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PHYTOSTABILIZATION OF ARTIFICIALLY HEAVYMETAL CONTAMINATED SOIL USING PGPR (PLANT GROWTH PROMOTING RHIZOBACTERIA)

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Abstract: **Phytoremediation is an emerging technology that uses plants and their associated microbes to clean up pollutants from the soil, water, and air. In order to select the plant growth-promoting rhizobacteria (PGPR) for phytoremediation of heavy metal contamination soil.The present investigation was carried out in soil sample collected from Nallampalli, Dharmapuri district, TamilNadu. Bacterial PGPR isolates in agricultural soil. The PGPR bacteria identify Based on biochemical characterization and specialized literature were identified of the genus *Pseudomonas* and *Bacillus* in the soil. The sterilized soil was treated with 1% of Cu, Mn, Pb heavy metal by spraying. After Cu, Mn, Pb contaminated soil and agricultural soil were put in pots. *Capsicum annum* was selected to PGPR inoculated and metal inoculated on pots which significantly analyzing the plant biomass and photosynthetic pigments of chlorophyll, carbohydrates and Protein component was reported on inoculation microbes under heavy metal and determined the contaminated level in cultivated soil (inoculation Bioinoculants) compared to control plants at 45 DAI (Day After Inoculation). Bioinoculants inoculated plant's fresh and dry weight were decreased under heavy metal treatment compared to microbes uninoculanted control plants. The results of this research showed that heavy metal metabolism was decrease in inoculation microbes on vegetable crops cultivated soil and heavy metal was degraded by microbes. Thus, the capacity of the microbes to survive and degrading of heavy metal and promoted to growth of vegetable crops on *Bacillus* spp. and *Pseudomonas* spp. were good candidates of environmental protector. Therefore, studying the behavior of heavy metal in the soil environment had great significance for its valid application. Phytostabilization is the process by which PGPR are stimulated to rapidly degrade the heavy metal contaminated soil to environmentally safe levels in soils.**

Keywords: **Phytostabilization, Degradation, Bioinoculants, *Solanum lycopersicum*, Environment.**

I. INTRODUCTION

Soil is an important resource that produces food and other raw materials for human. However, soil is often a sink for wastes, including heavy metals. Some heavy metals are essential for living organisms at lower concentrations e.g Co, Cu, Cr, Mn and Zn and are known as trace elements or micronutrients. The term toxic heavy metal includes those elements that are non-essential such as Cd, Pb, Ba, Hg and As (Park, *et al*., 2011). Slow growth and low biomass of plants in metal-contaminated soils may limit the efficiency of phytoremediation (Li and Ramakrishna, 2011).Heavy metals are not biodegradable and therefore once they are present in soil or water they tend to remain there for a long time unless they are actively removed (Sobukola, *et al*., 2010). Association of metals with various soil phases along with element uptake by plants has been studied by some researchers. They have found that the majority of metal uptake is attributed to loosely-bonded ions (Karbassi, *et al*., 2016). However, the slow growth and low biomass of plants in metal-contaminated soils may limit the efficiency of phytoremediation (Ma, *et al*., 2011a).Plant growth promoting rhizobacteria (PGPR) is a group

of bacteria that can be found in the rhizosphere (Ahmad, *et al*., 2008).One of the other most important effective factors in increasing plant yield is seed inoculation or priming with plant growth promoting rhizobacteria (PGPR) (Ashrafi and seiedi, 2010). **.** PGPR is proved to play in important role in degradation heavy metal soil residues. A good phytostabilization process and approach will involve strategic use of PGPR in an engineered way to achieve the best possible detoxification of heavy metal levels.

II. MATERIALS AND METHODS

Isolation and identification of rhizosphere bacteria

Agricultural land soils samples collected from Nallampalli, Dharmapuri district, TamilNadu. Soil samples were placed in plastic bags and stored at40C.Soil samples (10g) were taken into 250ml conical flask, to that 90ml of distilled water was added and kept in a rotary shaker for 15 min.1ml of soil suspension was serially diluted up to 10-8 dillutions.0.1 ml of sample was spread on nutrient agar plates and incubated at 370C for 24 hours. Experiment was carried out thrice to get a pure culture. the colonies were identified by performing Gram's staining and biochemical tests like Indole, MR, VP, Citrate. The colonies were identified on the basis of Bergey's manual of systematic Bacteriology (Rani, 2012)

Extra cellular enzyme activity of *Pseudomonas* Spp and *Bacillus* Spp.

Catalase activity (Ajay Kumar, *et al*., 2012)

48 hrs old test bacterial cultures were placed on a clean glass slide and 3% of H_2O_2 was dropped and mixed with tooth pick. Observation of bubble formation indicates the positive test for catalase.

Oxidase production (Gaby and Hadley, 1957)

The isolates were streaked on year extract mannitol agar plates and incubated for 3 days at 28^0 C. after incubation, a loopful of isolates was placed over oxidase disc (N, N- Tertra methyl –para-phenylediamine dihydrochloride). Development of blue or purple coloration was positive to oxidase production.

Urease activity (Mac Faddin, 2000)

The isolates were streaked on christensen's urea agar slants and incubated for 3 days at 28^0C. Observer the slant for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by a bright pink colour on the slant that may extend into the butt.

Phosphate solubilization tests (Chen, *et al*., 2006)

The bacterial isolates were streaked on pikovsakaya's agar medium. The presence of clearing zones around the bacterial colonies following incubation at 28± 2^0 C for 24 hours indicated positive for phosphate solubilization.

Qualitative detection of siderophore (plate assay) (Schwyn&Neilands, 1987)

The chrome azurol sulfonate (CAS) assay (universal assay-Schwyn&Neilands, 1987) was used since it is comprehensive, exceptionally responsive, and most convenient. The chrome azurol sulfonate assay agar was used. For the qualitative assay cultures were spot inoculated onto the blue agar and incubated at 37^0C/24-48 hours. The results were interpreted based on the colour change due to transfer of the ferric iron from its intense blue complex to the siderophore. The sizes of yellow-orange haloes around the growth indicated total siderophores activity.

Heavy metal tolerant test (Yogendra, *et al*., 2003)

Heavy metal tolerance by bacterial strains was detected by agar dilution method. Freshly prepared agar plates embedded with different heavy metals. Like Cu, Mn, Pb of 100mg/ml was inoculated with overnight grown cultures. Tolerance was observed by the appearance of the bacterial growth on the plates after the incubation at 37^0 C for 24 to 48 hrs.

Preparation of pot culture

The sterilized soil was treated with 1% of Copper (Cu), Manganese (Mn) and Lead (Pb) (1 liter distilled water and 10 ml) herbicide by spraying. After Cu, Mn, Pb contaminated soil and agricultural soil were put in pots The two types of vegetables seed of *Solanum lycopersicum*were obtained from the seeds were soaked in water over-night and surface sterile with 70% ethanol for 15 minutes and washed several times with sterile distilled water. After germination, the seedlings were thinned out to three in each pot. The pots were arranged over a slab in the green house. The plants were irrigated with nitrogen free, sterile tap water on alternate days. After 45 days, the plants were uprooted and scored for nodulation and measured root and shoot length uninoculated plants served as control.

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Analysis of Morphology Parameters from *Solanum lycopersicum*

Growth characteristics (cm)

The plant length was calculated at 45th day in heavy metal Copper (Cu), Manganese (Mn), Lead (Pb) treated soil plants and agricultural soil (inoculated bioinoculants) and control (non-inoculated bioinoculants).Two plants were taken each pot to measure the mean value for all the treated and control plants.

Fresh and dry weight (g)

Plants were collected from triplicates of leaf, stem and root length was recorded. Plants of each replicates were taken to consideration of observation and the respective treated with the control. The fresh weight was recorded and dried in the oven at 80^{0}C until constant dry weight was obtained.

III. RESULTS

Soil physico-chemical properties

Agricultural land soils samples collected from Nallampalli,Dharmapuri district, Tamilnadu. The collected soil (test and control) analyzed for physicochemical parameters such as soil texture, pH, Electrical conductivity, phosphorous, potassium, Nitrogen. These parameters were analysed by (using the standard procedure) soil testing laboratory, Department of Agriculture, Salem district, Government of Tamilnadu, India.

Biochemical characterization of *Pseudomonas aeruginosa* and *Bacillus cereus* isolates

Among two isolates were selected on the basis biochemical characterization, in which one was Gram positive.

Another was Gram negative, and another test was IMVC tests and enzyme activity by *P.aeruginosa* was higher compared to that of *B.cereus* as observed by positive results.**(Table.1)** and the Antibacterial sensitivity by both isolates as observed by positive results.

Both the isolates showed phosphate solubilising activity on Pikovaskaya's agar medium. The phosphate solubilising activity by *P.aeruginosa* was higher compared to that of *B.cereus* as observed by a clear zone around the inoculated strain, after 3 days.

The isolates showed Siderophore production activity on CAS agar medium. The Siderophore production activity by *P.aeruginosa* was higher compared to that of *B.cereus* as observed by a clear zone around the inoculated bacterial isolates**.**

Table 1: Biochemical characterization of *Pseudomonas* spp. and*Bacillus* spp

S.NO	Test	*Pseudomonas aeruginosa (Ps03)*	*Bacillus cereus.*
1	Gram staining	-	+
	Shape	Rod	Rod
2	Motility	+	+
3	**IMVic test**		
	Indole production	+	-
	Methyl red test	+	-
	Vogas-Proskaur	+	+
	Citrate utilization	+	+
4	**Extra-cellular enzymes**		
	Catalase activity	+	+
	Oxidase production	+	+
	Urease activity	+	+
5	**Heavymetal tolearnce**		
6	**Phosphate solubilization**	+	+
7	**Siderosphore production**	+	+

+ Positive - Negative

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Effect of heavy metals on bacterial growth

The growth of the strain measured as the optical density of culture supernant at the definite time intervals; during the incubation of 7 days. However, compared to the metal free culture medium, slight reductions in bacterial growth were observed in metal supplemented media.

Pot culture Studies

After 45 days of inoculation of isolates were compared to *Pseudomonas aeruginosa* and *Bacillus cereus.*All isolates were highly promoting the plant growth of leaf, shoot root compared to control. Then inoculation of *Pseudomonas* and *Bacillus*, and heavy metals (copper, lead and manganese) are inoculated to the pot in *Solanum lycopersicum* . After 45 days the results were observed for root, shoot and leaf growth of the plant. (Figure,1)

Growth characteristic of *Solanum lycopersicum*Shoot, Root length at 45 DAI (cm/Plant)

In the study present study, leaf, shoot and root length *Solanum lycopersicum* were analysed at 45 DAI of isolates treatment compared to control. All isolates were highly promoting the plant growth of leaf, shoot and root length compared to control. Then *Pseudomonas aeruginosa* and *Bacillus cereus* and increased in the leaf, shoot root length when compared to control plant. Length of shoot, root were significally increased in isolated increased in heavy metal S*olanum lycopersicum* treated Ps03+Ps04 Cu and Ps03+Ps04 .Mn increased shoot Root length increased *Solanum lycopersicum* treated Ps04 compared to control plants. (Figure,2)

Growth characteristic of *Solanum lycopersicum*andFresh weight at 45 DAI (g/Plant)

Fresh weight of leaf, shoot, root, were significally increased in isolated increased in heavy metal *Solanum lycopersicum*treated Ps03+Ps04 Cu and Ps03 Pb increased fresh weight compared to control plant. (Figure,3)

Growth characteristic of *Solanum lycopersicum* Dry weight at 45 DAI (g/Plant)

Dry weight of leaf, shoot, and root *Solanum lycopersicum*plant were significantly than isolates of heavy metals and corresponding control. The isolate of heavy metal was decreased in dry weight of shoot and root likewise fresh weigh. The maximum dry weight was observed in treated to *Solanum lycopersicum* Ps04 increase dry weight compared to control plant. (Figure,3)

Growth characteristic of *Solanum lycopersicum*Total chlorophyll and Carotenoids at 45 DAI (mg/g)

The inoculated of isolates are significantly degraded the heavy metals and increased chlorophyll a, b and total chlorophyll content. The highest increase of photosynthetic pigments such as such as chlorophyll a, b and total chlorophyll content was treated with *Solanum lycopersicum* Ps03+Ps04 Pb increased compared to control plant. Analysis carotenoids treated plant *Solanum lycopersicum* Ps04 compared to control plant

Control Ps04 Ps03 Ps03+Ps04 Cu Mn Pb

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Figure 1: Growth characteristic of *Solanum lycopersicum* at 45 DAI (cm/Plant)

Ps03+Cu Ps03+Mn Ps03+Pb Ps04+Cu Ps04+Mn Ps04+Pb Ps03+Ps04 Cu Ps03+Ps04 Mn Ps03+Ps04 Pb

Carbohytrade analysis of *Solanum lycopersicum*(mg/g)

DeterminationCarbohydrate content from heavy metal (Cu, Mn, and Pb) soil plant and control soil plants are presented in treated *Solanum lycopersicum*corbohytrade content increase the Ps03 Pb and Ps04 Pb increase carbohytrade content compared to control plant (Figure,4)

Protein analysis of *Solanum lycopersicum*(mg/g)

Protein plays a major role in plant growth and development. In this study, control soil plants and PGPR treated soil plant showed increased amount of protein (45days). *Solanum lycopersicum* Ps03+Ps04 Cu protein increase the compared to control plant (Figure,4)

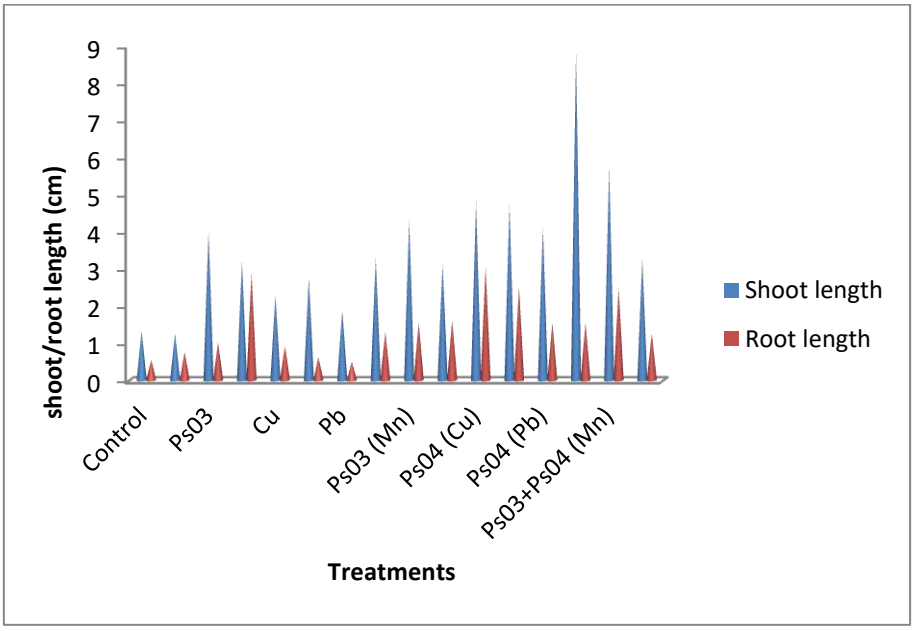

Figure 2: Growth characterization of *Solanum lycopersicum* L. (Shoot length, Root length) at 45 DAI (Cm/plant)

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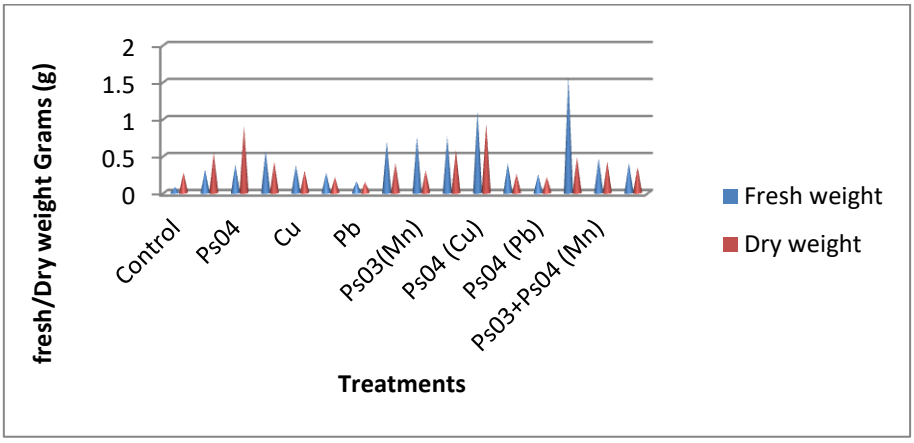

Figure 3: Growth characterization of *Solanum lycopersicum* L. (Fresh weight, Dry weight) at 45 DAI (g/plant)

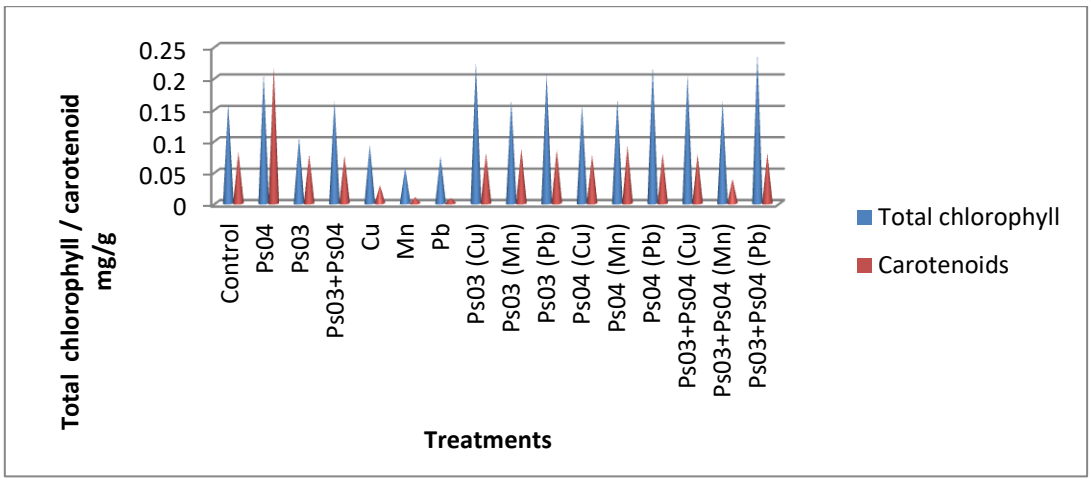

Figure 4: Photosynthetic pigments of *Solanum lycopersicum* L. Total chlorophyll and Carotenoid content at 45 DAI (mg/g)

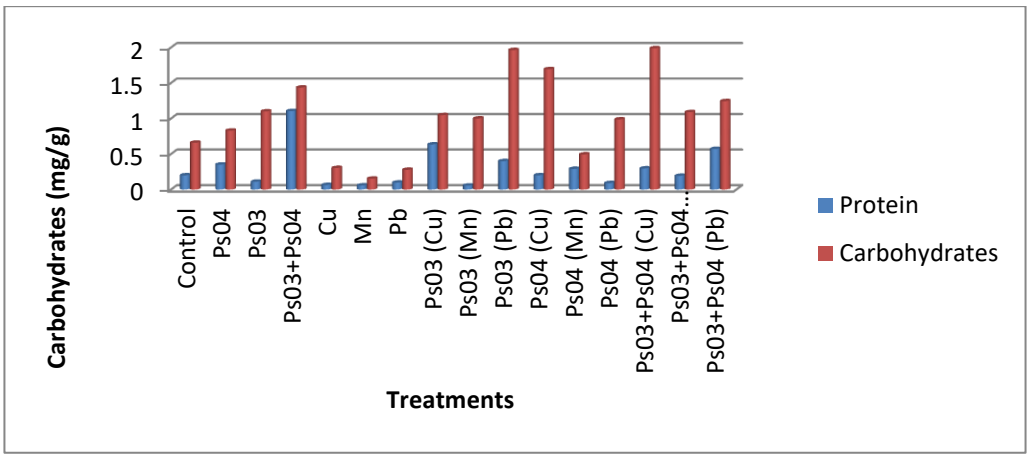

Figure 5: Analysis of Carbohydrates and protein estimation of *Solanum lycopersicum* L. on 45 DAI (mg/g)

IV. DISCUSSION

The present study was carried out in soil sample collected from Nallampalli,Dharmapuri district, Tamil Nadu.Culturing on different agar medium revealed presence of *Pseudomonas* and *Bacillus* bacteria. The classification of the *Pseudomonas* and *Bacillus* genus by specific physiological and biochemical characteristics is useful, but is not enough to distinguish all *Pseudomonas* species. Results confirmed that identification and characterization of the *Pseudomonas and Bacillus spp* can only be achieved by combining cultural, biochemical tests.

In Gram's staining, the morphology of isolated *Pseudomonas* strains showed Gram-negative, pink coloured, motile, rod shaped appearance. These findings agreed with the findings reported by earlier researchers (Tripathi, *et al*., 2011). Same result as found in the isolates which gives positive reaction for catalase activity, oxidase activity, citrate utilization, arginine hydrolysis and negative reaction for nitrate reductase activity (Bhojiya and Joshi, 2015). Similarly, reported that biochemical and morphological characterization was carried out. Similarly, (Remette, *et al*., 2006) revealed that pseudomonas have plant growth promoting properties. Isolated strains showed high ability of IAA production, phosphate solubilization and siderophore production.

Similarly, differences observed among the plant growth parameters and biomass of plants between control and mine soil. Biomass is an important factor considering the phytoremediation efficiency of plants (Ciura, *et al*., 2005). Resulted for bioinoclulated (PGPR) *Pseudomonas* and *Bacillus spp* increases the plant growth and plant biomass.

The changes of total chlorophyll and carotenoid concentration between the PGPR inoculated plant and control plant, contaminated plant. After 45 days reported as contain chlorophyll and carotenoid concentration observed of *Solanum lycopersicum* .PGPR inoculated plant increase of chlorophyll and carotenoid concentration compared to control plant and metal contaminated plant. Furthermore, decrease in total chlorophyll content under Mn exposure suggested the possibility of adverse effect on photosynthesis and plant metabolism as evidenced with findings of chlorosis, browning and necrosis of young leaves (Heniriques, 2003). Cd ions cause degradation of chlorophyll a more rapidly than chlorophyll b, resulting decreased ch a/b ratio (Kummerova, *et al*., 2010).

Phytostabilization studies imply that it is possible to develop new phytoextraction strategies with inoculation of plants used for phytoremediation with rhizobial microbes in order to enhance phytoextraction of metals from contaminated soils. When considering approaches to alter heavy metal mobilization, there are several advantages to the use of beneficial microbes rather than chemical amendments because the microbial metabolites are biodegradable, less toxic, and it may be possible to produce them in situ at rhizosphere soils.

V. SUMMARY

The present investigation was carried out in soil sample collected from Nallampalli, Dharmapuri district, Tamilnadu. Bacterial PGPR isolates in agricultural soil. The PGPR bacteria identify based on biochemical characterization and specialized literature were identified of the genus *Pseudomonas aeruginosa* and *Bacillus cereus*. in the soil.

The sterilized soil was treated with 1% of Cu, Mn, Pb heavy metal by spraying. After Cu, Mn, Pb contaminated soil and agricultural soil were put in pots.*Solanum lycopersicum* was selected to PGPR inoculated and metal inoculated on pots which significantly analyzing the growth character's. The heavy metal was reflected by statistical significant decrease in average morphological parameters and increased photosynthetic pigments, Carbohydrates and Protein on vegetable crops. Plant growth, shoot and root length, fresh weight, dry weight and photosynthetic pigments of chlorophyll, carbohydrates and Protein component was reported on inoculation microbes under heavy metal and determined the contaminated level in cultivated soil (inoculation Bioinoculants) compared to control plants at 45 DAI (Day After Inoculation).

Bioinoculants inoculated plant's fresh and dry weight were decreased under heavy metal treatment compared to microbes uninoculanted control plants. The results of this research showed that heavy metal metabolism was decrease in inoculation microbes on vegetable crops cultivated soil and heavy metal was degraded by microbes. Thus, the capacity of the microbes to survive and degrading of heavy metal and promoted to growth of vegetable crops on *Bacillus* spp. and *Pseudomonas* spp. were good candidates of environmental protector. Therefore, studying the behavior of heavy metal in the soil environment had great significance for its valid application.

Phytostabilization is the process by which PGPR are stimulated to rapidly degrade the heavy metal contaminated soil to environmentally safe levels in soils. PGPR is proved to play in important role in degradation heavy metal soil residues. A good phytostabilization process and approach will involve strategic use of PGPR in an engineered way to achieve the best possible detoxification of heavy metal levels.

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