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A Study on Microbe-Assisted Phytoremediation of Cadmium by Five Different Ornamental Plants

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Abstract*: Heavy metal pollution has become a serious environmental concern. Anthropogenic and industrial activities lead to the emission of heavy metals into the environment. Among the heavy metals cadmium is considered toxic because of its high mobility from the soil to plant system and it induces oxidative stress in plants. The aim of the present study is to determine the effect of cadmium tolerant rhizobacteria on ornamentals for phytoremediation of cadmium. Rhizobacteria were isolated from plants growing along the Cooumriver, Chennai. Fifteen rhizobacterial strains were isolated and screened for their cadmium tolerance. Five rhizobacterial strains which showed high resistance to cadmium were selected and identified as Bacillus sp. (four strains) and Escherichia sp. (one strain).The selected five rhizobacterial strains were also characterized for the plant growth promoting traits. Of the five strains one strain of Bacillus sp. was selected and used for the phytoremediation studies in combination with five ornamental plants namely Helianthus sp., Tagetes sp., Tithonia sp., Zinnia sp. and Cosmos sp. belonging to the family Asteraceae. Pot experiments were conducted to study the ability of these five ornamentals alone and in combination with PGPR to accumulate cadmium. Of the various treatments given, ornamental plants in combination with bacteria and cadmium performed better than the other treatments. Helianthus sp. thrived best in the soil artificially contaminated with cadmium. The cadmium content was analyzed using ICP-MS and the results showed that the cadmium accumulation was more in the presence of bacteria.

Keywords: Cadmium, PGPR, Bacillus sp., Helianthus sp., phytoremediation.

I. INTRODUCTION

Heavy metal pollution is becoming a global problem, although levels of pollution differ from place to place. The economic, agricultural and industrial developments are often polluting the environment [1]. Man's exposure to heavy metals comes from industrial activities like mining, smelting, refining and manufacturing processes [2]. Heavy metals such as cadmium, copper, lead, chromium, zinc and nickel are important environmental pollutants [3]. Moreover, the soil has been traditionally the site for disposal of the heavy metal wastes which needs to be treated. Cadmium is one such extremely toxic metal, commonly found in industrial workplaces related to metal plating, batteries, paint pigments, plastic stabilizers, photographic chemicals etc [4]. Municipal sewage, fuels and chemical fertilizers especially phosphate ones are among the major sources of cadmium [5], [6]. Cadmium is considered as a toxic metal because of its relatively high mobility in the soil-plant system [7], [8]. It is responsible for causing an oxidative stress and disruption of membrane composition and functioning [9], [10]. It is responsible for the disturbances of several physiological processes, such as photosynthesis, water relations, uptake of transport and utilization of several macro and micronutrients [11] - [13]. Consumption of food is the most common route for the exposure of cadmium in human beings. It affects central nervous system, immune system, reproductive system, lungs, kidneys and bones. High exposure can lead to lung and prostate cancer [4]. Phytoremediation is the use of hyperac cumulating plants and beneficial microbes to clean up metal contaminated soils through phytoextraction, phytovolatilization, phytodegradation, rhizodegradation, rhizofilteration and phytostabilization[14], [15]. Many studies have been made to demonstrate the potential of certain edible plants like Vignaradiata, Vignaunguiculata, Dolichusbiflorus, Brassica juncea, Eleusinecoracana and Zea mays in combating pollution caused by cadmium and other similar heavy metals in the soil. However, ultimately the economic value of the plant is lost as they have heavy metal accumulated in them and they are unfit for human or animal consumption.In the present study, an attempt was made to determine the potential of five selected ornamental plants namely Helianthus sp., Tagetes sp., Tithonia sp., Zinnia sp. and Cosmos sp. that belong to the family Asteraceae (Compositae) for phytoremediation of cadmium contaminated soil. Asteraceae members are reported to have the ability to tolerate heavy metals like lead, zinc, copper, nickel and they respond well in the contaminated sites when compared to other plants. In addition, rhizosphere strains isolated from contaminated sites were evaluated for their cadmium tolerance and PGPR characteristics. The synergistic effect of cadmium tolerant rhizosphere bacteria and the potential of selected ornamental plants in phytoremediation was further analyzed in the present study.

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II. MATERIALS AND METHODS

A. Rhizosphere Sample Collection

Rhizosphere soil samples were collected from the plants growing along the Cooum river bank. The selected plants were carefully uprooted and the excess of soil was removed by gentle shaking and roots were collected in sterile polythene bags.

B.Isolation of Rhizobacteria

The root samples were immersed in 25 ml of sterile distilled water in a sterile conical flask and kept in the shaker overnight. The soil particles were allowed to suspend in water thoroughly and 1 ml of this supension was serially diluted and plated on sterile nutrient agar for isolation of rhizobacteria.

C. Screening for Cadmium Tolerant Rhizobacteria

Cadmium tolerant ability of the rhizobacteria was visually estimated by minimal inhibitory concentration (MIC) method. MIC was determined by amending cadmium chloride of different concentrations (100 mg/L to 4000 mg/L) in the nutrient agar medium by agar dilution method [16]. The lowest concentration of cadmium chloride at which no growth occurred, when compared with the control was considered as MIC. All the experiments were carried out in replicates.

D. Biochemical Characterization of Cadmium Resistant Rhizobacteria

The cadmium tolerant rhizobacteria were identified based on gram staining and some standard biochemical tests like catalase, oxidase, motility, IMVIC test, gas production, urease test, triple sugar iron test using Bergey's Manual [17].

E. Characterization of Cadmium Tolerant Rhizobacteria for Plant Growth Promoting Traits

*1) Production of Indole Acetic Acid:*The bacterial strains were grown in Luria Bertani broth amended with 0.1 g/L of tryptophan for 24 h. After incubation, 2 ml of cell suspension was centrifuged at 1000 rpm for 10 min and 2-3 drops of orthophosphoric acid was added to the supernatant along with 4 ml of Salkowski's reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid). The tubes were kept at room temperature for 20 min. IAA production was indicated by the development of pink colour. Optical density was read at 530 nm and level of IAA production was estimated by using standard IAA [18].

2) Ammonia production: Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml of peptone water incubated at 28±2 C for 48-72 h and 0.5 ml of Nessler's reagent was added. Development of yellow to brown colour indicated a positive result [19].

3) Siderophore production: Bacterial strains were grown in Luria Bertani broth for 24 h at 28 C. The cells were pelleted by centrifuging the cultures at 10,000 rpm for 5 min. To the supernatant equal volumes of Chrome Azurol S (CAS) reagent was added and observed for the colour change. The change of colour from blue to orange indicated positive result [20 modified].

4) Phosphate solubilization: The bacterial isolates were grown on Pikovskaya medium and phosphate solubilization activity was indicated by the formation of a clear halo zone around the bacterial growth after 3 days of incubation at 28±2 C [21].

5) Hydrogen cyanide (HCN) Production: The isolates were screened for the production of HCN by using nutrient agar amended with 4.4 g/l glycine and the bacterial isolates were streaked onto the medium. A Whatman no.1 filter paper soaked in 2% sodium carbonate in 0.5% picric acid solution was placed on top of the agar surface. Plates were sealed with parafilm and incubated at 28±2 C for 4 days. Development of orange to red colour indicated HCN production [22].

F. Tolerance of Rhizobacteria to Other Heavy Metals

The cadmium tolerant bacterial isolates were also screened for resistance to other heavy metals such as zinc, mercury, lead and chromium. This was performed by amending various concentrations of zinc sulphate, mercuric chloride, lead acetate and potassium dichromate (100 mg/L to 2000 mg/L) in the nutrient agar medium by agar dilution method [16] and the bacterial isolates were streaked onto the medium and incubated at 37 C for 48 h. The growth of the bacterial isolates in various concentrations of these heavy metals indicated their tolerance level.

G. Soil Sterilization and Analysis

Soil used in pot experiments contained red soil, manure and sand. The soil was sterilized twice at 121 C under 15 psi for30 min. The soil sample used was analyzed for different soil characteristics and physiochemical properties of soil viz., pH, electrical conductivity, organic carbon, nitrogen, sulphur, potassium, manganese, iron, copper and zinc content.

H. Plant Materials Used in the Study

Five ornamental plants namely *Helianthus* sp., *Tagetes*sp., *Tithonia*sp., *Zinnia* sp. *and Cosmos* sp. belonging to the family Asteraceae (Compositae) were selected for the study. The seeds were purchased from the Agri Horticultural society, Chennai.

I. Phytoremediation-Seed Germination Assay

Seed germination assay was conducted to screen the ability of the selected ornamentals to grow in the soil artificially amended with cadmium. The seeds were surface sterilized with 0.1% mercuric chloride solution and then washed several times in sterile distilled water. The surface sterilized seeds were soaked in cadmium chloride solution of different concentrations (0, 2, 5, 10, 15, 20, 25 and 30 ppm) overnight and were sown in small cups containing sterile soil. Ten seeds per cup were sown. After sowing, the seeds were regularly watered with the respective concentration of cadmium chloride and control was watered with distilled water. All the assays were carried out in triplicates. After 3-5 days number of seeds germinated was counted for germination percentage.

$$Germination = \frac{Total\ number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sown} \times 100\%$$

J. Bacterization of Seeds

24 h culture of the bacteria of 0.1 OD was mixed with 1% Carboxymethylcellulose. The seeds were soaked in this mixture for 10 min and then sown in sterile soil.

K. Phytoremediation Using Pot Culture Experiment

The seeds of the selected plants (*Helianthus* sp.,*Tagetes*sp., *Tithonia*sp., *Zinnia* sp.) were surface sterilized with 0.1% mercuric chloride and then washed 4-5 times with sterile distilled water. The concentration of cadmium chloride which exhibited 50% growth inhibition of these plants were selected (*Helianthus sp.* and *Tagetes sp*.- 30 ppm; *Tithonia* sp.- 5 ppm and *Zinnia* sp.- 15 ppm) and the seeds were soaked overnight in the respective concentration of cadmium chloride and control was maintained with distilled water. The soaked seeds were sown in small polythene bags and four different treatments were given including the control.

T0- The plants were watered with distilled water and maintained as control;
T1- The seeds were treated with cadmium tolerant rhizobacteria and watered with distilled water;
T2- The plants were spiked with cadmium chloride solution;
T3- The seeds were treated with cadmium tolerant rhizobacteria and spiked with cadmium chloride solution.

L. Measurement of Growth Parameters and Statistical Analysis

The growth parameters such as root length, shoot length, fresh weight and dry weight of the plants were measured after 5 days and 10 days. The root and the shoot length was measured in cm and the fresh and dry weights were measured in grams. All the experiments were carried out in triplicates and the data was subjected to Least Significant Difference (LSD).

M. Drying of the Plant Sample for Cadmium Analysis

The harvested plants were washed thoroughly with tap water and then with distilled water to remove the soil particles adhering to it. The plants were then dried at 80 C for 48 h in an hot air oven. The dried plants were ground into powder using a mortar and pestle and then used for the analysis.

N. Cadmium Analysis

Cadmium content in the root and shoot tissues were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Sample preparation was done following the protocol of Ref 23. The cadmium in the plant tissues were expressed in parts per million (ppm).

O. Bioaccumulation Factor

Bioaccumulation factor (BAF) was determined by the ratio of metal concentration in plant to that of metal concentration in soil following the method of Ref 24.

$$BAF = \frac{Metal\ concentration\ in\ plant}{Metal\ concentration\ in\ soil}$$

P. Translocation Factor

Translocation factor (TF) was determined as the ratio of metal concentration in shoot to that of metal concentration in roots following the method of Ref 25.

$$TF = \frac{Metal\ concentration\ in\ shoot}{Metal\ concentration\ in\ root}$$

III. RESULTS

Fifteen bacterial strains were isolated from the rhizosphere of plants growing along the bank of Cooumriver. Cadmium tolerant ability of the rhizobacteria was estimated by minimal inhibitory concentration (MIC) method. The lowest concentration of cadmium chloride at which no growth occurred, when compared with the control was considered as MIC. The Minimum Inhibitory Concentration (MIC) of cadmium of each bacterial strain is given in Table I. Of the fifteen rhizobacterial strains isolated, five strains showed a MIC in the range of 1300 mg/L to 4000 mg/L.

TABLE I
MINIMUM INHIBITORY CONCENTRATION OF CADMIUM OF RHIZOBACTERIAL STRAINS

S.No	Bacterial strains	MIC of cadmium (mg/L)
1.	R1	100
2.	R2	200
3.	R3	1300
4.	R4	3000
5.	R5	100
6.	R6	200
7.	R7	900
8.	R8	800
9.	R9	900
10.	R11	900
11.	R14	600
12.	R15	600
13.	C2	4000
14.	C7	2700
15.	C8	2600

The five rhizobacterial strains which were highly resistant to cadmium were further identified by standard biochemical tests using Bergey's manual. The results of the biochemical tests are tabulated in Table II.Based on the results of biochemical tests the selected cadmium resistant rhizobacterial strains were identified using Bergey's manual as four strains of Bacillus sp. (R3, R4, C2 and C7) and one strain of Escherichia sp. (C8).

TABLE II
BIOCHEMICAL CHARACTERIZATION OF CADMIUM RESISTANT RHIZOBACTERIA

Biochemical characteristics	R3	R4	C2	C7	C8
Gram's stain	+	+	+	+	–
Cell size	3×1µm	1×0.5µm	4×1.5µm	1×0.5µm	1.5×0.5µm
Motility	+	+	+	+	+
Catalase activity	+	+	+	+	+
Oxidase activity	–	–	–	–	–
Indole production	–	–	–	–	+
Methyl red test	–	–	–	–	+
Vogesproskauer test	+	+	+	+	–
Citrate utilization	+	+	+	+	–
Urease	–	–	–	–	–
Triple Sugar Iron test	K/K	K/K	K/K	K/K	A/A
Gas production	–	–	–	–	+

(+) indicates presence; (-) indicates absence

The above mentioned cadmium resistant rhizobacteria were also screened for the plant growth promoting characteristics such as IAA production, ammonia production, siderophore production, phosphate solubilization and hydrogen cyanide (HCN) production. The results are tabulated in Table III and Table IV.

TABLE III
CONCENTRATION OF IAA IN FIVE BACTERIAL STRAINS

S.No	Name of the Bacteria	Concentration of IAA (µg/ml)
1.	Bacillus sp. (R3)	73.21
2.	Bacillus sp. (R4)	55.56
3.	Bacillus sp. (C2)	61.20
4.	Bacillus sp. (C7)	39.79
5.	Escherichia sp. (C8)	14.26

TABLE IV
PLANT GROWTH PROMOTING (PGP) CHARACTERISTICS OF CADMIUM RESISTANT RHIZOBACTERIA

PGP Characteristics	R3	R4	C2	C7	C8
Ammonia production	++	++	++	++	+
Siderophore production	+	–	–	–	–
Phosphate solubilization	+	+	–	–	–
HCN production	++	++	++	++	++

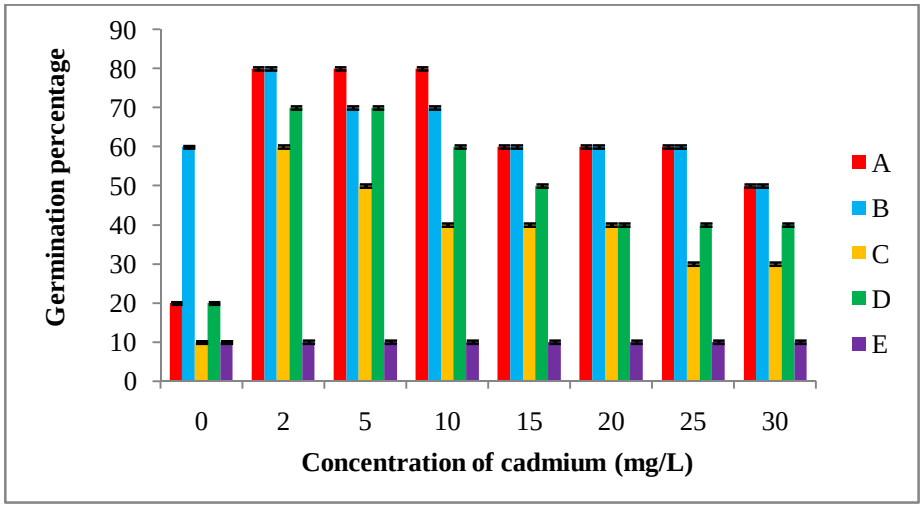
(++) indicates strong presence; (+) indicates presence; (-) indicates absence; R3, R4, C2 and C7- *Bacillus* sp.; C8- *Escherichia* sp.

The cadmium tolerant rhizobacteria were also screened for the tolerance to other heavy metals like zinc, mercury, lead and chromium from 100 mg/L to 2000 mg/L. All the strains were tolerant to zinc, lead and chromium upto 2000 mg/L. The strain R4 (Bacillus sp.) ws tolerant upto 900 mg/L and the other four strains were tolerant upto 100 mg/L.The soil used in the present study was analyzed for the physicochemical parameters such as pH, Electrical Conductivity (EC), Organic Carbon (OC), macronutrients and micronutrients. The results are tabulated in Table V.

TABLE V
PHYSICOCHEMICAL PARAMETERS OF THE SOIL USED FOR THE STUDY

S.No	Physicochemical parameters	Quantity
1.	pH	6.77
2.	EC	0.14
3.	Organic carbon (OC)	0.47%
4.	Nitrogen (N)	114.31 Kg/acre
5.	Phosphorus (P)	19.17 Kg/acre
6.	Potassium (K)	95.5 Kg/acre
7.	Calcium	421.87 mg/kg
8.	Magnesium	122.79 mg/kg
9.	Sodium	91.79 mg/kg
10.	Iron	16.65 mg/kg
11.	Manganese	9.78 mg/kg
12.	Copper	1.34 mg/kg
13.	Zinc	0.82 mg/kg
14.	Boron	0.53 mg/kg
15.	Sulphate	10.29 mg/kg
16.	Humus (HA)	94.06 Kg/acre
17.	Total minerals	228.98 Kg/acre

Five ornamental plants namely Helianthus sp., Tagetes sp., Tithonia sp., Zinnia sp. and Cosmos sp. belonging to the family Asteraceae were chosen for the study. Before using the seeds of the selected ornamentals for phytoremediation study, seed germination assay was conducted to determine the percentage of germination of those seeds in different concentrations of cadmium (0, 2, 5, 10, 15, 20, 25, 30 ppm). The results are shown in Fig 1. Since Cosmos sp. showed poor germination rate, the other four ornamental plants were selected for the phytoremediation studies.



A- Helianthus sp.; B- Tagetes sp.; C- Tithonia sp.; D- Zinnia sp. and E- Cosmos sp.
Fig 1: Germination percentage of the selected ornamental plants in different concentrations of cadmium (mg/L)

Pot culture experiment was carried out with the four ornamental plants namely Helianthus sp., Tagetes sp., Tithonia sp. and Zinnia sp. The seeds of these plants were given four different treatments including the control. The four different treatments include:
T0- plants watered with distilled water and maintained as control;
T1- seeds treated with cadmium tolerant rhizobacteria and watered with distilled water;
T2- plants spiked with cadmium chloride solution;
T3- seeds treated with cadmium tolerant rhizobacteria and spiked with cadmium chloride solution.
The growth parameters such as root length, shoot length, fresh weight and dry weight were measured after 5 days and 10 days in all the four plants. All the growth parameters measured in treated plants (T1, T2and T3) showed a gradual increase in their length and weight when compared with the control. Of the three treatments, the plants treated with cadmium and *Bacillus* sp. (T3) showed more increase in the growth parameters (root length, shoot length, fresh weight and dry weight) when compared with the control (T0) of the four plants. Helianthus sp. showed more growth than the other three ornamental plants (Fig 2, 3, 4 and 5).

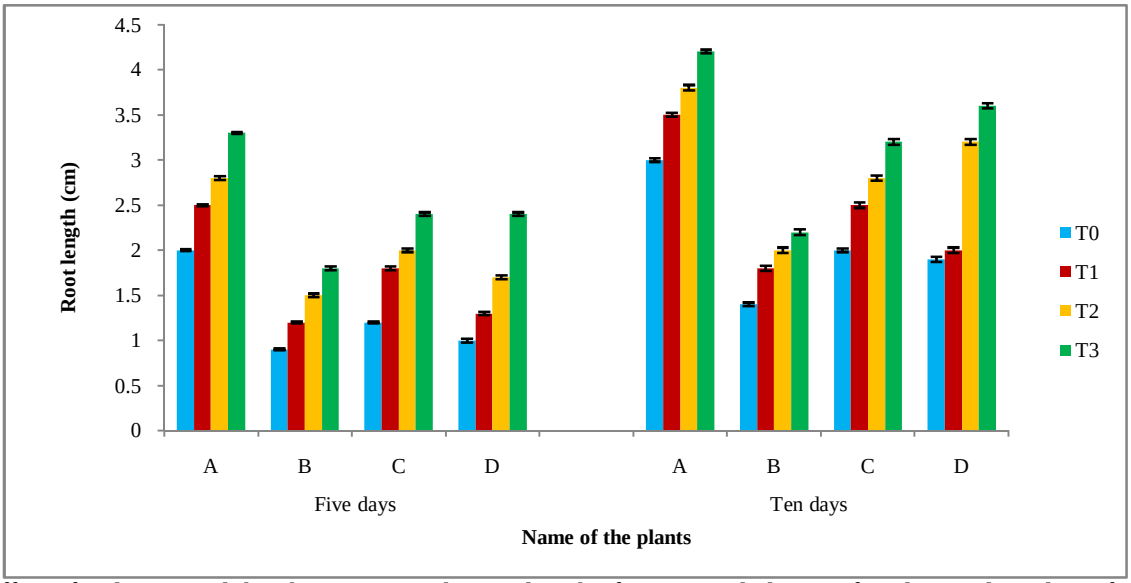


Fig 2: Effect of cadmium and rhizobacterium on the root length of ornamental plants at five days and ten days after germination

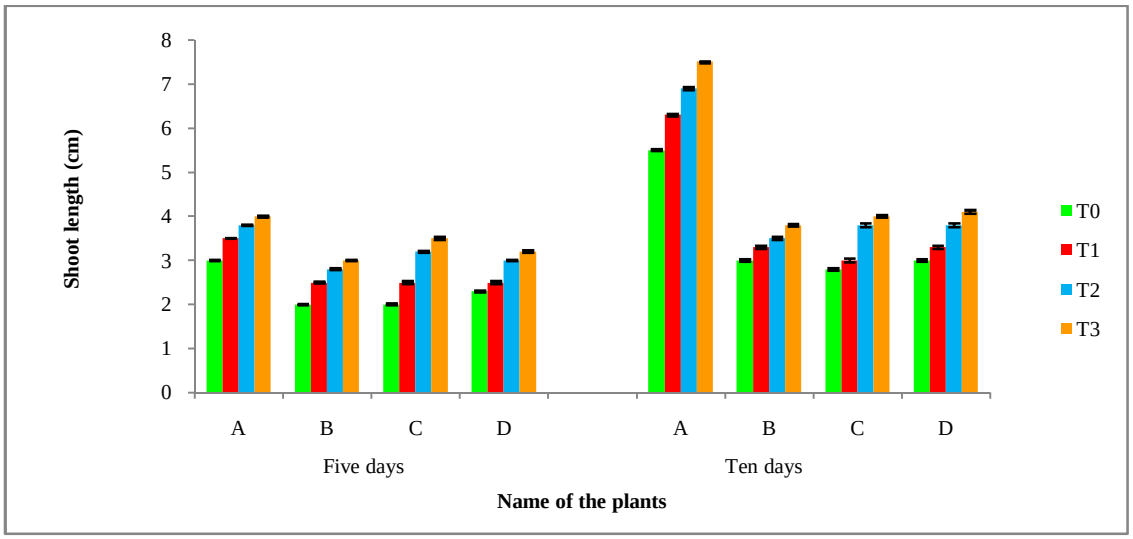


Fig 3: Effect of cadmium and rhizobacterium on the shoot length of ornamental plants at five days and ten days after germination

A- Helianthus sp.; B- Tagetes sp.; C- Tithonia sp.; D- Zinnia sp; T0- Control; T1- Treated with Bacillus sp.; T2- Treated with cadmium chloride solution and T3- Treated with Bacillus sp. and cadmium chloride solution.

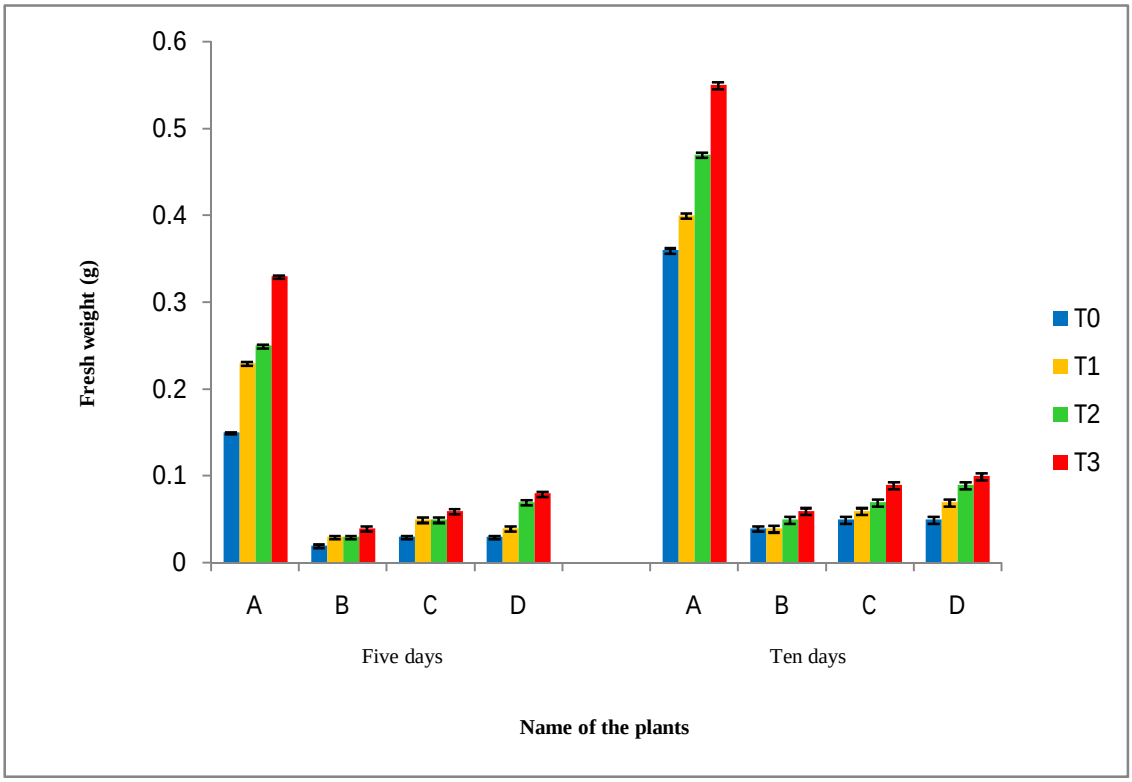


Fig 4: Effect of cadmium and rhizobacterium on the fresh weight of ornamental plants at five days and ten days after germination

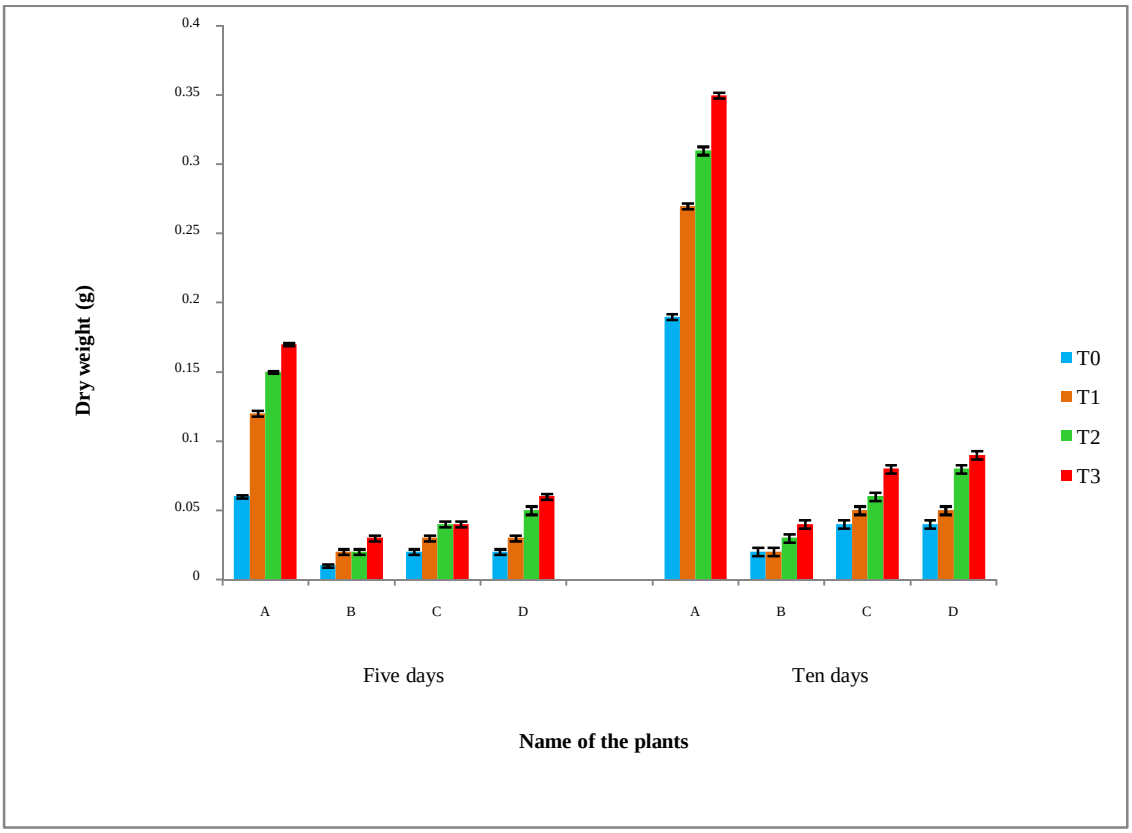


Fig 5: Effect of cadmium and rhizobacterium on the dry weight of ornamental plants at five days and ten days after germination

A- Helianthus sp.; B- Tagetes sp.; C- Tithonia sp.; D- Zinnia sp; T0- Control; T1- Treated with Bacillus sp.; T2- Treated with cadmium chloride solution and T3- Treated with Bacillus sp. and cadmium chloride solution.

The growth parameters measured was subjected to Least Significant Difference (LSD). There was a significant increase in the growth parameters (root length, shoot length, fresh weight and dry weight). Among the four plants chosen for the phytore mediation studies, Helianthus sp. was the only plant which thrived well in the cadmium contaminated soil. Hence the root and shoot tissues were analyzed separately for the presence of cadmium after ten days of growth using Inductively Coupled Plasma-Mass spectrometry (ICP-MS).The results showed that the cadmium level in the control soil and plant was below detectable limit. In the treated plants (T2 and T3) the cadmium accumulation was more in the root tissue than in the shoot tissue. From the above result Bioaccumulation Factor (BAF) and Translocation Factor (TF) in Helianthus sp. was calculated using the formula:

$$BAF = \frac{Metal\ concentration\ in\ plant}{Metal\ concentration\ in\ soil}; \qquad TF = \frac{Metal\ concentration\ in\ shoot}{Metal\ concentration\ in\ root}$$

The results showed that the translocation factor was less than one and the bioaccumulation factor was more than one for Helianthus sp. in both the treatments T2 and T3.

IV. DISCUSSION

Heavy metal contamination caused by anthropogenic and geological activities on the biosphere has become a global problem of great concern. Among the various heavy metals cadmium is highly toxic because it induces oxidative stress in plants and is easily translocated. Although, cadmium does not have a known essential role in the metabolism of higher plants, it gets accumulated into the plant tissues and thereby transferred to the other organisms in the food chain [26]. In recent years, less-invasive *in-situ* technologies are being adopted to remove or to minimize cadmium contamination from soil. Phytoremediation is considered to be one such appealing technology that uses vegetation and its associated microbiota, soil amendments and agronomic techniques [27]. Researches done involving edible plants for phytoremediation is considered to be a non-productive method as these plants cannot be used for human consumption. Hence, there is a need to identify potential plants that are not edible, for cadmium tolerance and phytoremediation. Therefore, in the present study an attempt was made to identify such economically important ornamental plants that can be used for both phytoremediation of cadmium as well as for their economic uses. A combined strategy involving both rhizobacteria with cadmium tolerance and Plant Growth Promoting (PGP) traits and ornamental plants for phytoremediation might enhance the uptake of cadmium. To evaluate this, five different ornamental plants were selected for the study namely Helianthus sp., Tagetes sp., Tithonia sp., Zinnia sp. and Cosmos sp. from the family Asteraceae. Rhizobacteria were isolated from rhizosphere of plants growing along the Cooum river bank. Fifteen bacterial strains were isolated of which five strains showed high resistance to cadmium between the range of 1300 mg/L to 4000 mg/L. One of the isolated rhizobacterial strainBacillus sp.(R_3) showed a MIC of 1300 mg/L which is in accordance with a study conducted by Ref 28, in which Bacillus cereus has showed a MIC of 1200mg/L. Plasmid-borne cadmium resistance and its uptake, transport has also been studied in Escherichia coli, Bacillus subtilis, Listeria monocytogenes, Bacillus cereus [29], [30] in recent years. Escherichia coli and Bacillus sp. were the most abundantly isolated bacteria and Bacillus cereus was reported as the most cadmium resistant bacteria with MIC of 6 mM[31]. Further, the selected strains were characterized for the presence of PGP traits. PGP traits help in stimulating plant growth by increasing cell division or cell elongation and this can be determined by assessing various growth parameters like root length, shoot length, fresh weight and dry weight. Bacillus sp. (R_3) strain was selected for further study as it possessed PGPR characteristics like production of IAA, Ammonia, HCN and Siderophore. It also showed positive result for phosphate solubilization. It exhibited multiple heavy metal resistance to other heavy metals like Zinc, Mercury, Lead and Chromium in addition to Cadmium. The ornamental plants were subjected to germination assay in the presence of Cadmium in different concentrations (0, 2, 5, 10, 15, 20, 25 and 30 ppm) to study their tolerance level. Helianthus sp., showed highest germination percentage of upto 80% in the presence of cadmium when compared to other ornamentals. As the concentration of Cadmium increased germination percentage decreased in the ornamental plants. The plants did not show any symptom of toxicity in the presence of cadmium and this indicated the tolerance ability of the Asteraceae members. The tolerance level of the Cosmos sp. was found to be poor and hence further experiments for phytoremediation studies were carried out with the other four ornamental plants. A pot culture experiment was conducted with the selected ornamental plants (Helianthus sp., Tagetes sp., Tithonia sp. and Zinnia sp.) to study the effect of Bacillus sp. (R3) on the growth parameters and cadmium accumulation by the plants. The various growth parameters studied were root length, shoot length, fresh weight and dry weight after five days and ten days of growth of plants under cadmium stress condition. Statistical analysis

showed that there was a significant increase in the growth parameters. The maximum increase in the growth parameters was observed in the Helianthus sp. Among the four treatments given, the maximum increase in the growth parameters was observed in T3 [cadmium and Bacillus sp. (R3)]. Various researchers have earlier reported that the metal resistant PGPRs are helpful in growth enhancement of both hyperaccumulator and non- hyperaccumulator plants in metal contaminated soil [32], [33]. Plant growth promoting bacteria (PGPB) possessing single or multiple traits such as alleviation of metal toxicity, alteration of metal availability (metal immobilizing or mobilizing bacteria), production of siderophores, IAA production and biochelator (organic acid- or biosurfactants-producing bacteria), fixation of nitrogen and solubilization of mineral nutrients (phosphate or potassium solubilizing bacteria) have been widely used as effective bioinoculants for microbe-assisted phytoremediation [34]- [39]. Siderophores of all the metabolites are extremely effective in solubilizing and increasing the mobility of a wide range of metals such as Cd, Cu, Ni, Pb, Zn and the actinides [40]. Due to their dual role of phytoremediating ability and growth promoting traits the microbial inoculants can lead to increased plant biomass and enhanced metal mobilization or immobilization in the soil.In the present study,Helianthus sp. thrived well in the artificially cadmium contaminated soil when compared to the other ornamentals. Hence, the cadmium content in the root and shoot tissues were analyzed separately after ten days of each treatment using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The cadmium content in T0 was found to be below detectable limit (BDL). The cadmium content was more in the root tissues compared to shoot tissues in the treatments T2 (57.45 ppm) and T3 (42.29 ppm). The concentration of cadmium in the shoot tissues was more in T3 than T2. This might be due to the presence of rhizobacterium and its ability to mobilize cadmium from root tissues to shoot tissues. The Plant Growth Promoting Rhizobacteria (PGPR) strain R3 (Bacillus sp.) performed significantly well in enhancing the growth of Helianthus sp. both in the presence and in the absence of cadmium. Similarly, in a study conducted by Ref 41 there was more accumulation of cadmium in the root tissues of two Vigna sp. namely Vignaunguiculata and Vignaradiata. Since roots are the first organs to receive the heavy metal ions from the soil via apoplastic transport, the concentration of metal in them is in an increased level [42], [43]. Metal concentrations in plant also varies with plant species and genotypes [44]. Bioaccumulation factor is an index of the ability of plant to accumulate a particular metal with respect to its concentration in the sediment [45]. The higher the BAF value the more suitable is the plant for phytoextraction[46]. Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1 and values S> 1 showing that the metals are stored in the stems and leaves. The bioaccumulation and the translocation factors wascalculated for the treatments T0, T2 and T3. In the present study, the Bioaccumulation Factor (BAF) value of Helianthus sp. in treatment T3 was more than T2 indicating that the uptake of cadmium is more in the presence of the rhizobacterium Bacillus sp (R3). Hence, microbe-assisted phytoremediation would prove to be an effective strategy in promoting cadmium accumulation when compared to other treatments. Of the five different ornamental plants chosen for the present study, Helianthus sp. was more cadmium tolerant. However, since the translocation factor was less than one in Helianthus sp. it is a non-hyperaccumulator of cadmium.

V. CONCLUSION

Helianthus sp. thrived well in the soil amended with cadmium. The combination of the PGPR strain R3 (Bacillus sp.) and the ornamental plant Helianthus sp. proved to be more efficient for phytoremediation of cadmium. Hence, Helianthus sp. can be recommended as a remediator for cadmium contaminated soil and is suitable for phytoextraction.

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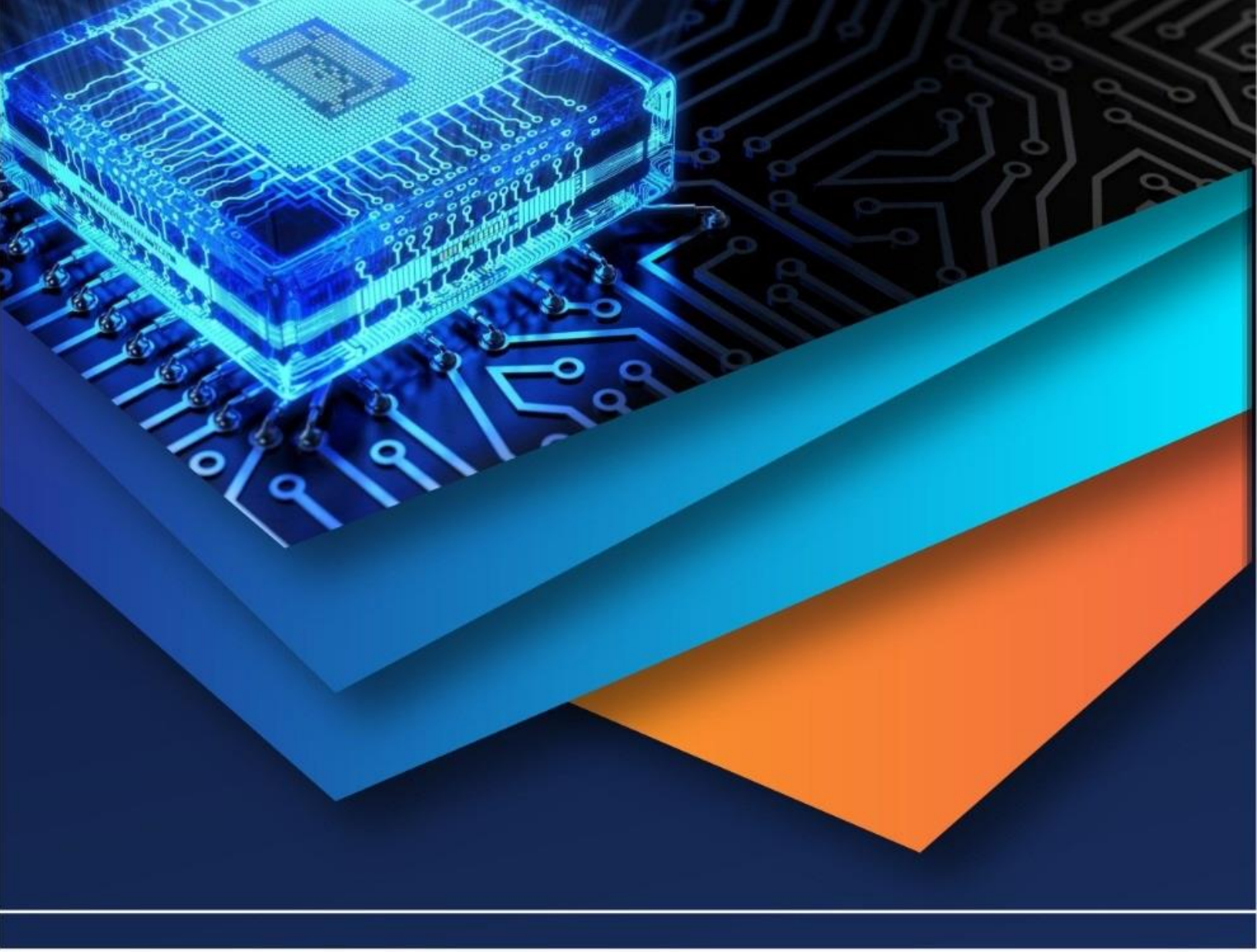
REFERENCES

[1] E.U. Ikhuoria and F.E. Okieimen, “Scavenging cadmium, copper, lead, nickel and zinc ions from aqueous solution by modified cellulosic sorbent,” Int. J. Environ. Stud., vol. 57, pp. 401-409, 2000
[2] B. Kavitha, P. Jothimani, S. Ponmani, and R. Sangeetha, “Phytoremediation of heavy metals- A review,” Int. J. Res. Stud. Biosci., vol. 1, pp. 17-23, 2013.
[3] United States Environmental Protection Agency (USEPA), Cleaning up the nation’s waste sites: Markets and technology trends, EPA/542/R-96/005, Office of solid waste and emergency response, Washington, DC., 1997
[4] R. Kathal, P. Malhotra, and V. Chaudhary, “Phytoremediation of cadmium from polluted soil,” J. Bioremediat. Biodegrad., vol. 7, pp. 376, 2016
[5] A. Baryla, P. Carrier, F. Franck, C. Coulomb, C. Sahut, and M. Havaux, “Leaf chlorosis in oilseed rape plants (Brassica napus) grown on cadmium- polluted soil: Causes and consequences for photosynthesis and growth,” J. stress physiol. Biochem., vol. 212, pp. 696-709, 2001

[6] M. Mejare and L. Bulow, "Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals," Trends Biotechnol., vol. 19, pp. 67-73, 2001
[7] M.P. Benavides, S.M. Gallego, and M.L. Tomaro, "Cadmium toxicity in plants," Braz. J. Plant Physiol., vol. 17, pp. 21-34, 2005.
[8] M.D. Groppa, M.P. Ianuzzo, E.P. Rosales, S.C. Vazquez, and M.P. Benavides, "Cadmium Modulates NADPH oxidase activity and expression in sunflower leaves," Biol. Plant., vol.56, pp. 167-171, 2012
[9] P.L. Gratao, C.C. Monterio, M.L. Rossi, A.P. Martinelli, L.E.P. Peres, and L.O. Medici, "Differential ultrastructural changes in tomato hormonal mutants exposed to cadmium," Environ. Exper. Bot., vol. 67, pp. 387-394, 2009
[10] S.M. Gallego, L.B. Pena, R.A. Barcia, C.E. Azpilicutea, M.F. Lannone, and F. Maria, "Unravelling Cadmium toxicity and tolerance in plants: insight into regulatory mechanisms," Environ. Exper. Bot., vol. 83, pp. 33-46, 2012
[11] U.J. Lopez- Chuken and S.D. Young, "Modellingsulphateenhanced Cadmium uptake by Zea mays from nutrient solution under conditions of constant free Cd2+ ion activity," J. Environ. Sci., vol. 22, pp. 1080 -1085, 2010.
[12] S.S. Gill, N.A. Khan, and N. Tuteja, "Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (LepidiumSativam L.)," Plant Sci., vol. 182, pp. 112-120, 2012
[13] S. Li, J. Yu, M. Zhu, F. Zhao, and S. Luan, "Cadmium impairs ions homeostasis by altering K^+ and Ca^{2+} Channel activities in rice root hair cells," Plant Cell Environ., vol. 35, pp. 1998-2013, 2012
[14] T. Lebeau, A. Braud, and K. Jezequel, "Performance of bioaugmentation- assisted phytoextraction applied to metal contaminated soils: a review," Environ. Pollut., vol. 153, pp. 497–522, 2008
[15] B.R. Glick, "Using soil bacteria to facilitate phytoremediation," Biotechnol. Adv., vol. 28, pp. 367–374, 2010
[16] C. Cervantes, J. Chavez, N.A. Cardova, P. de la Mora, and J.A. Velasco, "Resistance to metal by Pseudomonas aeruginosa clinical isolates," Microbios., vol. 48, pp. 159-163, 1986.
[17] Bergey's, Manual of Determinative Bacteriology 9th ed.,Williams and Wilkins Pub., MD: USA.
[18] J.M. Brick, R.M. Bostock, and S.E. Silverstone, "Rapid insitu assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane," Appl. Environ. Microbiol., vol. 57, pp. 535-538, 1991
[19] J.C. Cappuccino and N. Sherman, In: Microbiology, A Laboratory Manual, 3rd ed., Benjamin/Cummings Pub.Co., New York, 1992
[20] B. Schwyn and J.B. Neilands, "Universal chemical assay for the detection and determination of siderophores," Anal Biochem., vol. 160, pp. 47-56, 1987
[21] Gaur, A.C. 1990. Physiological functions of phosphate solubilizing micro-organisms. In: Gaur, A.C. (Ed.), Phosphate solubilizing micro-organisms as biofertilizers. Omega Scientific Publishers, New Delhi, 16-72
[22] H. Lorck, "Production of hydrocyanic acid by bacteria," Plant Physiol., vol. 1, pp. 142-146, 1948
[23] United States Environmental Protection Agency (USEPA), Method 200.8, Determination of Trace Elements in Water and Wastes by Inductively Coupled Plasma-Mass Spectrometry, Rev. 5.4, US Environmental Protection Agency, 1994
[24] H.W. Shin, M. Sidharthan, and K. Shin young, "Forest fire ash impact on micro-macro algae in the receiving waters of the east coast of South Korea," Ma. Pollut. Bull., vol. 45, pp. 203- 209, 2002
[25] S. Cui, Q. Zhou, and L. Chao, "Potential hyperaccumulation of Pb, Zn, Cu and Cd in endurant plants distributing in an old smeltery, northeast China," Environmental research, vol. 3, pp. 1-18, 2007
[26] T.W. Lane and F.M. Morel, "A biological function for cadmium in marine diatoms," Proc. Natl. Acad. Sci. U. S. A., vol. 97, pp. 4627-4631, 2000
[27] R.A. Wuana, P.A. Adie, J. Abah, and M.A. Ejeh, " Screening of Pearl Millet for phytoextraction potential in soil contaminated with cadmium and lead," Int. J. Sci. Technol., vol. 2, pp. 310-319, 2013
[28] H.U. Qing, D.O.U. Min-na, Q.I. Hong-yan, X.I.E. Xiang-ming, ZHUANG Guo-qiang, and YANG Min, "Detection, isolation and identification of cadmium-resistant bacteria based on PCR-DGGE," J Environ Sci., vol. 19, pp. 1114-1119, 2007
[29] S. Kamalakannan, S.Mahadevan, and R. Krishnamoorthy, "Characterization of a mercury-reducing Bacillus cereus strain isolated from the Pulicat Lake sediments, south east coast of India," Arch. Microbiology., vol. 185, pp. 202-211, 2006.
[30] M. Lebrun, A. Audurier, and P. Cossart, "Plasmid-borne cadmium resistance genes in Listeria monocytogenes are similar to cadA and cadC of Staphylococcus aureus and are induced by cadmium," J.Bacteriol., vol. 176, pp. 3040-3048, 1994
[31] F. Kafilzadeh, Y. Moghtaderi, and A.R. Jahromi, "Isolation and identification of cadmium-resistant bacteria in Soltan Abad river sediments and determination of tolerance of bacteria through MIC and MBC," Eur. J. Exp. Biol., vol. 3, pp. 268-273, 2013
[32] Y. Ma, M. Rajkumar, and H. Freitas, "Improvement of plant growth and nickel uptake by nickel resistant- plant growth promoting bacteria," J Hazard. Mater., vol. 166, pp. 1154-1161, 2009
[33] Q. Yang, S. Tu, G. Wang, X. Liao, and X. Yan, "Effectiveness of applying arsenate reducing bacteria to enhance arsenic removal from polluted soils by Pterisvittata L.,"Int. J. Phytoremediat., vol. 14, pp. 89-98, 2012
[34] Y. Ma, M.N.V. Prasad, M. Rajkumar, and H. Freitas, "Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils," Biotechnol. Adv., vol. 29, pp. 248-258, 2011a
[35] Y. Ma, R.S. Oliveira, F. Nai, M. Rajkumar, Y. Luo, I. Rocha, and H. Freitas, "The hyperaccumulator Sedum plumbizincicola harbors metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal contaminated soil, J. Environ. Manag., vol. 156, pp. 62–69, 2015 a
[36] Y. Ma, R.S. Oliveira, L. Wu, Y. Luo, M. Rajkumar, I. Rocha, and H. Freitas, "Inoculation with metal-mobilizing plant-growth-promoting rhizobacterium Bacillus sp. SC2b and its role in rhizoremediation," J. Toxicol. Environ., vol. 78, pp. 931–944, 2015 b
[37] Y. Ma, M. Rajkumar, I. Rocha, R.S. Oliveira, and H. Freitas, "Serpentine bacteria influence metal translocation and bioconcentration of Brassica juncea and Ricinuscommunis grown in multi-metal polluted soils," Front. Plant Sci., vol. 5, pp. 757, 2015 c
[38] M. Rajkumar, S. Sandhya, M.N.V. Prasad, and H. Freitas, H, "Perspectives of plant-associated microbes in heavy metal phytoremediation," Biotechnol. Adv., vol. 30, pp. 1562–1574
[39] M. Ahemadand M. Kibret, "Mechanisms and applications of plant growth promoting rhizobacteria: current perspective," J. King Saud Univ. Sci., vol. 26, pp. 1–20, 2014

[40] I.J. Schalk, M. Hannauer, and A. Braud, "Minireview new roles for bacterial siderophores in metal transport and tolerance, Environ Microbiol., vol. 13, pp. 2844-2854, 2011
[41] P.R. Chandra, A.K. Abdussalam, N. Salim, and J.T. Puthur, "Distribution of bio-accumulated Cd and Cr in two Vigna species and the associated histological variations," J Stress PhysiolBiochem., vol. 6, pp. 4-12, 2010
[42] B.B.M. Sridhar, S.V. Diehl, F.X. Han, D.L. Monts, and Y. Su, "Anatomical changes due to uptake and accumulation of Zn and Cd in Indian Mustard (Brassica juncea)," Environ Exper Bot., vol. 54, pp. 131-141, 2005
[43] M. Drazkeiwicz, A. Tukendorf, and T. Baszynski, "Age dependent response of maize leaf segments to cadmium treatment: effect on chlorophyll fluorescence and phytochelation accumulation," J. Plant physiol., vol. 160, pp. 247- 254, 2003.
[44] C.A. Grant, J.M. Clarke, S. Duguid, and R.L. Chaney, "Selection and breeding of plant cultivars to minimize cadmium accumulation," Sci. Total Environ., vol. 390, pp. 301-310, 200
[45] M. Ghosh and S.P. Singh, "A review on phytoremediation of heavy metals and utilization of it's by products," Appl. Ecol. Environ. Res., vol. 3, pp. 1-18, 2005
[46] M.J. Blaylock, D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, Y., B.D. Ensley, and I. Raskin, "Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents," Environ. Sci. Tech., vol. 31, pp. 860- 865, 1997.



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