

## Enhancing Heavy Metal Cleanup: Microbial-Assisted Phytoremediation by *Alternanthera ficoidea* (L.) P. Beauv

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In the era of sustainable development, phytoremediation has emerged as a promising, eco-friendly strategy for reclaiming heavy metal-contaminated environments. This study explores the phytoremediation potential of *Alternanthera ficoidea* for cadmium (Cd) and lead (Pb) and investigates the role of beneficial microbes—*Azotobacter* sp., *Pseudomonas fluorescens*, and *Trichoderma viride*—in enhancing metal uptake. Remarkably, the roots of plants treated with Cd + *Azotobacter* sp. exhibited a 173% increase in Cd accumulation compared to Cd-only treatments. Even more striking, Cd accumulation in the shoots surged by 1025% in plants treated with Cd + *T. viride*. Conversely, microbial inoculation significantly reduced Pb accumulation in both roots and shoots of *A. ficoidea*. Bioaccumulation (BCF) and translocation factor (TF) analyses revealed that microbial augmentation, particularly with *Azotobacter* sp., enhanced the phytostabilization capacity of *A. ficoidea* for Cd. Additionally, the species demonstrated inherent potential for Pb phytostabilization. These findings underscore the synergistic benefits of combining phytoremediation with microbial assistance for the sustainable detoxification of heavy metal-laden soils.

**Key words:** Heavy metals, Cadmium, Lead, *Azotobacter* sp., *P. fluorescens*, *T. viride*

Soil is an abiotic factor which is rudimentary for most of the plants as it acts as a substrate for anchorage and absorption of nutrients and minerals (Mishra *et al.*, 2016). Among the various pollutants that are released into the environment, heavy metals exert severe toxic effects. A metal species might be regarded a "contaminant" if it is present in an undesired location or in a concentration or form that has a negative impact on people or the environment (McIntyre, 2003). Normally heavy metal phytotoxicity in living organisms result from changes in numerous physiological processes caused at cellular/molecular level by inactivating enzymes, blocking functional groups of metabolically important molecules, displacing or substituting for essential elements and disrupting membrane integrity. The effect of heavy metal poisoning in plants is their interference with electron transport activities, especially that of chloroplast membranes resulting in the production of reactive oxygen species (ROS) (Vecchia *et al.*, 2005; Pagliano *et al.*, 2006).

The most common heavy metals that contaminate the environment are Cd, Cr, Hg, Pb, Cu, Zn, and As. Cadmium and lead, are the heavy metals that do not have any physiological function in living organisms and are considered as toxicants (Sinicropi *et al.*, 2010). Anthropogenic activities, such as the burning of fossil fuels, effluents generated from landfill sites, agricultural land, and mining waste, especially from zinc and lead mines, leads to increased cadmium contamination in the environment (Thompson & Bannigan, 2008). Cadmium is also used in the manufacturing of Ni-Cd batteries (Genchi, 2020) in cadmium plating baths, electrodes for storage batteries, catalysts, and ceramic glazes (Lokhande *et al.*, 2004).

In plants, cadmium can cause changes in the uptake of minerals through its effects on the availability of minerals from the soil (Moreno *et al.*, 1999). It has been shown to interfere with the uptake, transport, and use of several elements (Ca, Mg, P, and K) and water by plants (Das *et al.*, 1997). After prolonged exposure to Cd, the root becomes necrotic, decayed, and mucilaginous, which reduces the elongation of plant roots and shoots and results in leaf rolling and chlorosis (Abbas *et al.*,

2018). Chlorosis and stunted growth are the readily identifiable symptoms caused by toxicity of Cd in plants (Jali *et al.*, 2016), and it indirectly contributes to the production of Reactive Oxygen Species (ROS) in the chloroplasts of leaves (Gallego *et al.*, 2012).

Sources of Pb pollution, apart from natural weathering, are exhaust fumes of automobiles, chimneys of factories using Pb, effluents from the storage battery industry, mining and refining of Pb ores, metal plating and finishing operations, fertilizers, pesticides, and additives in pigments and gasoline (Eick *et al.*, 1999). In plants, Pb toxicity can result in stunted growth, chlorosis, and blackening of the root system. It inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status, and affects membrane structure and permeability (Sharma & Dubey, 2005). Many technologies such as the removal of contaminated material and chemical or physical treatment, are expensive and do not produce an attractive or long-lasting solution to clean up heavy metal-contaminated soil (Mulligan *et al.*, 2001; Cao *et al.*, 2002). However, phytoremediation can offer an affordable, durable, and aesthetically pleasing solution for the remediation of contaminated sites (Ma *et al.*, 2001).

Phytoremediation has the capability to provide a green, cost-effective, eco-friendly, and feasible application to address some of the world's environmental challenges (Newman *et al.*, 2023). 'Phytoremediation' consists of the Greek prefix *phyto* (plant), attached to the Latin root *remedium* (to correct or remove an evil) (Cunningham *et al.*, 1997). Phytoremediation utilizes many mechanisms including degradation (rhizo-degradation, phytodegradation), accumulation (phytoextraction, rhizofiltration), dissipation (phytovolatilization), and immobilization (hydraulic control and phytostabilization) to degrade, remove, or immobilize the pollutants (Pivetz, 2001).

Phytoextraction involves the uptake and accumulation of metals in plant shoots, which can subsequently be harvested and removed from the area (Anton & Mathe-Gasper, 2005; Nissim *et al.*, 2018). Phytostabilization is another application of phytoremediation in which plants are used to reduce

metal mobility in contaminated soils (Jung & Thornton, 1996; Rosselli et al., 2003). Phytovolatilization uses plants to absorb toxic elements from the soil, change them into less toxic volatile forms, and then release the altered forms into the atmosphere by transpiration through the leaves or foliage system of the plant (Mahar et al., 2016). Phytofiltration is the process of removing pollutants from contaminated surface waters or wastewaters by using plant roots (rhizofiltration), shoots (caulofiltration), or seedlings (blastofiltration) (Mesjasz-Przybyłowicz et al., 2004).

In the phytoremediation techniques adopted, the extent of heavy metal accumulation is measured based on two parameters: bioconcentration factor (BCF) and translocation factor (TF). BCF is calculated by dividing the quantity of heavy metal in root by that in soil (Yoon et al., 2006). Similarly, TF is calculated by dividing the quantity of heavy metal in shoot by that in root (Kumar & Maiti, 2014). If both BCF and TF values are greater than one, the plant is suitable for phytoextraction. If the BCF value is greater than one and the TF value is less than one, the plant is suitable for phytostabilization. If both these values are lower than one, the plant is not appropriate for phytoremediation.

Even though phytoremediation is an efficient method for environmental cleanup, the process typically takes a long time, and the application of it is restricted by the quality of the soil, the weather, and the phytotoxicity of the pollutants. Therefore, to ensure maximum metal harvest from contaminated sites in limited time, microbial-assisted phytoremediation can be applied, which helps in enhancing the pace of environmental cleanup. Microbes can reduce the metal phytotoxicity and stimulate plant growth indirectly by inducing the defense mechanisms, or directly by the solubilization of different mineral nutrients, the production of phytohormones, and the secretion of enzymes (Ma et al., 2016).

*Pseudomonas fluorescens* belongs to Plant Growth Promoting Rhizobacteria (PGPR), an important group of bacteria that play a major role in plant growth promotion, induced systemic resistance, biological control of pathogens, etc. Enhancement of plant growth promotion and reduction of various diseases have been exhibited

by various strains of *P. fluorescens* (Ganeshan & Kumar, 2005). *Trichoderma* spp. application to the plant rhizosphere promotes the growth of plant morphological traits such as root-shoot length, biomass, height, number of leaves, tillers, branches, fruits, etc. (Halifu et al., 2019; Sajeesh et al., 2015). Diazotrophs help in the solubilization of phosphates, which is necessary for ATP formation, signal transduction, membrane biosynthesis, and nodule formation. This indirectly mitigates or improves the process of heavy metal uptake in plants (Graham & Vance, 2000).

*Alternanthera ficoidea* (L.) P. Beauv., commonly called as 'Fig Joyweed,' which belongs to the family Amaranthaceae, is a common and widespread plant throughout the tropics and is being increasingly regarded as an invasive weed (Abbasi & Tauseef, 2018). Even though a number of studies have been conducted to identify the heavy metal phytoremediation potential of this plant, to the best of our knowledge, no studies have been conducted with the aim of enhancing the heavy metal phytoremediation potential of *A. ficoidea* using microbes like *Pseudomonas fluorescens*, *Trichoderma viride*, and *Azotobacter* sp. Taking all these factors into account, the present study was conducted to analyse the effect of the microbes *Pseudomonas fluorescens*, *Trichoderma viride*, and *Azotobacter* sp. on Cd and Pb accumulation in *A. ficoidea*. Additionally, the effect of these heavy metals and nanoparticles on growth parameters, moisture content, and tolerance index were also evaluated to identify the stress response of *A. ficoidea*.

## MATERIALS AND METHODS

### Plant Material

The plant material used for the present study was *Alternanthera ficoidea* (L.) P. Beauv., of the family Amaranthaceae.

### Experimental Design

Plant materials were collected by field exploration from Devagiri, Kozhikode, and they were acclimatized in the Botanical Garden of St. Joseph's College (Autonomous), Devagiri, Kozhikode, Kerala, India, for a period of one month. The stem cuttings from these plants were transferred into grow bags containing one

kilogram of garden soil. The plants were left to root and grow in this mixture for a period of 2 weeks. After rooting, the plants were subjected to heavy metal and microbial biofertilizers (Table 1).

### Experimental Procedure

The treatments and treatment combinations included control plants (to which no heavy metals or microbial fertilizers were provided), Cd-treated plants (to which 100 mg/kg CdCl<sub>2</sub> was provided), Pb-treated plants (to which 100 mg/kg PbCl<sub>2</sub> was provided), *Azotobacter* sp.-treated plants (to which 800 mg/kg *Azotobacter* sp. was provided), *P. fluorescens*-treated plants (to which 800 mg/kg *P. fluorescens* was provided), *T. viride*-treated plants (to which 800 mg/kg *T. viride* was provided), autoclaved *Azotobacter* sp.-treated plants (to which 800 mg/kg autoclaved *Azotobacter* sp. was provided), autoclaved *P. fluorescens*-treated plants (to which 800 mg/kg autoclaved *P. fluorescens* was provided), autoclaved *T. viride*-treated plants (to which 800 mg/kg autoclaved *T. viride* was provided), Cd + *Azotobacter* sp.-treated plants (to which 100 mg/kg CdCl<sub>2</sub> and 800 mg/kg *Azotobacter* sp. were provided), Cd + *P. fluorescens*-treated plants (to which 100 mg/kg CdCl<sub>2</sub> and 800 mg/kg *P. fluorescens* were provided), Cd + *T. viride*-treated plants (to which 100 mg/kg CdCl<sub>2</sub> and 800 mg/kg *T. viride* were provided), Pb + *Azotobacter* sp.-treated plants (to which 100 mg/kg PbCl<sub>2</sub> and 800 mg/kg *Azotobacter* sp. were provided), Pb + *P. fluorescens*-treated plants (to which 100 mg/kg PbCl<sub>2</sub> and 800 mg/kg *P. fluorescens* were provided), Pb + *T. viride*-treated plants (to which 100 mg/kg PbCl<sub>2</sub> and 800 mg/kg *T. viride* were provided), and the duration of treatment was one month. All the treatments and treatment combinations are provided in Table 1.

### Growth Parameters

After a month of treatment, plant samples were carefully harvested and washed with distilled water. The root and shoot lengths were measured with a graduated scale. The fresh and dry weights of roots and shoots were recorded using an electronic weighing balance. For dry weight measurements, the weighed samples were dried in a hot air oven at 100°C for 1 h, and later the temperature was set at 60°C, in a hot air oven, until the

weight attained a constant value.

### Moisture Content

The moisture content was determined by measuring the fresh and dry weights of the plants (Lokhande *et al.*, 2011). Tissue moisture content percentage was calculated using the following equation:

$$\text{Moisture content (MC) \%} = \frac{[(\text{Fresh Weight} - \text{Dry Weight}) / \text{Fresh Weight}] \times 100}{1}$$

### Tolerance Index (TI)

The tolerance index (TI) of *Alternanthera ficoidea* under heavy metal and microbial biofertilizers was estimated using the given formula (Rabie, 2005):

$$\text{TI} = (\text{Dry weight of treated plants} / \text{Dry weight of control plants}) \times 100$$

### Accumulation of Cd and Pb

The dried plant samples were ground into a powder. The preparation of the samples for the heavy metal analysis was carried out following Allan's (1969) method. Accurately weighed 0.5 g of powdered roots and shoots from each treatment were digested with 40 ml of nitric acid (HNO<sub>3</sub>). After the mixtures were dried out by evaporation, they were extracted using distilled water. The solutions were then boiled, filtered, and made up to 50 ml. The metal ion concentrations were analysed by atomic absorption spectrophotometer at KFRI (Kerala Forest Research Institute), Peechi, Thrissur, Kerala.

### Evaluation of Phytoremediation Parameters

The parameters determining the phytoremediation efficiency of the plants, BCF, TF, and BAF, were calculated by the following equations:

- $\text{BCF} = (\text{Heavy metal in root}) / (\text{Heavy metal in soil})$  (Yoon *et al.*, 2006)
- $\text{TF} = (\text{Heavy metal in shoot}) / (\text{Heavy metal in root})$  (Kumar & Maiti, 2014)

$$\text{BAF} = (\text{Heavy metal in whole plant}) / (\text{Heavy metal in soil}) \text{ (Aladesanmi et al., 2019)}$$

## RESULTS

### Effect of *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on Cd, and Pb accumulation in *Alternanthera ficoidea*

**Effect on Cd Accumulation:** Cadmium (Cd) accumulation in the control and microbe-only treated *A. ficoidea* plants was below detectable levels, indicating an uncontaminated soil. Cd accumulation was highest in the roots of Cd + *Azotobacter* sp. treated plants and in the shoots of Cd + *T. viride* treated plants. Relative to Cd-only treated roots, Cd + *Azotobacter* sp. increased Cd accumulation by 173%, *T. viride* by 78%, and *P. fluorescens* by 52%. For shoots, Cd + *T. viride* increased Cd accumulation by 1025%, *Azotobacter* sp. by 170%, whereas *P. fluorescens* reduced it by 82% (Figure 1).

Bioconcentration factor (BCF) values were below one, except for Cd + *Azotobacter* sp. (1.325), indicating limited accumulation. The Bioaccumulation factor values (BAF) exceeded one in both Cd + *Azotobacter* sp. (2.41) and Cd + *T. viride* (5.72) treated plants, while TF value exceeded one only in Cd + *T. viride* treated plants (5.6). The BCF order was: Cd + *Azotobacter* sp. > Cd + *T. viride* > Cd + *P. fluorescens* > Cd. TF values followed: Cd + *T. viride* > Cd > Cd + *Azotobacter* sp. > Cd + *P. fluorescens*. Bioaccumulation factor (BAF) was highest in Cd + *T. viride* (Figure 2).

**Effect on Pb Accumulation:** Lead (Pb) accumulation was also below detection in control and microbe-only treatments. The highest root Pb accumulation occurred in Pb-only treated plants (865 mg/kg). In microbial co-treatments, root Pb levels decreased by 87% (*Azotobacter* sp.), 57% (*P. fluorescens*), and 51% (*T. viride*). Shoot Pb was undetectable in Pb + *Azotobacter* sp. and Pb + *P. fluorescens*, and reduced by 70% in Pb + *T. viride* (Figure 3).

All Pb-treated plants had BCF >1, with highest in Pb-only treatment. BCF ranking: Pb > Pb + *T. viride* > Pb + *P. fluorescens* > Pb + *Azotobacter* sp. All treatments had TF <1, highest in Pb-only (0.089) (Figure 4).

### Effect on Growth Parameters

**Cd and Microbial Influence:** Root length increased by 3% only in *Azotobacter* sp. treated plants. Cd treatment reduced root length by 69%. Cd + *Azotobacter* sp., Cd + *P. fluorescens*, and Cd + *T. viride* improved root length over Cd alone by 40%, 58%, and 56%, respectively.

Shoot length slightly increased by 1% in *P. fluorescens* treatment. Cd reduced shoot length by 37%. Cd + *Azotobacter* sp., Cd + *P. fluorescens*, and Cd + *T. viride* improved shoot length by 20%, 22%, and 24% over Cd.

Fresh root weight decreased across treatments. Compared to Cd, Cd + *Azotobacter* sp., Cd + *P. fluorescens*, and Cd + *T. viride* increased root fresh weight by 18%, 22%, and 46%, respectively. Dry root weight increased by 40%, 30%, and 50% in the same order.

Shoot fresh weight increased by 29%, 27%, and 19%, while shoot dry weight increased by 40%, 30%, and 50% for Cd + *Azotobacter* sp., Cd + *P. fluorescens*, and Cd + *T. viride*, respectively, over Cd (Table 2).

**Pb and Microbial Influence:** Only *Azotobacter* sp. increased root length by 3% over control. Pb reduced root length by 59%. Pb + *Azotobacter* sp. and Pb + *T. viride* improved root length by 15% and 24% over Pb. No change in Pb + *P. fluorescens*.

Shoot length increased only in *P. fluorescens* treatment (1%). Pb reduced it by 33%. Pb + *Azotobacter* sp., Pb + *T. viride*, and Pb + *P. fluorescens* improved it by 24%, 24%, and 18% over Pb.

Fresh root weight decreased across treatments. Compared to Pb, Pb + *Azotobacter* sp., Pb + *P. fluorescens*, and Pb + *T. viride* reduced root fresh weight by 28%, 9%, and 6%, respectively. However, dry weights increased by 90%, 81%, and 54%, respectively.

Shoot fresh weight decreased in all treatments. Compared to Pb, Pb + *Azotobacter* sp., Pb + *P. fluorescens*, and Pb + *T. viride* reduced shoot fresh weight by 37%, 17%, and 16%. Shoot dry weight increased by 70%, 62%, and 46%, respectively (Table 3).

### Effect of heavy metals (Cd, & Pb) and *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the moisture content of *Alternanthera ficoidea*

Moisture content of roots of control and treated plants in their decreasing order are: Pb treated > Cd treated > Cd + *T. viride* treated > Cd + *P. fluorescens* treated > Cd + *Azotobacter* sp. treated > *T. viride* treated > autoclaved *P. fluorescens* treated > autoclaved *T. viride* treated > autoclaved *Azotobacter* sp. treated > *P. fluorescens* treated > Pb + *T. viride* treated > Pb + *Azotobacter* sp. treated > *Azotobacter* sp. treated > Pb + *P. fluorescens* treated > Control *A. ficoidea* plants.

The moisture content of shoots of control and treated plants in their decreasing order is as follows: Autoclaved *P. fluorescens* treated > Autoclaved *Azotobacter* sp. treated > Pb treated > *T. viride* treated > Autoclaved *T. viride* treated > *Azotobacter* sp. treated > *P. fluorescens* treated > Cd treated > Cd + *P. fluorescens* treated > Cd + *T. viride* treated > Pb + *T. viride* treated > Pb + *Azotobacter* sp. treated > Cd + *Azotobacter* sp. treated > Pb + *P. fluorescens* treated > Control *A. ficoidea* plants (Figure 5).

### Effect of heavy metals (Cd, & Pb) and *Azotobacter*

### sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the tolerance index of *Alternanthera ficoidea*

Tolerance Index (TI) is a measure of relative tolerance of plants to metal toxicity (Ismail *et al.*, 2013). The plants having TI > 60% or TI > 1 show increased metal tolerance potential (Lux *et al.*, 2004). In the present study, tolerance index of plants decreased in the order: Control > *Azotobacter* sp. treated > *P. fluorescens* treated > *T. viride* treated > Autoclaved *Azotobacter* sp. treated > Autoclaved *P. fluorescens* treated > Autoclaved *T. viride* treated > Pb + *Azotobacter* sp. treated > Pb + *P. fluorescens* treated > Pb + *T. viride* treated > Cd + *Azotobacter* sp. treated > Cd + *P. fluorescens* treated > Pb treated > Cd + *T. viride* treated > Cd treated *A. ficoidea* plants (Figure 6).

### Visual toxic effects of heavy metals (Cd, & Pb) and *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* in *Alternanthera ficoidea*

The plants did not exhibit any visual toxic symptoms either in the leaves or in the stem. All plants grew well in the contaminated environment without wilting. Moreover, chlorosis was not observed.

**Table 1.-** Various Treatment Combinations of Heavy Metals and Microbes

Plant	Treatment Combinations	Cd (mg/kg)	Pb (mg/kg)	<i>Azotobacter</i> sp. (mg/kg)	<i>P. fluorescens</i> (mg/kg)	<i>T. viride</i> (mg/kg)
<i>A. ficoidea</i>	Control	-	-	-	-	-
	<i>Azotobacter</i> sp.	-	-	800	-	-
	<i>P. fluorescens</i>	-	-	-	800	-
	<i>T. viride</i>	-	-	-	-	800
	Autoclaved <i>Azotobacter</i> sp.	-	-	800	-	-
	Autoclaved <i>P. fluorescens</i>	-	-	-	800	-
	Autoclaved <i>T. viride</i>	-	-	-	-	800
	Cd	100	-	-	-	-
	Cd+ <i>Azotobacter</i> sp.	100	-	800	-	-
	Cd+ <i>P. fluorescens</i>	100	-	-	800	-
	Cd+ <i>T. viride</i>	100	-	-	-	800
	Pb	-	100	-	-	-
	Pb+ <i>Azotobacter</i> sp.	-	100	800	-	-
	Pb+ <i>P. fluorescens</i>	-	100	-	800	-
	Pb+ <i>T. viride</i>	-	100	-	-	800

**Table 2.** Effect of Cd, *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the growth parameters of *Alternanthera ficoidea*

SI No	Treatments	Root length (cm)	Shoot length (cm)	Fresh weight (g)		Dry weight (g)	
				Root	Shoot	Root	Shoot
1	Control	20.33±6.80	54.33±2.08	1.43±0.18	11.02±4.81	0.84±0.15	6.66±3.03
2	Cd	6.16±2.02	34.00±8.71	0.54	3.60±1.78	0.10±0.05	0.43±0.22
3	<i>Azotobacter</i> sp.	21.00±5.00	53.33±11.54	1.24±0.36	10.3±8.96	0.32±0.09	1.21±0.94
4	<i>P. fluorescens</i>	11.33±3.21	55.00±5.00	1.2±0.12	8.92±1.76	0.29±0.05	1.05±0.11
5	<i>T. viride</i>	17.00±15.62	51.66±7.63	1.14±0.49	8.89±2.64	0.25±0.13	1.02±0.13
6	Autoclaved <i>Azotobacter</i> sp.	11.16±6.04	48.00±6.00	1.10±0.09	6.93±1.87	0.26±0.13	0.71±0.12
7	Autoclaved <i>P. fluorescens</i>	11.00±2.64	49.00±5.56	1.04±0.52	6.75±3.40	0.24±0.02	0.66±0.24
8	Autoclaved <i>T. viride</i>	9.33±4.16	48.5±23.33	1.02±0.13	6.41±1.80	0.24±0.03	0.75±0.34
9	Cd + <i>Azotobacter</i> sp.	8.66±2.51	41.00±11.00	0.64±0.39	4.65±2.38	0.14±0.02	0.67±0.21
10	Cd + <i>P. fluorescens</i>	9.75±1.76	41.66±12.74	0.66±0.11	4.58±1.99	0.13±0.03	0.62±0.39
11	Cd + <i>T. viride</i>	9.66±2.08	42.33±19.13	0.79±0.34	4.29±1.52	0.15±0.07	0.59±0.33

**Table 3.** Effect of Pb, *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the growth parameters of *Alternanthera ficoidea*

SI No	Treatments	Root length (cm)	Shoot length (cm)	Fresh weight (g)		Dry weight (g)	
				Root	Shoot	Root	Shoot
1	Control	20.33±6.80	54.33±2.08	1.43±0.18	11.02±4.81	0.84±0.15	6.66±3.03
2	Pb	8.33±2.08	36.00±5.19	0.66±0.12	4.52±2.23	0.11±0.05	0.50±0.17
3	<i>Azotobacter</i> sp.	21.00±5.00	53.33±11.54	1.24±0.36	10.3±8.96	0.32±0.09	1.21±0.94
4	<i>P. fluorescens</i>	11.33±3.21	55.00±5.00	1.2±0.12	8.92±1.76	0.29±0.05	1.05±0.11
5	<i>T. viride</i>	17.00±15.62	51.66±7.63	1.14±0.49	8.89±2.64	0.25±0.13	1.02±0.13
6	Autoclaved <i>Azotobacter</i> sp.	11.16±6.04	48.00±6.00	1.10±0.09	6.93±1.87	0.26±0.13	0.71±0.12
7	Autoclaved <i>P. fluorescens</i>	11.00±2.64	49.00±5.56	1.04±0.52	6.75±3.40	0.24±0.02	0.66±0.24
8	Autoclaved <i>T. viride</i>	9.33±4.16	48.50±23.33	1.02±0.13	6.41±1.80	0.24±0.03	0.75±0.34
9	Pb + <i>Azotobacter</i> sp.	9.66±3.51	44.66±11.01	0.85±0.37	6.11±1.80	0.21±0.11	0.85±0.28
10	Pb + <i>P. fluorescens</i>	8.33±2.08	42.66±6.42	0.72±0.65	5.32±2.29	0.2±0.11	0.81±0.48
11	Pb + <i>T. viride</i>	10.33±3.51	44.66±5.50	0.70±0.30	5.27±1.61	0.17±0.16	0.73±0.13

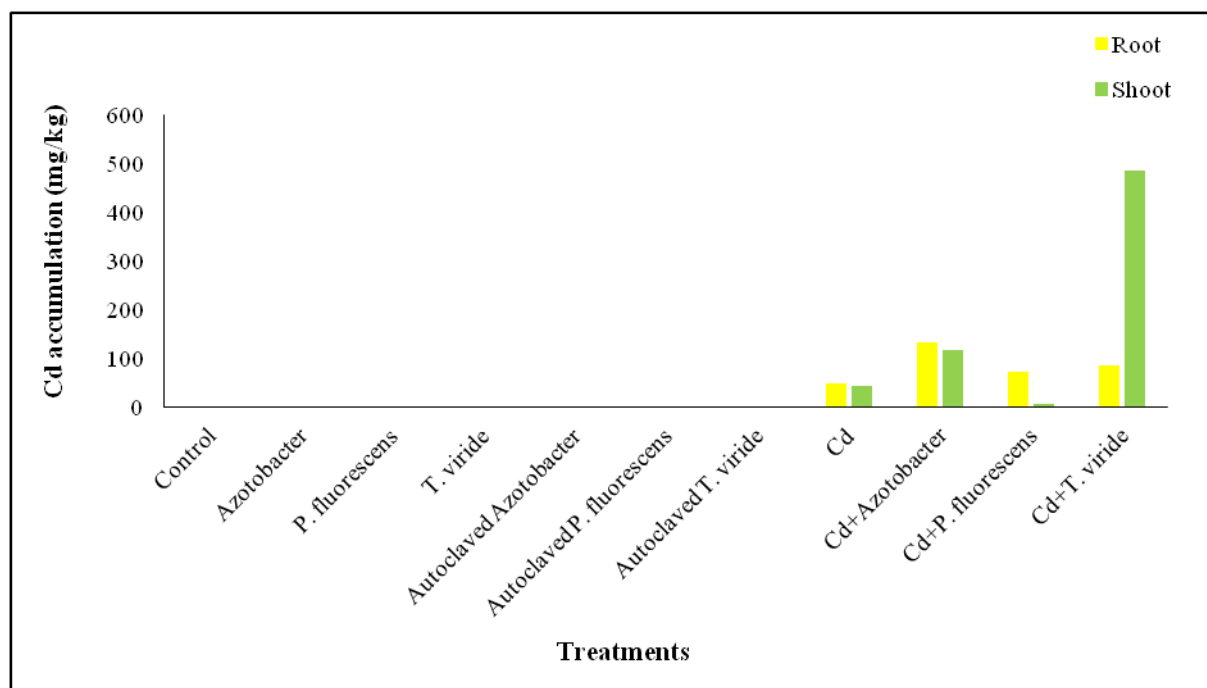


Figure 1. Effect of *Azotobacter* sp., *P. fluorescens* and *T. viride* on Cd accumulation in *Alternanthera ficoidea*

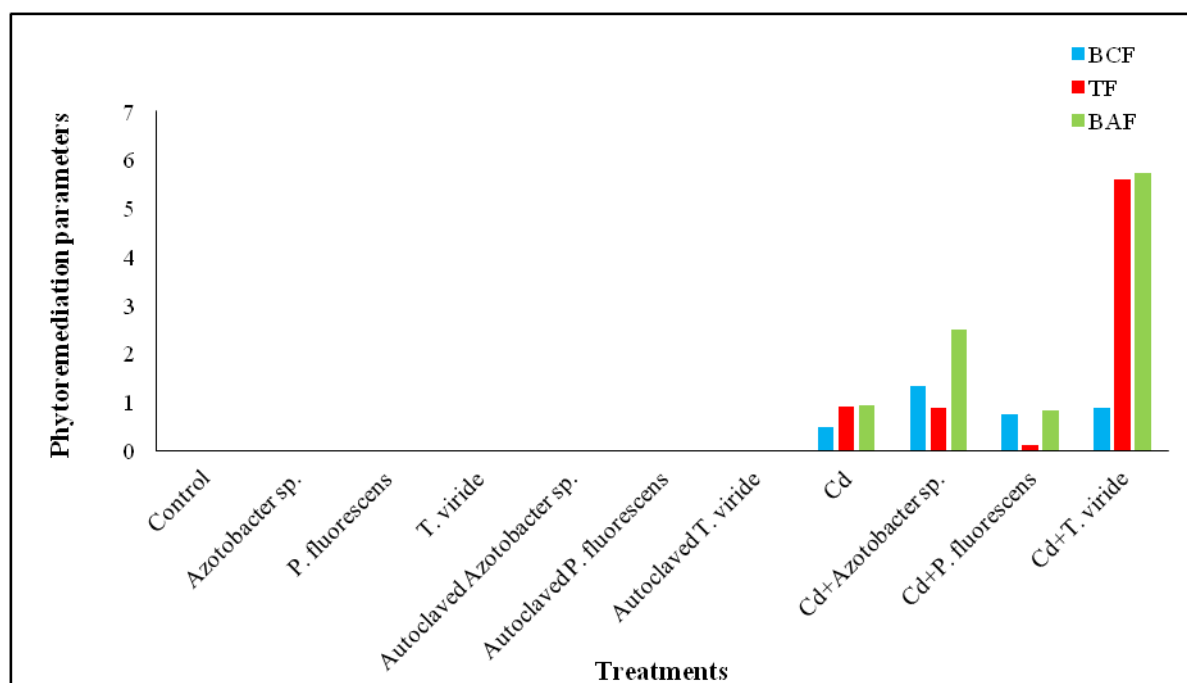


Figure 2. Effect of *Azotobacter* sp., *P. fluorescens* and *T. viride* on BCF, TF and BAF values of Cd in *A. ficoidea*



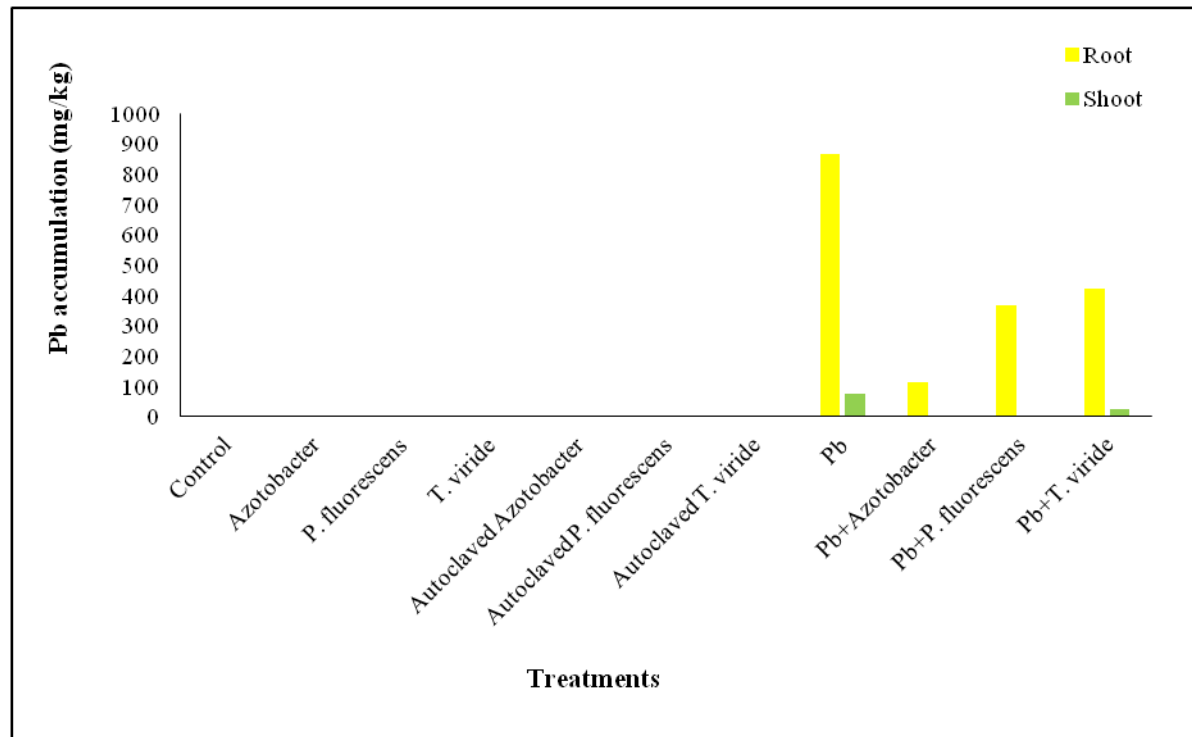


Figure 3. Effect of *Azotobacter* sp., *P. fluorescens* and *T. viride* on Pb accumulation in *A. ficoidea*

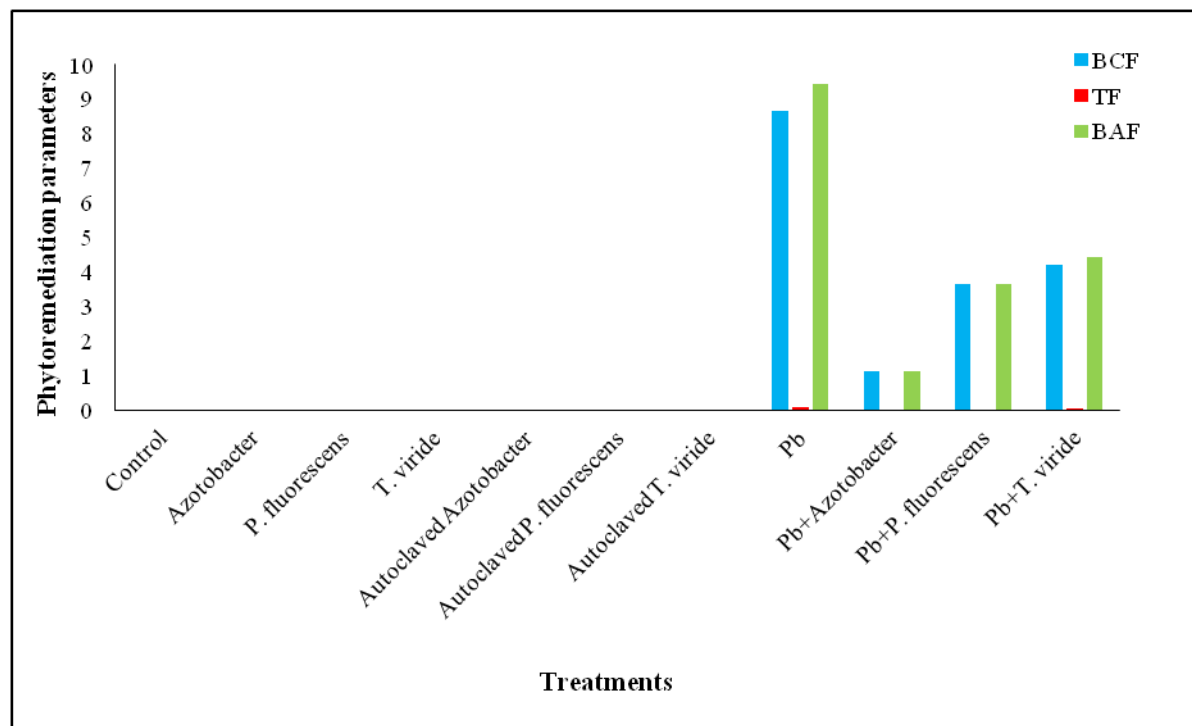


Figure 4. Effect of *Azotobacter* sp., *P. fluorescens* and *T. viride* on BCF, TF and BAF values of Pb in *Alternanthera ficoidea*

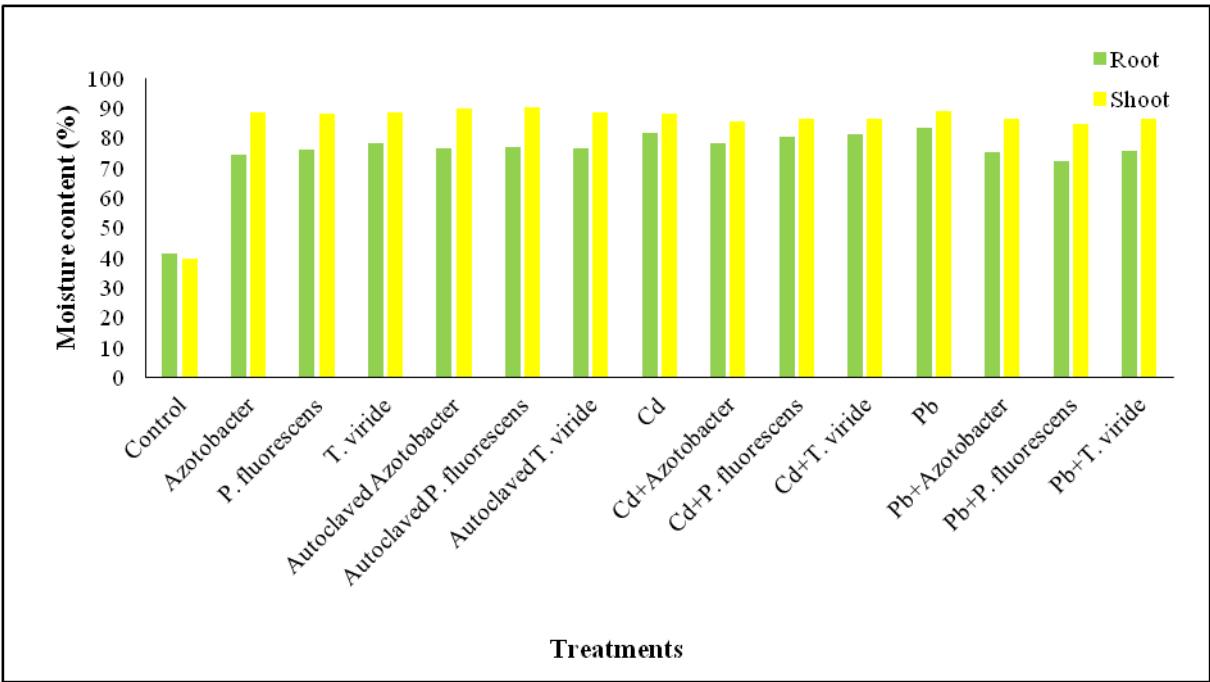


Figure 5. Effect of heavy metals (Cd, & Pb) and *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the moisture content of *Alternanthera ficoidea*

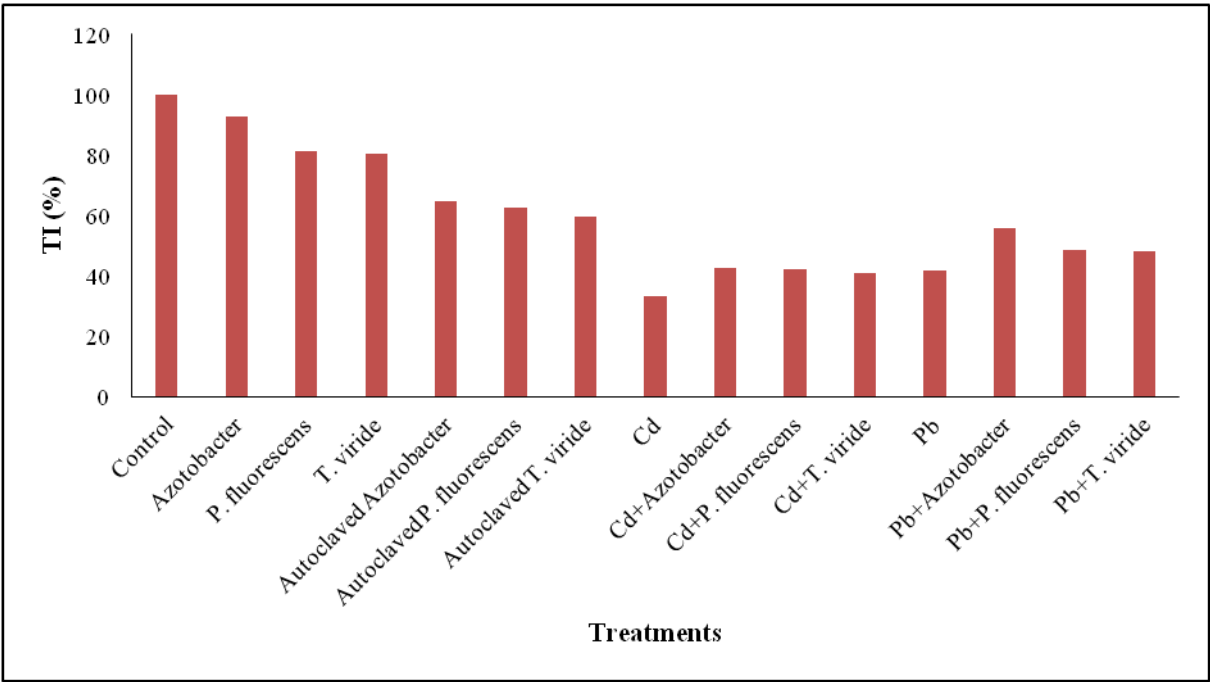


Figure 6. Effect of heavy metals (Cd, & Pb), *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the tolerance index of *Alternanthera ficoidea*

DISCUSSION

Effect of *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on Cd accumulation in *Alternanthera ficoidea*

Studies have shown that *Trichoderma*, *Azotobacter* sp. and *Pseudomonas* have mediated the enhanced accumulation of Cd in plants (Panwar *et al.*, 2011; Song *et al.*, 2015). Similarly, the present study has also witnessed enhanced accumulation of Cd in Cd + *Azotobacter* sp. and Cd + *T. viride* treated plants. However, decreased accumulation of Cd was observed

in the shoots of Cd + *P. fluorescens* treated *A. ficoidea*. This may be because *Pseudomonas* might have immobilized Cd in the root or rhizosphere, thereby causing a reduction in the uptake of Cd by plants (Wu et al., 2022).

#### **Effect of *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on Pb accumulation in *Alternanthera ficoidea***

Plants with BCF values greater than one and TF values lesser than one are potential phytostabilizers (Yoon et al., 2006). Hence, the present study reports *A. ficoidea* as a suitable plant for Pb phytostabilization. The highest BAF value was observed in *A. ficoidea* plants treated solely with Pb. This confirms that the application of microbes has decreased the accumulation of Pb in the plant. The effective role played by microbes in reduction of metal uptake is mainly due to immobilization that results in binding the metal to root by complex formation, which further prevents its translocation towards shoot (Khanna et al., 2019).

#### **Effect of heavy metals (Cd, Pb) and *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the growth parameters of *Alternanthera ficoidea***

##### **Effect of Cd and *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the growth parameters of *Alternanthera ficoidea***

The present study observed a significant decline in the growth parameters of Cd treated plants when compared to the control plant. Similar results due to Cd toxicity in plants were reported by Ci et al. (2009) and Faizan et al. (2012). Shukla et al. (2003) discussed the reduction in growth parameters due to the interference of Cd in nutrient uptake and altered levels of various biochemical constituents like proteins, amino acids, etc. The results obtained in the present study may also be due to these reasons. Enhanced growth of Cd + microbe treated plants compared to Cd-only treated *A. ficoidea* may be due to the stress-mitigating effect of microbes on the plants. The results of the present study are corroborative with the report of Rawat et al. (2012), who observed that *Trichoderma* could improve plant growth under stressful conditions.

#### **Effect of Pb and *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the growth parameters of *Alternanthera ficoidea***

The present study observed decreased growth parameters in Pb treated *A. ficoidea* compared to the control plants. Various studies have reported growth reduction in plants subjected to heavy metal stress. A study conducted by Hussain et al. (2021) found that Pb stress significantly halted growth (stem and root length), biomass (fresh and dry), and total water content in *Persicaria hydropiper* compared to plants grown in Pb-free medium. Ekmekçi et al. (2009) reported that plant growth retardation by metal stress is due to water deficit resulting from disturbance of water balance. The same reason may be applicable to the present case also. However, application of microbes enhanced the growth parameters of Pb-stressed *A. ficoidea* plants. The results of the present study align with the report of Rawat et al. (2012), who observed that *Trichoderma* could improve plant growth under stressful conditions. Further studies are required to clarify the reasons behind such responses in plants.

#### **Effect of heavy metals (Cd, & Pb) and *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the moisture content of *Alternanthera ficoidea***

With the excess concentration of heavy metals, decrease in transpiration and moisture content of the plants were observed by Kastori et al. (1992). However, our study revealed contradictory results. Further studies are required to clarify the reasons for obtaining such results.

#### **Effect of heavy metals (Cd, & Pb) and *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* on the tolerance index of *Alternanthera ficoidea***

The current study observed decreased TI in Cd treated, Pb treated, Cd + microbe treated and Pb + microbe treated plants compared to the control plants. This indicates that heavy metals have imposed stress on *A. ficoidea* plants. However, compared to the heavy metal-only treated plants, heavy metal + microbe treated *A. ficoidea* plants exhibited enhanced TI. This points out

the heavy metal stress-relieving ability of the microbes *Azotobacter* sp., *P. fluorescens* and *T. viride* on *A. ficoidea* plants.

#### **Visual toxic effects of heavy metals (Cd, & Pb) and *Azotobacter* sp., *Pseudomonas fluorescens* and *Trichoderma viride* in *Alternanthera ficoidea***

The concentration of heavy metal in the soil might be too low for the plant to exhibit toxicity symptoms or it may be because the plant is tolerant towards heavy metal stress. Further studies are required to explore the reason behind this observation.

### **CONCLUSION**

In the era of sustainability, phytoremediation plays a major role to rebuild the polluted areas. Phytoremediation is a natural way of finding a solution to reduce the increasing pollution in soils. The present study was conducted to investigate the accumulation efficiency of the heavy metals Cadmium (Cd) and Lead (Pb) by the microbial assistance of *Azotobacter* sp., *P. fluorescens* and *T. viride* in *Alternanthera ficoidea*. The effect of heavy metals and the microbes on the growth parameters, moisture content and tolerance index were also analysed.

The results revealed that *A. ficoidea* could accumulate Pb effectively in its root and hence can be used as an efficient plant for Pb phytostabilization. Application of microbes *Azotobacter* sp., *P. fluorescens* and *T. viride* enhanced the accumulation of Cd in the roots of *A. ficoidea* plants with respect to plants treated with Cd alone. Upon applying the microbe *Azotobacter*, plants treated with Cd + *Azotobacter* sp. showed Cd phytostabilization potential. Accumulation of lead was decreased in the roots and shoots of all plants that were treated with microbes. Therefore, we identified that *Azotobacter* sp., *P. fluorescens* and *T. viride* can be used for enhancing the Cd accumulation in *A. ficoidea*. However, opposite results were observed in the case of Pb. The findings demonstrated that *Azotobacter* sp., *T. viride*, and *P. fluorescens* could decrease the accumulation of Pb in *A. ficoidea* probably by immobilizing the heavy metal in the rhizosphere, thus helping in decreasing the risk of food chain contamination.

The growth parameters of plants treated with Cd + *Azotobacter* sp., Cd + *P. fluorescens* and Cd + *T. viride*, Pb + *Azotobacter* sp., Pb + *P. fluorescens* and Pb + *T. viride* increased remarkably compared to Cd only and Pb only treated plants respectively. This may be due to the stress mitigating effect of microbes on the heavy metal stressed plants. This inference is further supported by the results on tolerance index whereby the tolerance index of heavy metal + microbe treated *A. ficoidea* plants enhanced compared to the heavy metal only treated plants.

The study revealed the stressful conditions exerted by heavy metals and the stress relieving effect provided by microbes on heavy metal stressed *A. ficoidea*. However, more studies are required to find out the reasons behind these kinds of responses. Moreover, various anatomical, physiological, biochemical and molecular factors associated with the change in the growth parameters, moisture content and tolerance index need to be identified. Additionally, further studies can be conducted to find out an effective microbe for enhancing the Pb accumulation in *A. ficoidea*.

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### **CONFLICTS OF INTEREST**

The authors declare that they have no potential conflicts of interest.

### **REFERENCES**

- Abbas, T., Rizwan, M., Ali, S., Adrees, M., Zia-ur-Rehman, M., Qayyum, M. F., ... & Murtaza, G. (2018). Effect of biochar on alleviation of cadmium toxicity in wheat (*Triticum aestivum* L.) grown on Cd-contaminated saline soil. *Environmental Science and Pollution Research*, 25, 25668-25680
- Abbasi, S. A., & Tauseef, S. M. (2018). Use of the

- terrestrial weed *Alternanthera ficoidea* in treating greywater in soil-less SHEFROL® bioreactors. *Water Science and Technology*, 77(8), 2005-2013.
- Aladesanmi, O. T., Oroboade, J. G., Osisiogu, C. P., & Osewole, A. O. (2019). Bioaccumulation factor of selected heavy metals in *Zea mays*. *Journal of Health and Pollution*, 9(24), 191207.
- Allan, J. E. (1969). The preparation of agricultural samples for analysis by Atomic Absorption Spectrometry, *Varian Techtron Bulletin*.
- Cao, Y. C., Jin, R., & Mirkin, C. A. (2002). Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. *Science*, 297(5586), 1536–1540.
- Ci, D., Jiang, D., Dai, T., Jing, Q., & Cao, W. (2009). Effects of cadmium on plant growth and physiological traits in contrast wheat recombinant inbred lines differing in cadmium tolerance. *Chemosphere*, 77(11), 1620–1625.
- Cunningham, S. D., Shann, J. R., Crowley, D. E., & Anderson, T. A. (1997). Phytoremediation of contaminated water and soil. *ACS Symposium Series*, 664, 2–17.
- Dalla Vecchia, F., Rocca, N. L., Moro, I., De Faveri, S., Andreoli, C., & Rascio, N. (2005). Morphogenetic, ultrastructural, and physiological damages suffered by submerged leaves of *Elodea canadensis* exposed to cadmium. *Plant Science*, 168(2), 329–338.
- Das, P., Samantaray, S., & Rout, G. R. (1997). Studies on cadmium toxicity in plants: A review. *Environmental Pollution*, 98(1), 29-36.
- Eick, M. J., Peak, J. D., Brady, P. V., & Pesek, J. D. (1999). Kinetics of lead adsorption/desorption on goethite: Residence time effect. *Soil Science*, 164(1), 28-39
- Ekmekçi, Y., Tanyolaç, D., & Ayhan, B. (2009). A crop tolerating oxidative stress induced by excess lead: Maize. *Acta Physiologiae Plantarum*, 31, 319–330.
- Faizan, S., Kausar, S., & Perveen, R. (2012). Variation in growth, physiology, and yield of four chickpea cultivars exposed to cadmium chloride. *Journal of Environmental Biology*, 33(6), 1137.
- Gallego, S. M., Pena, L. B., Barcia, R. A., Azpilicueta, C. E., Iannone, M. F., Rosales, E. P., ... & Benavides, M. P. (2012). Unravelling cadmium toxicity and tolerance in plants: Insight into regulatory mechanisms. *Environmental and Experimental Botany*, 83, 33-46.
- Ganeshan, G., & Manoj Kumar, A. (2005). *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. *Journal of Plant Interactions*, 1(3), 123–134.
- Genchi, G., Carocci, A., Lauria, G., Sinicropi, M. S., & Catalano, A. (2020). Nickel: Human health and environmental toxicology. *International Journal of Environmental Research and Public Health*, 17(3), 679.
- Graham, P. H., & Vance, C. P. (2000). Nitrogen fixation in perspective: An overview of research and extension needs. *Field Crops Research*, 65(2-3), 93-106.
- Guidi Nissim, W., Palm, E., Mancuso, S., & Azzarello, E. (2018). Trace element phytoextraction from contaminated soil: a case study under Mediterranean climate. *Environmental Science and Pollution Research*, 25, 9114-9131.
- Halifu, S., Deng, X., Song, X., & Song, R. (2019). Effects of two *Trichoderma* strains on plant growth, rhizosphere soil nutrients, and fungal community of *Pinus sylvestris* var. *mongolica* annual seedlings. *Forests*, 10(9), 758
- Hussain, F., Hadi, F., & Rongliang, Q. (2021). Effects of zinc oxide nanoparticles on antioxidants, chlorophyll contents, and proline in *Persicaria hydropiper* L. and its potential for Pb phytoremediation. *Environmental Science and Pollution Research*, 28(26), 34697–34713.
- Jali, P., Pradhan, C., & Das, A. B. (2016). Effects of cadmium toxicity in plants: A review article. *Scholars Academic Journal of Biosciences*, 4(12), 1074–1081.
- Jung, M. C., & Thornton, I. (1996). Heavy metal contamination of soils and plants in the vicinity of a lead-zinc mine, Korea. *Applied Geochemistry*, 11(1–2), 53–59.
- Kastori, R., Petrović, M., & Petrović, N. (1992). Effect of

- excess lead, cadmium, copper, and zinc on water relations in sunflower. *Journal of Plant Nutrition*, 15(11), 2427–2439.
- Khanna, K., Jamwal, V. L., Gandhi, S. G., Ohri, P., & Bhardwaj, R. (2019). Metal-resistant PGPR lowered Cd uptake and expression of metal transporter genes with improved growth and photosynthetic pigments in *Lycopersicon esculentum* under metal toxicity. *Scientific Reports*, 9(1), 5855.
- Kumar, A., & Maiti, S. K. (2014). Translocation and bioaccumulation of metals in *Oryza sativa* and *Zea mays* growing in chromite asbestos contaminated agricultural fields, Jharkhand, India. *Bulletin of Environmental Contamination and Toxicology*, 93(4), 434–441.
- Lokhande, B. J., Patil, P. S., & Uplane, M. D. (2004). Studies on cadmium oxide sprayed thin films deposited through non-aqueous medium. *Materials Chemistry and Physics*, 84(2-3), 238–242.
- Ma, J. F., Goto, S., Tamai, K., & Ichii, M. (2001). Role of root hairs and lateral roots in silicon uptake by rice. *Plant physiology*, 127(4), 1773–1780.
- Ma, Y., Oliveira, R. S., Freitas, H., & Zhang, C. (2016). Biochemical and molecular mechanisms of plant-microbe-metal interactions: Relevance for phytoremediation. *Frontiers in Plant Science*, 7, 918.
- Mahar, A., Wang, P., Ali, A., Awasthi, M. K., Lahori, A. H., Wang, Q., & Zhang, Z. (2016). Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. *Ecotoxicology and Environmental Safety*, 126, 111–121.
- Máthé-Gáspár, G., & Anton, A. (2005). Phytoremediation study: Factors influencing heavy metal uptake of plants. *Acta Biologica Szegediensis*, 49(1–2), 69–70.
- Mesjasz-Przybyłowicz, J., Nakonieczny, M., Migula, P., Augustyniak, M., Tarnawska, M., Reimold, W. U., & Głowacka, E. (2004). Uptake of cadmium, lead, nickel and zinc from soil and water solutions by the nickel hyperaccumulator *Berkheya coddii*. *Acta Biologica Cracoviensia Series Botanica*, 46, 75–85.
- Mishra, R. K., Mohammad, N., & Roychoudhury, N. (2016). Soil pollution: Causes, effects, and control. *Van Sangyan*, 3(1), 1–14.
- Moreno, J. L., Hernández, T., & Garcia, C. (1999). Effects of a cadmium-contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil. *Biology and Fertility of Soils*, 28(3), 230–237.
- Mulligan, C. N., Yong, R. N., & Gibbs, B. F. (2001). An evaluation of technologies for the heavy metal remediation of dredged sediments. *Journal of Hazardous Materials*, 85(1–2), 145–163.
- Newman, L., Ansari, A. A., Gill, S. S., Naeem, M., & Gill, R. (2023). Phytoremediation. In *Phytoremediation: Management of Environmental Contaminants*, Volume 7
- Pagliano, C., Raviolo, M., Dalla Vecchia, F., Gabbrielli, R., Gonnelli, C., Rascio, N., Barbato, R., & La Rocca, N. (2006). Evidence for PSII donor-side damage and photoinhibition induced by cadmium treatment on rice (*Oryza sativa* L.). *Journal of Photochemistry and Photobiology B: Biology*, 84(1), 70–78.
- Panwar, B. S., Kádár, I., Biró, B., Rajkai-Végh, K., Ragályi, P., Rékási, M., & Márton, L. (2011). Phytoremediation: Enhanced cadmium (Cd) accumulation by organic manuring, EDTA and microbial inoculants (*Azotobacter* sp., *Pseudomonas* sp.) in Indian mustard (*Brassica juncea* L.). *Acta Agronomica Hungarica*, 59(2), 117–123.
- Pivet, B. E. (2001). Phytoremediation of contaminated soil and ground water at hazardous waste sites (EPA/540/S-01/500). *U.S. Environmental Protection Agency, Office of Research and Development, Office of Solid Waste and Emergency Response*.
- Rawat, L., Singh, Y., Shukla, N., & Kumar, J. (2012). Seed biopriming with salinity-tolerant isolates of *Trichoderma harzianum* alleviates salt stress in rice: Growth, physiological, and biochemical characteristics. *Journal of Plant Pathology*, 353–365.
- Rosselli, W., Keller, C., & Boschi, K. (2003). Phytoextraction capacity of trees growing on a

- metal-contaminated soil. *Plant and Soil*, 256(1), 265–272.
- Sajeesh, P. K. (2015). Cu-Chi-Tri: A triple combination for the management of late blight disease of potato (*Solanum tuberosum* L.) (Doctoral dissertation, GB Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand).
- Sharma, P., & Dubey, R. S. (2005). Lead toxicity in plants. *Brazilian Journal of Plant Physiology*, 17, 35-52.
- Shukla, U. C., Singh, J., Joshi, P. C., & Kakkar, P. (2003). Effect of bioaccumulation of cadmium on biomass productivity, essential trace elements, chlorophyll biosynthesis, and macromolecules of wheat seedlings. *Biological Trace Element Research*, 92, 257–273.
- Sinicropi, M. S., Caruso, A., Capasso, A., Palladino, C., Panno, A., & Saturnino, C. (2010). Heavy metals: Toxicity and carcinogenicity. *Pharmacologyonline*, 2, 329-333.
- Song, N., Ma, Y., Zhao, Y., & Tang, S. (2015). Elevated ambient carbon dioxide and *Trichoderma* inoculum could enhance cadmium uptake of *Lolium perenne* explained by changes of soil pH, cadmium availability, and microbial biomass. *Applied Soil Ecology*, 85, 56–64.
- Thompson, J., & Bannigan, J. (2008). Cadmium: Toxic effects on the reproductive system and the embryo. *Reproductive Toxicology*, 25(3), 304-315.
- Wu, S., Zhou, Z., Zhu, L., Zhong, L., Dong, Y., Wang, G., & Shi, K. (2022). Cd immobilization mechanisms in a *Pseudomonas* strain and its application in soil Cd remediation. *Journal of Hazardous Materials*, 425, 127919.
- Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment*, 368(2-3), 456-464.