± 0.8^{tu}	35.22^{b}
A7	0.01 ± 0.0^{x}	$37.6 \pm 1.9^{n-r}$	49.8 ± 0.9^{bc}	$42.6 \pm 0.8^{g-l}$	$40.9 \pm 1.3^{j-m}$	$39.2 \pm 1.7^{m-p}$	32.5 ± 0.5^{st}	34.70^{b}
A3A4	0.03 ± 0.0^{x}	$44.6 \pm 2.1^{e-h}$	53.7 ± 0.8^{a}	$44.3 \pm 1.5^{e-i}$	$44.6 \pm 0.8^{e-h}$	$43.3 \pm 1.3^{f-k}$	35.1 ± 1.0^{rs}	37.97^{a}
A3A7	0.02 ± 0.0^{x}	$41.4 \pm 1.1^{i-m}$	52.4 ± 0.6^{ab}	$45.3 \pm 0.5^{e-g}$	$45.3 \pm 1.1^{e-g}$	$44.5 \pm 1.1^{e-i}$	$36.5 \pm 0.8^{p-r}$	37.96^{a}
A4A7	0.03 ± 0.0^{x}	46.8 ± 1.6^{cd}	54.5 ± 0.6^{a}	$45.1 \pm 0.4^{e-g}$	$43.8 \pm 0.7^{e-j}$	$45.5 \pm 1.0^{e-g}$	36.0 ± 0.9^{qr}	38.83^{a}
A3A4A7	0.04 ± 0.0^{x}	46.4 ± 0.8^{de}	55.1 ± 1.4^{a}	$46.0 \pm 0.6^{d-f}$	$45.4 \pm 1.0^{e-g}$	$44.8 \pm 0.4^{e-g}$	35.8 ± 0.5^{r}	39.10^{a}
Mean (Cd)	0.02^{e}	40.20^{c}	51.23^{a}	42.64^{b}	42.39^{b}	40.88^{c}	33.076^{d}	

Values in each column represent the mean ± standard error of three replicates. Those marked with different superscripts in each of the column show significant difference at $P \leq 0.05$ as analyzed by LSD. The mean Cd analysis is within the row

The effects of the bacteria strains on root Cd uptake showed that the inoculation of triple A3A4A7 and double A4A7 were significantly higher than all other bacterial treatments. The effects of the single strain inoculations were lower and significantly different from the combination treatments. A CK value of 42.07 was significantly lower than all other bacterial treatments. Generally, the pattern of the root Cd content in terms of the bacterial application was as follows: cocktails > single > no bacteria. On the other

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hand, the highest mean root Cd value of 78.83 mg/kg was obtained at 200 mg/kg whereas the lowest mean root Cd value of 0.045 mg/kg was recorded at 0 mg/kg Cd concentration. Maximum cadmium uptake in root of 83.5 mg/kg was recorded at the interaction of 200 mg/kg Cd and the consortium of the triple bacteria inoculation (*Table 7*).

Table 7. *Concentrations of Cd in roots of P. giganteum grown in different concentrations of Cd contaminated soil, under the application of different bacteria strains and their cocktails*

Treatments	**Root Cd (mg/kg)**							
Conc. (mg/kg)	**0**	**25**	**50**	**75**	**100**	**150**	**200**	**Mean (Bacteria)**
CK	0.02 ± 0.0^{a}	30.0 ± 1.1^{z}	48.5 ± 0.8^{u}	48.6 ± 1.3^{u}	50.1 ± 1.4^{st}	55.3 ± 0.8^{u}	$61.8 \pm 0.7^{k-n}$	42.07^{e}
A3	0.02 ± 0.0^{a}	37.6 ± 0.9^{y}	49.7 ± 3.4^{u}	$56.1 \pm 1.4^{q-t}$	69.9 ± 1.3^{g}	69.7 ± 2.6^{h}	$78.8 \pm 0.7^{c-e}$	51.16^{d}
A4	0.04 ± 0.0^{a}	38.9 ± 0.4^{xy}	$55.4 \pm 0.9^{r-t}$	$58.4 \pm 0.9^{o-q}$	62.5 ± 1.1^{fg}	$72.2 \pm 0.9^{j-m}$	$81.0 \pm 1.0^{a-c}$	52.64^{c}
A7	0.03 ± 0.0^{a}	36.5 ± 0.7^{y}	54.2 ± 0.5^{t}	$57.2 \pm 1.2^{p-s}$	65.1 ± 0.4^{hi}	$65.2 \pm 0.5^{h-j}$	$80.6 \pm 1.6^{b-d}$	51.28^{d}
A3A4	0.06 ± 0.0^{a}	41.7 ± 0.9^{w}	$59.4 \pm 0.6^{n-p}$	$63.0 \pm 0.2^{i-l}$	71.4 ± 0.2^{f}	73.8 ± 0.6^{fg}	$80.6 \pm 0.6^{b-d}$	55.72^{b}
A3A7	0.05 ± 0.0^{a}	41.2 ± 1.2^{wx}	$58.1 \pm 1.1^{o-r}$	$61.9 \pm 0.2^{k-n}$	73.0 ± 0.1^{e}	77.1 ± 0.1^{f}	$81.0 \pm 0.2^{a-c}$	56.07^{b}
A4A7	0.06 ± 0.0^{a}	44.4 ± 1.1^{v}	$60.4 \pm 0.6^{l-o}$	$64.2 \pm 0.3^{h-k}$	70.1 ± 0.3^{de}	77.9 ± 0.4^{g}	83.1 ± 0.5^{ab}	57.21^{a}
A3A4A7	0.07 ± 0.0^{a}	45.5 ± 0.1^{v}	$60.0 \pm 0.3^{m-o}$	$64.8 \pm 0.1^{h-j}$	73.1 ± 0.3^{de}	78.1 ± 0.1^{f}	83.5 ± 0.2^{a}	57.89^{a}
Mean (Cd)	0.045^{g}	39.504^{f}	55.746^{e}	59.313^{d}	71.196^{b}	66.412^{c}	78.83^{a}	

Values in each column represent the mean ± standard error of three replicates. Those marked with different superscripts in each of the column show significant difference at $P \leq 0.05$ as analyzed by LSD. The mean Cd analysis is within the row

P. giganteum chlorophyll content responses against Cd-induced oxidative stress in different bacteria inoculation

The chlorophyll content of plants under triple strain inoculation was significantly higher (32.52) than all other bacterial treatments. There was no significant difference in the chlorophyll contents of the double strain inoculated plants which were however significantly higher than the single strain inoculated plants. The CK showed a significantly least value of 23.21. On the other hand, the chlorophyll contents of the plants had an inverse relationship with the concentration of Cd with the highest chlorophyll content of 46.76 being recorded at 0 mg/kg Cd concentration and the lowest value of 16.63 recorded at 200 mg/kg Cd concentration. The chlorophyll content significantly differed from each other at the various Cd concentrations. The interactions of the bacteria strains and the Cd revealed the maximum mean chlorophyll content was 55.1 recorded in plants inoculated with A3A4A7 strains at 0 Cd concentration, whereas the minimum chlorophyll content of 15.2 was recorded from plants at 200 mg/kg Cd supplied with no bacteria application (*Table 8*).

Effect of cadmium on bioaccumulation and translocation factors

Bioaccumulation factor (BAF) and Translocation factor (TF) are widely used to determine the uptake and translocations of heavy metals into growing plant tissues. BAF is used to measure the efficiency of plant species in accumulating heavy metals from soil environment into its tissues. On the other hand, TF is a measurement of the efficiency of a plant's ability to translocate metals accumulated from its roots to its shoots (Ladislas et al., 2012).

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The BAF for shoot at 25 mg/kg Cd were all above 1.0, whereas the BAF values for shoot at 50 mg/kg were all above 1.0 with the exceptions of the CK and A3 inoculations. Above 50 mg/kg Cd treatments, the BAF values progressively decreased below 1.0 as Cd concentration increased. Overall, shoot BAF values were in the range of 0.13 (CK, 200 mg/kg) to 1.87 (A4A7, 25 mg/kg) (*Figure 1a*).

Table 8. *Chlorophyll content response of P. giganteum plants under different concentrations of Cd (mg/kg soil) under the application of different bacteria strains and their cocktails*

Treatments								
Conc. (mg/kg)	**0**	**25**	**50**	**75**	**100**	**150**	**200**	**Mean (Bacteria)**
CK	35.6 ± 0.99^{g}	30.1 ± 1.71^{hi}	$23.3 \pm 0.69^{n-q}$	$21.7 \pm 0.47^{p-s}$	$19.8 \pm 0.78^{r-u}$	16.4 ± 0.84^{wx}	15.2 ± 0.58^{x}	23.21^{e}
A3	41.8 ± 1.48^{f}	36.3 ± 1.14^{g}	26.7 ± 0.91^{kl}	$23.6 \pm 1.05^{m-p}$	$22.3 \pm 0.66^{o-r}$	$19.3 \pm 0.81^{s-v}$	16.1 ± 0.40^{wx}	26.60^{d}
A4	42.4 ± 1.24^{f}	40.0 ± 1.79^{f}	26.8 ± 0.52^{kl}	$24.4 \pm 0.73^{l-o}$	$23.1 \pm 0.35^{o-q}$	$20.3 \pm 0.47^{r-t}$	16.5 ± 0.44^{wx}	27.69^{c}
A7	41.2 ± 1.53^{f}	35.4 ± 1.10^{g}	$25.9 \pm 0.41^{k-m}$	$23.2 \pm 0.49^{n-q}$	$22.0 \pm 0.29^{o-r}$	$18.4 \pm 0.21^{t-w}$	16.5 ± 0.69^{wx}	26.12^{d}
A3A4	52.5 ± 1.24^{bc}	47.4 ± 1.04^{e}	30.2 ± 0.79^{hi}	$25.9 \pm 0.44^{k-m}$	$24.4 \pm 0.23^{l-o}$	$22.0 \pm 0.95^{o-r}$	$17.0 \pm 0.27^{v-x}$	30.99^{b}
A3A7	51.3 ± 1.18^{c}	48.4 ± 1.19^{de}	$29.3 \pm 0.64^{h-j}$	$25.7 \pm 0.27^{k-n}$	$23.5 \pm 0.55^{m-p}$	$21.3 \pm 0.67^{p-s}$	$17.1 \pm 0.19^{v-x}$	31.40^{b}
A4A7	54.0 ± 0.78^{ab}	47.5 ± 1.15^{e}	$29.8 \pm 1.32^{h-j}$	27.4 ± 1.19^{jk}	$23.0 \pm 0.67^{o-q}$	$21.0 \pm 0.58^{q-s}$	$17.0 \pm 0.58^{v-x}$	31.43^{b}
A3A4A7	55.1 ± 1.15^{a}	50.6 ± 0.99^{cd}	30.8 ± 1.45^{h}	$28.1 \pm 0.82^{i-k}$	$23.7 \pm 0.59^{m-p}$	$21.9 \pm 0.74^{p-r}$	$17.4 \pm 0.35^{u-x}$	32.52^{a}
Mean (Cd)	46.76^{a}	41.98^{b}	27.90^{c}	25.04^{d}	22.77^{e}	20.13^{f}	16.63^{g}	

Values in each column represent the mean ± standard error of three readings. Those marked with different superscripts in each of the column show significant difference at $P \leq 0.05$ as analyzed by LSD. The mean Cd analysis is within the row

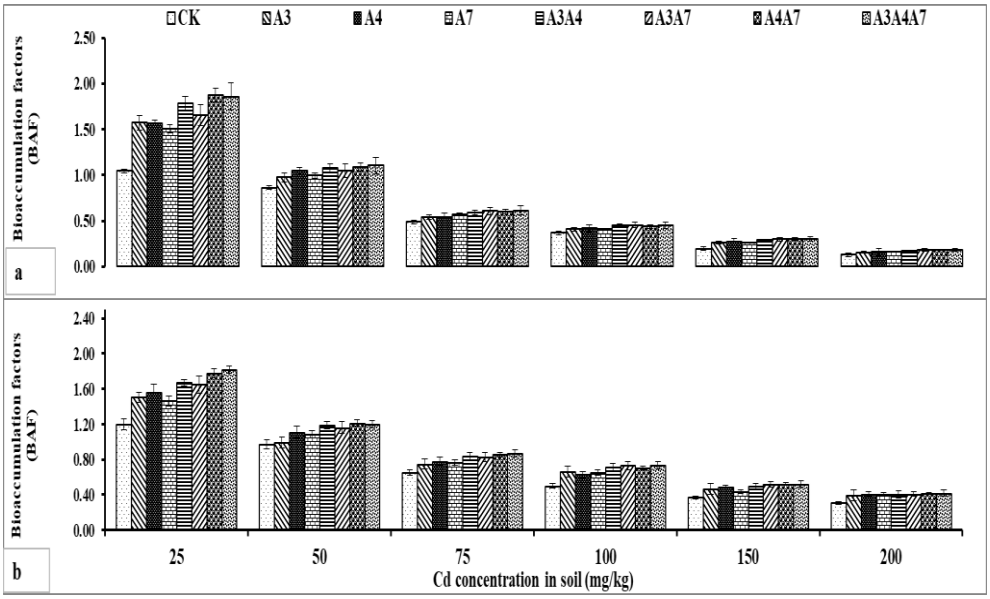

Figure 1. *Bioaccumulation factors (BAF) of Cd for (a) shoot and (b) root of P. giganteum plants grown on metal contaminated soils and under different bacteria strains and their cocktails with standard error shown as error bars*

The root BAF presented in *Fig. 1b* followed a similar pattern as the shoot BAF, decreasing with increasing Cd concentration. However, the root BAF values at the corresponding Cd treatments were slightly higher than the shoot. All treatments up to

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50 mg/kg had values 1.0 and above except the CK (no bacteria application) at 50 mg/kg. Overall, root BAF values were in the range of 0.31 (CK, 200 mg/kg) to 1.82 (A4A7, 25 mg/kg). BAF values for the treatments with no bacteria inoculation were lowest for all Cd treatments for both shoot and root.

The highest TF values for each of the bacteria treatment was recorded at 25 mg/kg Cd, however, above 25 mg/kg Cd, it then decreased progressively with increasing Cd content in the soil. Overall, the values of TF were in the range of 0.30 (CK, 0 Cd supplied) to 1.07 (A3A4, 25 mg/kg Cd supplied) (*Figure 2*).

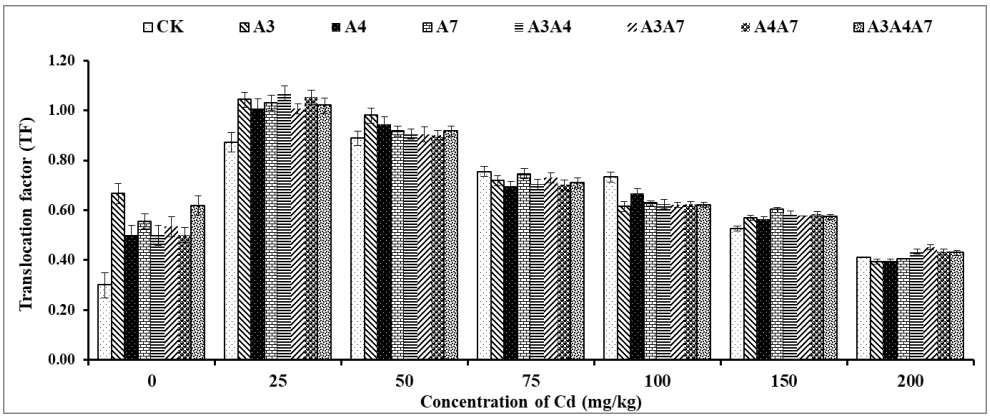

Figure 2. *Translocation factor (TF) of Cd for P. giganteum plants grown on metal contaminated soils and under different bacteria strains and their cocktails with standard error shown as error bars*

Discussion

Several studies have enumerated the beneficial effect of PGP rhizobacteria in promoting plant growths under various abiotic stresses whereby the bacteria act as biosorption for soil toxins and assist in the removal of pollutants from the soil (Glick et al., 2007; Glick, 2012; Saxena et al., 2019).

Although single strain inoculations promoted biomass production, the levels were not as high as in double or triple inoculations. As a result, double and triple could be the preferred combinations in *P. giganteum* growth promotion whereby A4A7 and A3A4A7 had the highest values. It could therefore be inferred that the combined traits from each of the bacterium played a considerable role in growth promotion as explained by the additive hypothesis (Bashan and Holguin, 1997). The additive hypothesis explains the synergistic mechanism of PGPB in achieving higher growth and phytoremediation abilities of consortium bacteria application over single strain application. Several studies have shown that *Klebsiella pneumonia* and *Klebsiella sp* produce high quantities of plant growth-promoting phytohormones such as IAA and ACC deaminase which may have been responsible for the observed high biomass (Qin et al., 2014).

The exposure of plants to excess levels of Cd could have inhibited physiologically active enzymes (Gadd, 2007), inactivated photosystems (Ghori et al., 2019; Zhu et al., 2019), affected mineral absorption and metabolism (Janas et al., 2010). Cd toxicity could have severely damaged various metabolic activities in plants leading to reduced height and weight, and leaf chlorosis (Chowdhury et al., 2018). Leaves of plants exposed to moderate to high Cd concentration without PGPB assistance turned yellow

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whereas plants under the 0 mg/kg Cd (no Cd added) and soil supplied with low metal concentrations were healthy. This is because, plant Cd uptake is largely influenced by the availability of metals, explaining why higher quantities of metals are absorbed into the root tissues of the plants at higher Cd supplied when the plants were growing well under the assistance of PGPB. However, the content of metals in shoots initially increased then starts decreasing as the concentration of Cd supplied continued to increase. This is partly because the excess Cd supplied affected the photosynthetic ability of the plant which in turn reduced the quantity of metals translocated to the shoot of the plant. Plant heights and weights significantly increased at low concentrations of Cd, and then progressively reduced as the quantity of Cd increased. This observation is in line with the findings of Jadia and Fulekar (2008) and Yang et al. (2018), who showed that low quantities of applied Cd elongated the root and shoot of sunflower but at higher concentrations, significantly reduced germination percentage and plant growth especially root and shoot elongation. Many authors, (Tewari et al., 2002; Zhou and Qiu, 2005; Gajewska and Skłodowska, 2007; Alaboudi et al., 2018) observed that different plant species grown in high Cd contaminated soils showed a visible effect on growth and metabolism and described symptoms including stunted growth, reduced biomass, and yellowing of leaves. Such characteristic effects were observed in the *P. giganteum* plants grown under the high Cd concentrations without PGPB.

The extraction of heavy metals from soil by plants is usually a slow process. In order to speed up the process, studies have suggested environmentally friendly approaches such as the use of PGPB and their synergistic effects. From our results, the average concentrations of Cd in shoot of plants inoculated with double strain at 0, 25, and 200 mg/kg Cd were significantly higher than those inoculated with single strain by 51.9%, 12.9%, and 11%, respectively. Similarly, in roots, the average concentrations of Cd in the double strain inoculated plants over the single strain inoculated plants were 53.3%, 11.3%, and 1.7% higher, respectively for 0, 25, and 200 mg/kg Cd. This indicates that a cocktail of the bacterial mixture is better than using single strain culture and their synergistic effect is key in Cd remediation whereby they boost the growth and Cd absorption by host plants. This is because each strain possesses unique characteristics such as Cd resistance, the synthesis of ACC deaminase enzymes, IAA, and siderophore productions and it could be inferred that the combined effects of these traits produced the observed positive results. For instance, *Klebsiella pneumonia* produce antioxidants and exhibit strong Cd tolerance abilities which enable high survival in Cd stressed environment and promote plant growth through the secretion of IAA, ACC deaminase, and other PGP physiological traits (Pramanik et al., 2017). Results from our study correlate the findings of Kang et al. (2016), who reported that the synergistic effect of *Viridibacillusarenosi* B-21, *Sporosarcina soli* B-22, *Enterobacter cloacae* KJ-46, and *E. cloacae* KJ-47 had better resistance and efficiency in the remediation of Pb, Cd, and Cu compared with using single strain culture after 48 h.

Heavy metals such as Cd are known to inhibit the transport of electron in photosynthetic pathway (Monni et al., 2001). The synthesis of chlorophyll in the plant is also directly inhibited by Cd through a misstepping of an enzymatic process or by reducing the efficiency of an essential nutrient (Duan et al., 2018). There was significant reduction in chlorophyll content in the leaves of plants in our study as Cd concentrations increased above 25 mg/kg. At all Cd concentrations, chlorophyll contents were high in treatments with cocktail bacteria inoculations. Numerous studies have demonstrated that the decrease in photosynthetic rate with an increasing amount of

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Cd may be a result of inhibition of the chlorophyll biosynthesis and the photochemical reactions (Song et al., 2019) as well as the disturbance in the activity of enzymes involved in CO_2 fixation (Krantev et al., 2008). However, as evidenced by this study and by other numerous studies (Tak, 2015; Pan et al., 2016; Chiboub et al., 2018), PGPB can improve oxidative stress thereby improving the synthesis of chlorophyll, improve other physiological and biochemical stresses imposed by heavy metals, and improve the adaptability of remediation plants to heavy metal pollution.

According to McGrath and Zhao (2003), the efficiency at which a plant can extract pollutants from the soil is determined by two key factors: biomass production and metal hyperaccumulating capacity. The high biomass production of the *P. giganteum* plant is noted by Zhanxi and Zhanhua (2001) as high as 300 tons (green) material annual yield per hectare is achieved. In terms of metal hyperaccumulating capacity, our study characterized the plant based on the understanding that firstly, for a plant to qualify as a hyperaccumulator of a particular metal, its BAF and TF values should be greater than 1 (Mirza et al., 2010). BAF for both shoot and roots were all > 1 at 25 mg/kg Cd supplied even without the contribution of bacteria, indicating that *P. giganteum* could be a suitable hyperaccumulator of Cd at levels 25 mg/kg. However, to sustain this hyperaccumulative ability up to 50 mg/kg, the plants required the concerted effort of PGPB.

According to our study, TF values at 25 mg/kg were > 1 in plants assisted with PGPB. At same Cd concentration of 25 mg/kg, the TF values in plants without PGPB were less than 1, showing the role the bacteria are playing in the plant's hyperaccumulative characteristics. At same Cd concentrations, root BAF were generally higher than shoot BAF indicating that the mobilization and storage of the metal in aerial plant parts is reduced. This observation was also noted by Xue et al. (2013) who also found that Cd accumulated more in the roots of soybean than in the shoots. Xu et al. (2018) observed similar findings and asserted that in higher plants, roots are the first organs with contact to Cd, and hence, the roots strongly retain more of the Cd with just about 2 % of the accumulated Cd translocated to leaves. It is however necessary to note that, the uptake and translocation of metal from roots to shoots is strongly linked to the speciation of the metal in question, the soil pH, and other factors. Since the goal of the phytoremediation process is to reduce heavy metal concentrations in contaminated soil to acceptable levels within a reasonable time frame, high root BAF values are appreciated. Our results agree with the view that plants possessing greater shoot biomass compensate for lower ability to concentrate metals in shoots in phytoextraction techniques (Ebbs et al., 1997).

Overall, even without PGPB assistance, *P. giganteum* is a hyperaccumulator at lower concentrations of Cd. At moderate concentrations, BAF and TF values were greater than 1 only with the assistance of PGPB, and at high concentrations, the toxicity effects of the metals on both the plants and the bacteria reduced its metal accumulative capacity. Thus, BAF and TF increase as a function of Cd supplied up to 25 mg/kg, after which the factors start decreasing reaching a low of 0.31 (root) and 0.13 (shoot) for BAF and 0.39 for TF, all at 200 mg/kg. These relationships, as seen in *Figures 1 and 2*, were also observed by Sabeen et al. (2013) who found the translocation and bioaccumulation factors of *Arundo donax* L. increased with increasing concentration of Cd up to 500 μg/g, after which it decreased at higher concentration of Cd in soil.

Biological approaches at remediating polluted lands are catching up with a green-conscious society that is trying hard to reduce the excessive use of chemicals in its

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agricultural and environmental clean up activities. This study is a contribution to knowledge in this direction that seeks to reduce pollution and could be further utilized in the production of biofertilizer for the enhancement of plant growth and remediation of Cd polluted lands.

Conclusion

The study employed the use of *P. giganteum* in Cd remediation at different concentrations with the help of three bacteria strains and their combinations. As Cd concentrations progressively increased, plant growth parameters, including plant height, chlorophyll contents, and fresh and dry weights of shoots and roots significantly reduced. However, when the plants were inoculated with PGPB combinations, the measured growth parameters were enhanced. Although significant differences were observed between single and consortium bacteria application, there was no significant difference in growth parameters when either a double or triple consortium was applied. The study also revealed that, as the concentration of the metal increased, the effect differences of either a single, double, or triple strain application became similar. Overall, it has been asserted that high plant biomass can substitute for a relatively low metal accumulation capacity, resulting in the eventual accumulation of a large amount of heavy metal. Therefore, it is recommended that the synergistic abilities of these strains could be utilized in association with *P. giganteum* for Cd remediation. Many different heavy metals pollute our land and multiple contaminations are often common. A study to understand how Jujuncao in association with bacteria could remediate other heavy metals or their multiple contaminations needs to be undertaken.

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