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Heavy Metal Degradation Plant Growth Promoting Rhizobacteria (PGPR): Assess their Bioremediation Potential and Growth Influence of Vigna mungo L. Growing on Cadmium Contaminated Agriculture Soil

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Abstract

Heavy metal contamination in agriculture soil devotes serious of crosscutting issue worldwide. Numerous microbial strains have been frequently used to deduce the heavy metal contaminated in the agriculture soil. Herein, the present investigation demonstrates that the effects of inoculate of beneficial microbes on plant growth and heavy metals reducing ability as known bioremediation in contaminated soil. Therefore, two bacterial strains such as Bacillus subtilis and Pseudomonas fluorescens are isolated from rhizosphere soil and inoculate on Vigna mungo L. grown in cadmium (Cd) contaminated soils. Before the inoculation, the bacterial strains are conformed by biochemical test and molecular identification tools. Afterwards, the plant inoculated with isolates describes as experimental plant compared with non-inoculated control plants. The obtained results show the appreciable plant growth with decreased level of heavy metal concentration available at experiential group of plant growing on the cadmium (Cd) contaminated soils, while compared to control group. Moreover, inoculation of V.mungo L, particularly, P. fluorescens and B. subtilis appears to be an effective ways for enhancing the short term rhizoremediation potential of plant growth and lowering losses in the plant biomass and degreasing above ground tissue contamination. The results of the study indicate that metal mobilizing (Plant Growth Promoting Rhizabacteria) PGPR's could be used as an effective inoculant for improving the bioremediation in heavy metal polluted soil, as well as for the reclamation of heavy metal contaminated soil, which also favourable to growing economically important agriculture plants.

Keywords: *Bioremediation, Cadmium, Contaminated soil, Agriculture, Vigna mungo L.*

I.INTRODUCTION

In recent years, widespread industrialization and the various anthropogenic activities deposit a large quantity of the heavy metal elements and dust particles onto the agriculture soil, which severely cause the agriculture productivity from the year to year **[1].** On other words, heavy metals are considering as a major pollutant in the environment, which cause serious of side effects and health problem in living community including plant and animals **[2].** Typically, non-degradable and toxic nature of heavy metal ions accumulation on agricultural soils deduce soil quality and inhibits plant growth by hampering essential plant function and metabolic processes **[3].** Among the heavy metals, cadmium (Cd) is one of the important heavy metals that are more toxic and carcinogenic agent in nature and severely cause major organs while body inhalation. Moreover, Cd causes no opposing effect on growth and developmental stage of plant due to their huge bioaccumulation index in plants grown in soils **[4].** Soil and crop interaction assists to enter the Cd in the food-chain as results potential food safety and human health risks **[5].**Therefore, Cd contamination and accumulation in agriculture soil is particular serious in crop production. The application of the ecological remediation methods, such as bioremediation appears an excellent low-cost method for reclaiming the heavy metals from polluted soil. The use of heavy metal tolerant or metal accumulating specie that are important energy crops in such process is becoming a very promising opportunity as renewable energy source, avoiding the utilization of farmland for the production of such non-edible biomass **[6,7].**Phytoextraction refers to the plants' ability to import soil contaminants through their roots, and to heavy metal accumulate these compounds in the above ground tissues **[8,9].** The use of heavy metal resistant or tolerant microorganisms for the decontamination of heavy metals from contaminated soil has attracted growing attention because of several problems associated with pollutant removal using

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conventional methods **[10]**.Rhizosphere is the nutrient-rich part of the soil surrounding plant roots and is a habitat for millions of soil microbes that thrive on root exudates, termed rhizodeposits **[11].** Plant growth promoting rhizobacteria (PGPR) can enhance plant growth and biomass by employing multiple mechanisms and assuring the availability and uptake of certain macronutrients as well as micronutrients to the plant growth **[12].** In the contrast, PGPR and nitrogen fixing bacteria have other plant-beneficial properties such as bioremediation of soil contaminated with trace elements by increasing plant tolerance to abiotic stresses and limited availability [**13]**. Therefore, beneficial plant-microbe interactions in the rhizosphere can be influence plant vigour and soil fertility. These beneficial effects of the PGPR have direct and indirect mechanisms and performance on plant growth **[14].** Typically, bioinoculants are the plant growth promoting microorganisms which by several mechanisms augment plant growth. The application of bioinoculants into the soil improves the production of antibiotics and the biodegradation of soil organic matter, increase nutrient supply; enhance plant tolerance to environmental stresses on plant growth. Hence, bioinoculants have been adopted as a clean and efficient soil conditioner or amendment to improve the quality of soil nutrient and fertility by agriculturists and plant biologists **[15].**

Herein, the present work includes the effects of isolate two bacterial strains such as *Bacillus subtilis* and *Pseudomonas fluorescens* from rhizosphere and inoculate on economically important crop *Vigna mungo* L. growing in cadmium (Cd) contaminated soils. Then, assess metal tolerant and growth efficiency of Rhizosphere inoculated *Vigna mungo.* L. Furthermore, assess rate of heavy metal accumulation in *V. mungo* L. and the bioremediation ability of PGPR's in cadmium contaminated agriculture soil.

II. MATERIALS AND METHODS

A.Soil Collection, Preparation, and Characterization

Cadmium contaminated soil collected from both mining area of Salem District, Tamil Nadu, India. Soil collected from 0-20cm depth using an auger and collected soils were passed through 2 mm sieve to remove the large debris, dusts and stones. Soil samples were stored aseptically in sterile plastic polythene bags for further analysis. Afterward, the experimental and control soil were used to investigate physicochemical parameters and heavy metal analysis using standard protocols.

B.Isolation and identification of rhizosphere bacteria

Rhizosphere soil was collected from agricultural land from Dharmapuri district, Tamil Nadu, India. The collected soil samples were tightly packed in plastic polythene sterile bags and stored at 4°C for the further use. The bacteria colonies were obtained from the soil samples by serial dilution technique and grown Nutrient Agar medium at 37°C for 24h for isolation purpose. After the completion of incubation period, the bacterial strains were isolated by pure culture techniques.

C.Identification of Bacterial Strains by Using Biochemical Analysis

The morphological and structural properties of isolated bacterial strains were confirmed by staining and biochemical analysis using to determine their structural and biochemical properties **[16].** As well as, the biochemical investigation comprises Methyl red staining, VP test, Citrate utilization, Indole production of the isolates, which were performed by standard microbiological techniques and protocols **[17].**

D.Catalase Activity

To investigate catalytic activity of bacterial strains, forty-eight hours old test bacterial culture was placed on a clean glass slide. Then, 3% of H_2O_2 was dropped on the old bacterial culture and

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mixed with toothpick. The presence of bubble formation indicates the positive result for catalase activity of bacterial strains.

E.Oxidase Production

To determine oxidase production, the isolated bacterial strains were streaked on year extract mannitol agar plates and incubation for 3 days at 28° C. After the end of incubation time, a loopful of bacterial colony was placed over oxidase disc containing N, N-Tertra methyl-para-phenylediamine dihydrochloride. The appearance of blue or purple colour indicating the ability of oxidase production by bacterial strains.

F.Urease Activity

To assess the urease activity, bacterial strains were streaked and grown on Christensen's urea agar slants and the streaked culture plates were incubation for 72 h at 28°C. Afterward, the culture plates were kept under observation at particular period of time (every 6 and 24h), continuous to 6 day. The Urease activity was confirmed by the appearance of bright pink colour on the slant that may extend into the butt **[18].**

G.Phosphate Solubilization

The bacterial isolates were screened for phosphate solubilization according to the standard method **[19].**For this, Pikovsakaya's agar medium amended with tri calcium phosphate was prepared and loop full of fresh bacterial culture was placed on to the plates. Then, the plates were incubated at 28± 2°C for 3-4 days. Afterward, the phosphate solubilization was confirmed by a clear halo appearance around the bacterial colony.

H. Siderophore Production

Siderophore production performance by bacterial strains were assessed by using Chromazurol S (CAS) universal blue agar plates **[20].** Actively growing bacterial cultures were spot inoculated on the CAS blue agar plate and incubated at 30° °C for 48h. Subsequently, the formation of yellow-orange halo zone around the bacterial colony indicates the production and release of the siderophores by bacterial strains on the agar plates.

I.Cadmium (Cd) tolerance Test

To determine Cd tolerant efficiency of bacterial strains, the agar dilution method was carried out to investigate Cd tolerant of selected bacterial isolates **[21].** In this method freshly growing cultures were streaked on Cd (Cadmium nitrate) amended agar plates at different concentration ranging from 25-100 µg/L. Then, the Cd resistance was determined by the viability counting of selected bacteria after the 3 to 4 days of incubation. Then, the minimal inhibitory concentration (MIC) was estimated and recorded.

J. Salt and pH-tolerant test

Salt tolerant of bacterial isolate was investigated by inoculation of each bacterial strains in Nutrient Agar medium with different concentration of NaCl such as 0.5%, 5%, 10%,15%, 20%, 25% and 30%, respectively and incubation for 48 h at 28± 2° C. As well as, pH-tolerant of bacterial strains was investigated in the plat count agar medium with different acetic conditions ranging from 2-12, which was adjusted by using 1N NaOH and 4N HCl. After 48 h incubation, Salt and pH-tolerant growth efficiency of bacterial strains was estimated and recorded by colony counting unit.

K. Effect of Temparature on bacterial isolates

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Optimum growth conditions of bacterial isolates were determined with respect to temperature. To determine optimum temperature, isolates was grown in NB at different incubating temperatures viz., 20,25,30,37and 42°C.After 24h incubation, their absorbance was measured at 600nm using spectrophotometer.

L. FTIR Analysis

FTIR analysis bacterial strains were prepared according to **[22].** Briefly, the bacterium was grown in the presence and absence of 1mM Cd^{2+} for 24 h at 37°C. The bacterial cells were harvested by centrifugation at 3000 rpm for 10min and washed several times with saline solution (0.9% NaCl, pH 6.5).Then bacterial pellets were freeze-dried overnight and their infrared spectra were recorded on a FTIR spectrophotometer in the region 4000-500/cm.

M. Antibiotic Sensitivity Test

The susceptibility of bacterial strains against antimicrobial agents was testified by antibiotic disk method established by **[23].** Mueller- Hinton medium was poured into petri plates, after the solidification, it autoclaved at 15 lbs (121° C) for 15 min. Afterward, dipped a sterilized swab into the 24 h old culture broth and applied on the surface of the agar plate, then, completely allowed to dry for about 5 min. Then, the commercially available different antibiotic disk with treated with Streptomycin[25], Chloramphenicol[25], Kanamycin[25] were placed on the agar with the help of sterilized forceps and incubation at 30°C for 24h or until bacterial growth. Then, the zone of incubation was measured and recorded.

N.Molecular identification of bacterial Strains

To determine molecular identification at species level, the pure culture of selected strains was analysed in PAR Life science, Trichy, Tamil Nadu. The molecular identification was used to characterization of the selected bacteria by gene sequencing of 16S rRNA gene using 27 Forward and 1492 Reverse primer. The experimentally identified bacterial sequences were searched in BLAST and NCBI nucleotide database centre to compare the similarity between sequence **[24].** The obtained sequencing of 16S rRNA gene similar with *Pseudomonas fluorescens* (MK478897) and *Bacillus subtilis* (MK483262).

O. Pot experiments

For the pot experiment, the collected soils of Rhizosphere and Cd contaminated agriculture soil were sterilized and placed into plastic pots, and the moisture content of the soils has maintained at approximately 70% of water holding capacity. Healthy black gram (*Vigna mungo* L.) seeds were obtained from Tamil Nadu, Agriculture University (Coimbatore), and sterilized in 20% sodium hypochlorite for 10 min, then the sterilized seeds were washed with three times in deionized water. After the seedling, each plot was inoculated with the 2mL of bacterial suspension (*Pseudomonas fluorescens*-PF01, *Bacillus subtilis*-BS01). The control was bacterial free suspension. The plants were grown in under the greenhouse conditions. Every week, 1 mL of same bacterial suspension were diluted into 1ml of distilled water and inoculated into plant growing soil. After 45 days, plant biomass (fresh and dry weight), shoot and root length and chlorophyll contents was assessed.

P. Measurement of Growth parameters

Every time interval (7 days) until 45 days, the shoot and root length were measured by using vernier caliper. Then, the plant seed sowing was carefully collected and measured.

Q. Estimation of Fresh weight and Dry weights

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The fresh and dry weight of plant parts were recorded until 45 days and units are expressed in gram per plant (g/plant). The fresh weight of the plant parts were calculated using electronic weight balance immediately after the harvesting. The dry weight was calculated after overnight drying the plant parts at 65°C using hot air oven.

R. Photosynthetic pigment assay

Fresh leaves (0.150 g) were collected from each treatment and control group of plant and the chlorophyll a and chlorophyll b were determined according to the previous literature **[25].**

S. Heavy metal accumulation of plant samples

To analysis heavy metal accumulation of plants, the experimental plant was collected and thoroughly washed three times with deionised water to remove surface dust and soil particles. Subsequently, the washed plant parts were dried at 80°C. After completion dry, the plant parts weighed and cut into <0.5 mm size. The plant samples (200mg) was taken then digested in aqua regia (H_2 SO_4: HNO_3 : $HClO_4$) in ratio 1:3:1 using glass beakers on a hot induction plate. After that, digested samples were cooled and filtered using 0.22µm nylon syring filters. The samples were further diluted using double distilled water to make up the final volume upto 50ml.These samples were analysed for using atomic absorption spectrophotometry **[26].**

T. Estimate the Heavy Metal Concentration in Soil Samples

Total heavy metal concentration of some interest metal was estimated according to the method established by **[27].** using atomic absorption spectrophotometer and conditions as described above.

U. Mass production of bacterial isolates

(a). Bacillus subtilis

The prepared nutrient broth was sterilized at 1.1kg/cm^2 pressure for 20 min, and one loopful of *Bacillus subtilis* was inoculated and incubated at 37°C for 24 h, which was describe as mother culture. Then, 100 mL of mother culture was transferred to 1 L of sterilized nutrient broth kept in a fermenter. The inoculated bacteria growth was incubated for 72 h for bacterial harvesting. Then, the harvested samples were mixed with 2 kg of sterilized peat soil amended with 250g calcium carbonate, dried in shade and packed in polythene bags.

(b). *Pseudomonas fluorescens*

Pseudomonas fluorescens inoculated and multiplied in sterilized King's broth.The pH of the substrate (talc) is adjusted to pH7 by adding calcium carbonate 150g/kg and incubated the bacterial strain for 2 days afterward, 400 mL of *Pseudomonas fluorescens* suspension was added into 1kg of substrate containing 5g of carboxymethyl cellulose. Then, the mixed samples were collected and packed in polythene covers for further uses.

V. Soil Microbial Population (CFU/ 1g of soil)

To calculate soil microbial population, microbial population in the soil samples was estimated by the dilution plate's techniques. Then, the colony forming units per gram soil was measured and recorded.

Statistical analysis

The value of each experiment was independently conducted and performed in triplicate, and the data were assessed using a mean ± standard error and students ttest. The significant was statistically considered if p* <0.05.

III .RESULTS

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Physio-chemical properties of collected the soil samples such as cadmium contaminated soil and rhizosphere as a control soil were testified to the quantitative assessment of the presented metal elements and minerals, which were list out in **table1.** Detailed studies proved the estimated Cd level was about 1.5 mg and 12 mg / 1 g of soils in the normal and contaminated soil, respectively. Therefore, the present findings showed the Cd presented rate was differing between normal and Cd contaminated soils.

Table 1. Physicochemical analysis of heavy metal contaminated and normal soil samples

Physicochemical Properties	**pH**	**EC**	**N**	P	K	**Cu**	**Mg**	**Ca**	**Cd**	**Zn**
Contaminated soil	8.72	0.88	01.28	0.260	01.06	16	335	298	**12.5**	310
Normal soil	7.64	0.2	0.45	02.25	01.02	7.6	210	129	**0.5**	260

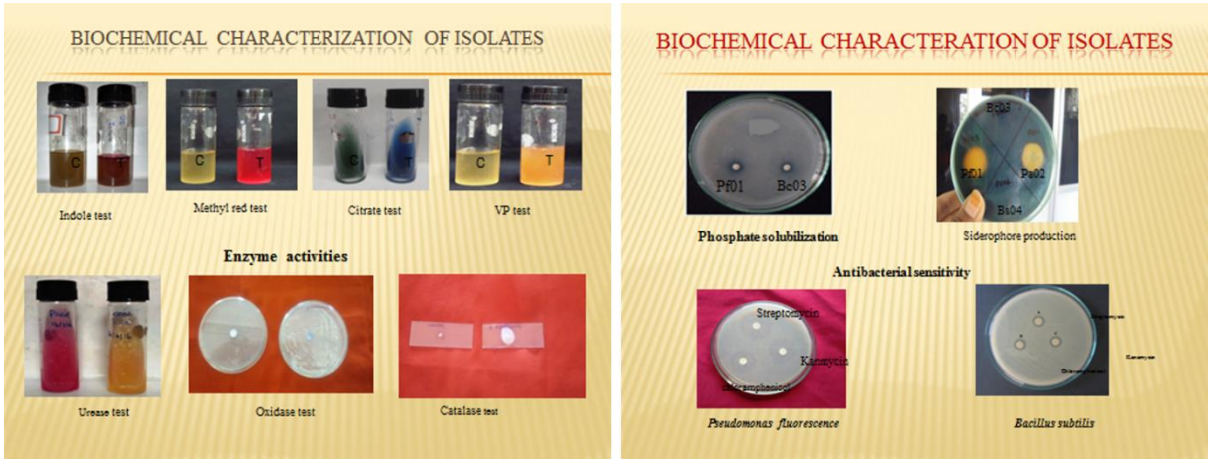

Figure 1. The results findings of bio-chemical characterization of isolated bacterial strains

The bacterial strains isolated from the rhizosphere soil; among them two beneficial strains were identified as *Pseudomonas fluorescens* (PF01) and *Bacillus subtilis* (BS01), according to the preliminary screening and molecular identification study. Two bacterial strains such as PF01 and BS01 were further used to assess the reduction ability to heavy metal toxicity in plant growing on Cd polluted soil. The obtained results showed both strains have enzyme production activity (citrate, catalase, urease and oxidase production), and phosphate solubilizing properties. Moreover, gram positive nature of PF01 exhibits tryptophanase activity and produce stable acids, acetoin, and siderophore, it proved by indole, methyl red (MR) and Voges-Proskauer (VP) test. But, in case of gram negative BS01 strain lack of tryptophanase activity and produce stable acids, acetoin, and siderophore. Therefore, PF01 had better biochemical properties while compared to BS01 strain. The presented biochemical parameters of both bacterial strains were list out in **figure 1 and table 2**.

Table 2. Biochemical analysis to identification of bacterial strains

Test	Gram staining	Indole	MR test	VP test	Citrate	Catalase	Oxidase	Urease	Phosphate solubilization	Siderophore production
PF01	+	+	+	+	+	+	+	+	+	+
Bs01	-	-	-	+	+	+	+	+	+	-

(+) – Positive, (-) Negative

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The pure culture technique was carried out isolate the bacterial strains, which was used to further studies. The different was showed between the PF01 and BS01 isolated and grown bacterial shown in **figure 2**.

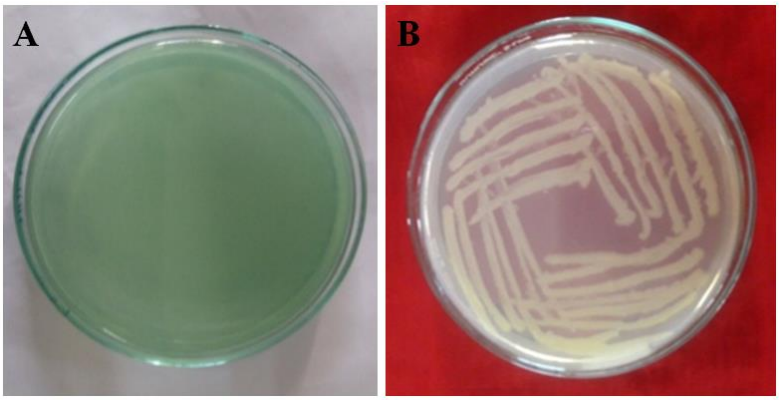

Figure 2. Pure culture technique for the bacterial strain's isolation.

Then, the Cd tolerant of both bacterial strains were assessed at the Cd concentration ranging from 25-200 µg/mL, respectively. The growth progression of both strains was not affected, and it indicates that the PF01 and BS01 exhibit more Cd tolerance efficacy even at the lower concentration (25 and 50 µg/mL). Slightly effects of growth inhibit occurring in 75 and 100 µg/mL Cd dissolved agar medium streaked with bacterial strain. In addition, the zone of inhibition was higher in PF01, when treated with 75 µg/mL, as well as, zone of inhibition in BS01 was highly noticed at 100 µg/mL, respectively. Therefore, these findings proved the both bacterial strains can able to well-grow efficiency in Cd contaminated soils (12.5 mg/1 g of soil). When compared to BS01, PF01had minimal antibody resistant's showed **table 3**.

Table 3. The values of Cadmium tolerant efficiency of PF01 and BS01 at different Cd concentration

Treatments	PF01	BS01
Control	0	0
Cd 25µg/l	0.3 mm	0.5 mm
Cd 50µg/l	0.75 mm	1 mm
Cd 75µg/l	1.27 mm	1.7 mm
Cd 100µg/l	2 mm	2.4 mm

As well as, the salt tolerant efficiency of bacterial isolate was estimated from 0.5-30 % of NaCl concentration. The obtained findings showed that the appreciable growth progression of both PF01 and BS01 was noticed from 0.05% and 15 % of NaCl concentration. In this contrast, the growth inhibits was noticed at increased concentration from 20-30% of NaCl concentration. Therefore, the bacterial strains of PGPR unable to be grown in higher salt concentration (25 and 30 %), while compared to lower concentration (0.05-15%) (**Table 4**).

Table 4. The sustainability of PF01 and BS01on different salt concentrations

	Salt concentration						
S.No	**0.5%**	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
PF01	+++	+++	++	+	-	-	-

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BS01	+++	+++	++	++	+	-	-

("+" or "-" sign indicate the presence and absence of bacteria growth)

Then, the pH tolerant efficacy of PF01 and BS01 was determined at acetic concentration ranging from pH 2-12. The obtained result displayed the experimental bacterial strains can able to noticeable grown at pH 6, 8 and 10, respectively. In addition, the growth inhibits was noticed at strong acetic conditions (pH 2 and 4) and strong alkaline condition (pH 12) acetic conditions, respectively. The obtained results clearly point outed the PGPR class of bacteria can able to survive at weak base to weak alkaline (pH 6-10) condition shown in **table 5**. Hence, the both bacterial strains can able to grow Cd contaminated soil (as weak alkaline condition).

Table 5. The growth effects of PF01 and BS01on different pH-conditions

	pH values					
	2	**4**	**6**	**8**	**10**	**12**
PF01	-	-	++	+	+	-
BS01	-	-	++	++	+	-

("+" or "-" sign indicate the presence and absence of bacteria growth)

The obtained results clearly point outed the PGPR class of bacteria can able to survive at temperature (25-37°C) condition shown in **table 6**. Hence, the both bacterial strains can able to grow Cd contaminated soil

Table 6. Effect of Various Temperature on the growth of bacterial isolates

	Various temperature				
	20°C	**25°C**	**30°C**	**37°C**	**42°C**
PF01	1.523	1.680	1.744	1.804	1.654
BS01	1.344	1.553	1.525	1.471	1.456
Cd+ PF01	2.199	2.622	2.770	2.655	1.186
Cd+ BS01	1.7528	2.739	2.768	2.498	2.462

The FTIR analysis confirmed that the presence of carboxyl group, amino and phosphate moieties in bacterium and this analysis conformed our assumption regarding binding of Cd^{2+} with bacterium. It is clear that the peaks attributed to amide linkage, appearing at1665 and 1533/cm are shifted to 1661 and 1409/cm in *Bacillus subtilis* and another one appearing at1664 and 1541/cm are shifted to 1662 and 1543/cm in *Pseudomonas fluorescens* respectively ,in the presence of Cd^{2+}**(Figure.3)**

The next investigation focusing about the antibiotic sensitivity evaluation of PF01 and BS01 against commercially available different antibiotic disk such as Streptomycin[25], Chloramphenicol[25] and Kanamycin[25]. The results revealed that the significant growth of PF01 and BS01 inhibition of was noticed against above mentioned three antibiotics. Moreover, increased zone of inhibition was observed at 25 mm and 29 mm, respectively, while treated with kanamycin[25] treated PF01 and BS01 group. Moreover, antibiotics effects on PF01 and BS01 were displayed as 14 mm and 16 mm for streptomycin[25] and 21 mm and 23 mm for chloramphenicol[25], respectively, as shown in **table 7**.

Table 7. Assess the zone of inhibition after the treated with three different antibiotics for Antibiotic Sensitivity test against PF01 and BS01.

S.No.	**Commercial**	**Inhibition zone diameter (mm)**

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	antibiotic disc	PF01	BS01
1.	Streptomycin	14	16
2.	Chloramphenicol	21	23
3.	Kanamycin	25	29

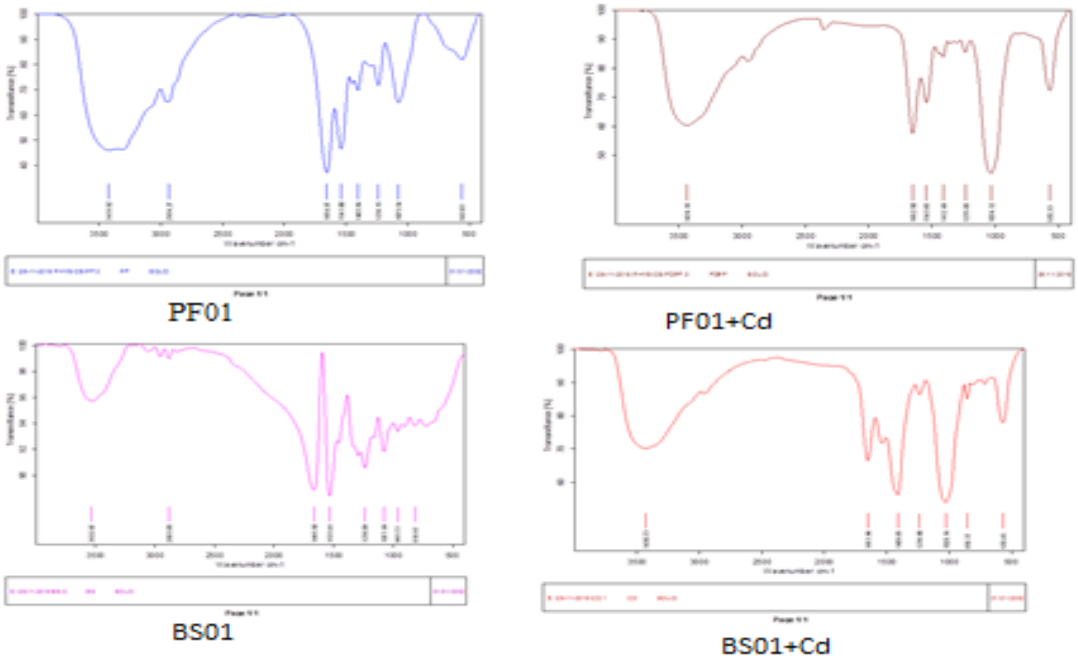

Figure .3. FTIR analysis PF01 and BS01 in the absence and presence of Cd^{2+}

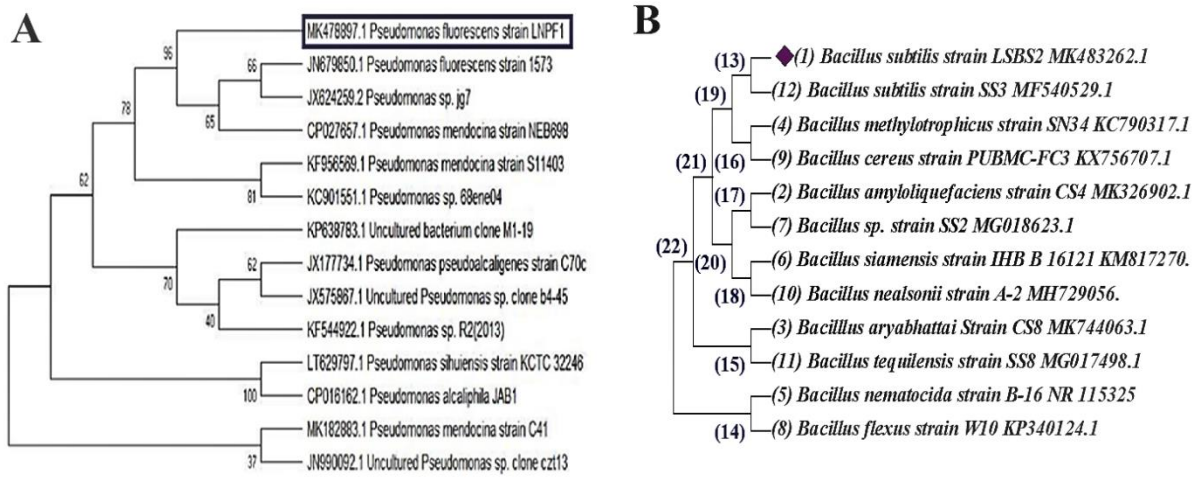

Figure 4. Phylogenic analysis used to identification of (A) *Pseudomonas fluorescens* (PF01) and (B) *Bacillus subtilis* (BS01)

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Isolated the bacterial strains from rhizosphere soil was identified on the basis partial 16S rDNA sequences analysis using molecular tool. The results also ensure the isolated microbial strains were identified as *Pseudomonas fluorescens* (PF01) and *Bacillus subtilis* (BS01) shown in **(Figure 4).**

The pot study was carried out to determine the effects of reducing ability of heavy metal toxicity by experiment microbes (figure 5). Seed were grown on both soil conditions such as cadmium (Cd) contaminated soil (experimental group) and the normal soil (Control). Afterward, the experimental set up was divided into 5 groups, Group I (Normal soil), Group II (Cd contaminated soil), Group III (PF01 inoculated Cd soil), Group IV (BS01 inoculate Cd soil) and Group IV (PF01+BS01 inoculated Cd soil). After 45 days, the plant growth and development were calculated by measuring the length of the root and shoot of compared to control and experimental plant. The results revealed the shoot and root height was measured as 28.4±0.98 and 8.9±0.66 g in PF01+BS01 treated group, which was higher than other groups such as BS01 (22.2±0.8 and 6.8±0.69 g) and PF01 (25.7± 0.9 and 7.5±0.42 g). Therefore, the combination of PF01+BS01 inoculate plant groups showed significantly higher shoot and root height, while other shown in **figure 5** and **table 8.**

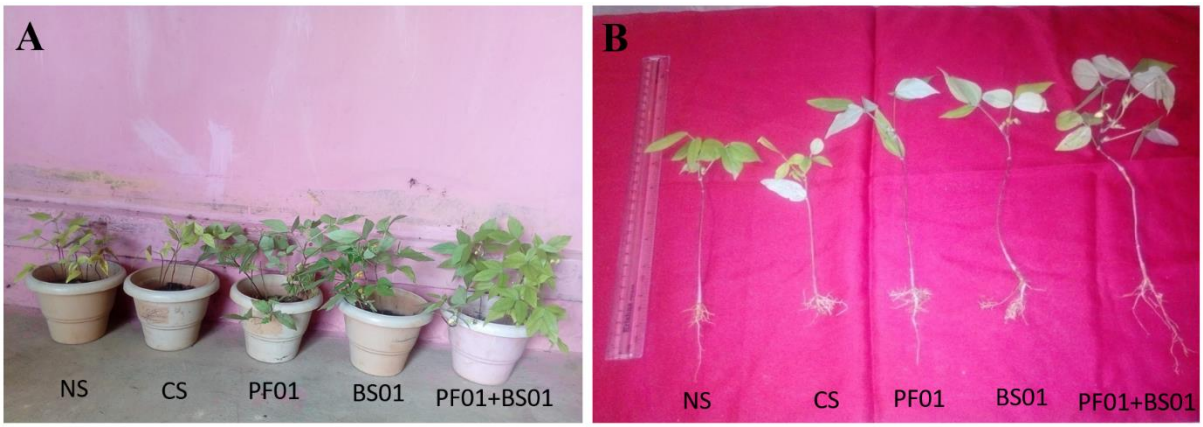

Figure 5. Investigation of plant growth in with or without bacterial inoculate.

Table 8. Determination Growth parameters(shoot and root length) of plant growing on control and treated group of soil after 45 days.

Treatments	**Shoot length (cm/plant)**	**Root length (cm/plant)**
Control	17.6±1.2	6.3±0.2
Contaminated soil	10.4 ±0.63	3.2±0.29
PF01	25.7± 0.9	7.5±0.42
BS01	22.2±0.8	6.8±0.69
PF01+BS01	28.4±0.98	8.9±0.66

At the meantime, fresh weight and dry weight of plant and photosynthetic pigments such as chlorophyll and carotenoid were assessed after 45 days treatment in shown **table 8** and **table 9**. The result findings showed PF01+BS01 effective influence the plant pigments and weight than others. The fresh weight of PF01+BS01 was observed as about 9.7 g and dry weigh was about 1.8 g, respectively. As well as, pigment such as chlorophyll and carotenoids were about 1.165±0.008 (mg/g) and 0.091±0.02 mg/g in PF01+BS01 treated plant groups. Then, the chlorophyll and carotenoids content in PF01 were noticed as 1.184±0.006 and 0.061±0.06 (mg/g). As well as, BS01 had increased chlorophyll and carotenoids contents, which was notices as 0.805±0.44 and 0.058±0.04 mg/g, respectively. The bacterial inoculate improved photosynthetic pigment, total root length and fresh weight and dry weights, while compared with control groups after the 45 days treatment.

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As in figure 6, The mass production of bacterial strains was shown in different colours. The *Pseudomonas fluorescens* appear in white in colour and *Bacillus subtilis* occurs at green colour due to its fluorescent properties.

Figure 6. Mass production of *Pseudomonas fluorescens* (PF01) and *Bacillus subtilis* (BS01).

Table 9. Determination of plant biomass (Fresh and dry weight) of plant parts after 45 days of treatment

Treatments	**Fresh weight (g/plant)**	**Dry weight (g/plant)**
Control	5.4	0.6
Contaminated soil	4.2	0.5
PF01	7.5	1.2
BS01	6.2	0.8
PF01+BS01	9.7	1.8

Table 10. Quantitative analysis of total chlorophyll and carotenoids of *Vigna mungo. L* grown on different soil condition.

Treatments	***(Vigna mungo. L)***	
	Total Chlorophyll (mg/g)	Carotenoids (mg/g)
Rhizospheric soil (control)	0.065±0.067	0.035±0.05
Contaminated soil	0.033±0.003	0.016±0.044
PF01	1.184±0.006	0.061±0.06
BS01	0.805±0.44	0.058±0.04
PF01+ BS01	1.165±0.008	0.091±0.02

After 45 days, the soil samples were subjected to assesses the quantitative amount of heavy metals and minerals accumulation in plant treated with different inoculates. The

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heavy metal accumulation in plant was treated group such as PF01, BS01, PF01+BS01 were calculated as bout 1.48mg/g,2.685 mg/g, 1.962mg/g, 1.673mg/g, 1.765mg/g respectively. Subsequently, Cd accumulation was highly occurring in plant, while treated with PF01 while compared to other groups (**Table.11**).

Table 11. Accumulation level of Cadmium in control and treated plants.

S.No	Treatments	Cd level in plant (mg/g)
1.	Normal soil (control)	1.48
2.	Contaminated soil	2.685
3.	PF01	1.962
4.	BS01	1.673
5.	PF01+BS01	1.765

After 45 days incubation, the soils samples with bacterial strain were testified to determine the quantitative analysis of heavy metals. The results showed the combinational PF01+BS01 inoculates deduce the heavy metal concentration (0.8 mg/g) in Cd contaminated soil, while compared with others such as BS01 (2.2 mg/g), and PF01 (1.4 mg/g). Therefore, the obtained results proved the bacterial inoculation effectively deduced Cd concentration after the 45 days shown in **table 12**.

Table 12. Heavy metal analysis of different control and experimental soil sample after 45 days

Physicochemical Properties (mg/g)	
Heavy metal analysis	(mg/g)
Normal soil (control)	0.5
Contaminated soil	10
PF01	1.4
BS01	2.2
PF01+BS01	0.8

The quantitative analysis of bacterial population in different soil population was estimated after 45 days shown in table 12. The obtained finding revealed more colony forming unit (CFU) was noticed at PF01+BS01 (8.2 $X10^7$ CFU/g soil) due to their combinational growth. As well as, the population was observed in PF01 and BS01 inoculated soil was estimated as 6.2 $X10^7$ and 4.8 $X10^7$ (CFU/g soil). Hence, the obtained result proved rhizosphere microbes inoculated soil used as a bio fertilizers to increase the agriculture crop productivity and used to the bioremediation of heavy metals in Cd contaminated soil shown in **table 13**.

the

and

Treatments	Total bacterial population in soil (CFU/g soil)
(Normal soil) Control	1.5 $X10^7$
PF01	6.2 $X10^7$
BS01	4.8 $X10^7$
PF01+BSO1	8.2 $X10^7$

Table 13. Determine total bacterial population in treatment control group of soil after 45 days**.**

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IV.DISCUSSION

Potential for bioremediation depends upon interactions among the soil, heavy metals, bacteria and plants. The roots of plants interaction with a large number of different microorganisms that are major determinants of extent of the phytoremediation. Bacterial genera such as *Bacillus, Pseudomonas* and *Bravi bacillus* are well known to promote the plant growth and yield in different non-leguminous plants **[28].** The isolates were screened for their plant growth promoting activities *viz.*, Indole production, MR-VP, Citrate, Phosphate solubilization, other lytic enzymes like Catalase, Urease and Oxidase activities. The isolated rhizobacterial characterization by assess their structural and morphological features, selected medium for their growth, determine their sustainability on salt concentration and pH was done as described in Bergy's Manual of Systematic Bacteriology **[29].** Plant growth on agricultural rhizospheric soil is influences by several environmental factors. Beneficial soil microorganisms can be a significant component for achieve the yield. Alignment of soil microbes not only bacteria, and also arbuscular mycorrhizal fungi, that can form interactions with the majority of plant species. These beneficial microbes have been shown to enhance plant growth by improving water and nutrient intake and to enrich plant tolerance under biotic and abiotic stresses, such as heavy metal, drought, and salinity **[30].** Proteobacteria are the supreme metal-tolerant microbes in seriously polluted soils **[31]** and Another one is *Actinobacteria* important of phylum was noticed in rice rhizosphere soil [**32**] these two phyla were start to assist as the dynamic bacterial fraction in heavy metal-polluted soil **[33].** Species in *Actinobacteria* are extensively dispersed in soil, water, and compost and play essential roles in defeating pathogenic microorganisms and degrading intractable compounds **[32**] and also this bacterial phylum dominant in metal contaminated soils and has been generally used to indicate heavy metal contamination**[33].**In the present studies, *Vigna mungo* L. grown on cadmium contaminated soil and further inoculated with *B.subtilis* and *P.fluorescens*, which showed that results influence the plant growth parameter and development. Afterward, determine their effect on the heavy metal degradation in cadmium contaminated soil are known bioremediation. The beneficial microorganism is effectively used as a biofertilizer as well as heavy metal deducing agent in agriculture soil, therefore this good strategy to clean environment [**34, 35]** reported that Zn and Cd contamination could significantly diminution the *Pseudomonas* richness in rhizosphere soils; however, they are highly resistant as Zn concentration pre-eminent. *Rhodobacter* and *Stenotrophomonas* are also the dominant genera, to resist metal concentration and could show passive or active absorption of metals; like wish *Steroidobacter* was found in the Cd contaminated soil and to reduce Cd concentration in polluted soil **[36].** In addition, the use of PGPR in the phytoremediation of metals and development of crops production has also limitations, most important of which is the difficulty to achieve similar results under other field, soil, and or plant conditions, as suggested by Parnell **[13].** Plants inoculated with plant growth promoting rhizobacteria assist to increased plant biomass and influence development of plants against heavy metal contaminated soil, where the metal content exceeds limit of plant tolerance. Therefore, Rhizosphere microorganisms are the best choice to product the agriculture plant growth due to their heavy metal resistance properties. The PGPR promote plant growth by produce plant hormones like auxins, cytokinins, and gibberellins, and to prevent ethylene production **[37]** and it can mineralization of organic phosphate and/or other nutrients. Habitually, PGPR have been used as hydroponic solutions to act like a buffer and to inhibit the disposal of metals for uptake. Additionally, PGPR was added to soil to increase the disposal of heavy metals for acceptance by the plant and to simplify their drive to plant roots and the accretion of heavy metals **[38].** Many of the previous studies have also found no changes or reductions in plant growth and yield with rises in the heavy metal uptake of different plants **[39].**

V.CONCLUSION

In the present, PGPR species of *Pseudomonas fluorescens* (PF01) and *Bacillus subtilis* (BS01) helpful for reduction of leaching of cadmium (Cd) heavy metals in agriculture environment. The obtained results revealed the used PF01 and BS01 minimization of toxicity of cadmium and enhanced of *Vigna mungo L.* growth under metal stress condition. The isolated PF01 and BS01strains was confirmed by various biochemical study such as enzyme like activity enzyme production activity (catalase, urease

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and oxidase production), phosphate solubilizing properties, siderophore production activity and gram staining technique. As well as, phylogenic analysis also used to confirms the isolated strains. The isolated and identified bacterial strains used as a bio-inoculate to assess the metal degradation efficiency and *Vigna mungo L.* plant growing on Cd contaminated agriculture. The result findings confirmed the PF01 and BS01 strains could able to deduce heavy metal concentration in agriculture soil and evoke *Vigna mungo L.* plant root and shoot length. Therefore, the present proved the combination or alone PF01 and BS01 as an effective agent to improve soil fertility and crop production under heavy metal stress condition. The synergistic interactions between plants and plant growth promoting rhizobacteria along with Phytoremediation and soil bio augmentation are important strategies to clean-up heavy metal-contaminated soil.

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