



BIOCHEMICAL BASIS FOR THE PHYTOREMEDIATION POTENTIAL OF *PARTHENIUM HYSTEROPHORUS* L. AGAINST CADMIUM CHLORIDE (CdCl₂) IN THE SOIL: A FRAMEWORK OF POT EXPERIMENT

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Abstract: Development in environmentally friendly development owing to hazardous metal contamination is severely hampered. Arguably the most harmful elements to plants and human beings is cadmium (Cd). Cd represents a key contributor to the overall degradation of the environment. It may be found in both naturally occurring and man-made substances. "Phytoremediation" the employment of plants to remove such toxins from the environment proves to be a potential substitute for the existing remediation techniques. The *Parthenium hysterophorus* L. represents one such unique plant. The species is recommended for the reason of its quick spread throughout India as well as the rest of the world plus its inedibility by herbivore. Given the foregoing, the research objective of the study was to demonstrate phytoremediation potential of *Parthenium hysterophorus* L. in Cd contaminated soils employing pot experiments. During the pot experiment, four different concentrations of cadmium (5, 10, 15, and 20 mg/kg) have been established. At the 30th, 60th, and 90th harvested days following the treatment, sample were taken to determine the plant's potential for phytoremediation. The results indicated that cadmium stress diminished development (root and shoot lengths) and biomass variables, and that cadmium hyperaccumulator ability was evident based on the bioaccumulation coefficient (BAC), bioconcentration factor (BCF), and translocation factor (TF). As a result, it can be said that *Parthenium hysterophorus* L. is capable of handling cadmium contamination effectively and serves as a vitally significant and economically advantageous measure for sustainable and environmental preservation.

Index Terms - Cadmium chloride, Biochemistry, *Parthenium hysterophorus* L., Phytoremediation.

I. INTRODUCTION

Towards the improvement of livelihood efficiency, various kinds of material beings are being included into routine. This has led the introduction of heavy metals, a phenomenon that in turn raises the diversity and uncertainty of their makeup in the surrounding environment, particularly so in nations that are rapidly industrializing. As a result, environmental destruction brought through contaminants such as heavy metals has been recognised as one of the primary environmental problems that poses a threat to the sustainability of development (Kushwaha et al., 2018). Through both man-made and natural channels, such as residential and agriculture usage, metal extracted from deposits and melted down operations, as well as other manufacturing processes, heavy metals enter the atmosphere, groundwater, and other water sources. They do not biodegrade and last for a very long time in the atmosphere (Usman et al., 2020). A few of several heavy metals, cadmium (Cd) is a non-degradable, poisonous minor, redundant metal, frequently and abundantly present metal. The ecology is seriously harmed by the transport of Cd through food chains (Parveen et al., 2023). The amounts of cadmium in soil as well as the groundwater especially serve as essential for ensuring wholesome food supplies as well as secure water for consumption, however cadmium levels increase due to geogenic and human causes. The usage of Cd containing fertilisers, emissions of combustible pollutants, and excavation are some significant anthropogenic sources of cadmium. In addition to human-caused sources, cadmium is additionally integrated into sulphides, carbonates, as well as phosphorites, causing higher levels of Cd in related varieties of rock (Andreas et al., 2019).

The exposure to cadmium poses serious detrimental effects for impacted organisms such as animals and plants (O'Connor et al., 2019). The World Health Organisation advised that the tolerated every month cadmium consumption be 25 g.kg⁻¹ body weights while recommending that the amount of Cd in water used for drinking shouldn't be higher than 3 g.L⁻¹ (Dutta et al., 2021). Because of interference with calcium along with additional nutrients, prolonged Cd toxicity results in renal tubular failure, osteomalacia and osteoporosis (Aoshima, 2016). According to Khan et al., (2017), being exposed to Cd has been additionally linked to problems with the metabolism of glucose, cancer, infarction of the brain, and cardiovascular disease. Along with the

adverse impacts that the aforementioned metal upon human health, Cd can relocate to culinary sections of plants, impair the growth and development of crops, and pose a danger to the nutritional value of food (Parveen et al., 2023). The majority of plants typically display obvious Cd toxicological signs whenever the overall cadmium (Cd) levels in soils surpasses 8 mg.kg^{-1} , the accessible levels of Cd rises to $>0.001 \text{ mg.kg}^{-1}$, while the amount of Cd within plant tissues approaches $3\text{--}30 \text{ mg.kg}^{-1}$. The Cd poisoning affects stomata conductance, function of enzymes, crop growth, the processes of photosynthesis, the development of seeds, and the intake of minerals (Dutta et al., 2021).

It is believed that there are numerous strategies that have been arrived at for cleaning up contamination from heavy metals in environments, including soil igniting, soil cleansing, and application of electric field (Sheoran et al., 2016, DalCorso et al., 2019). Although there are chemical as well as physical procedures to get rid of metal pollution, they require a lot of work. Chemical methods of treatment have been very costly and produce additional contaminants (Usman et al., 2019). As a result, the development of novel innovations becomes essential, and the investigation of various bio-based methods, such as bioremediation, followed. The application of naturally occurring substances is less expensive, safer, it produces minimal or no negative environmental consequences. Bio-augmentation, bioventing, composting, and phytoremediation are all types of bioremediation techniques. The capacity for plants to extract and endure enormous amounts of heavy metals was proved by a novel plant-based method called phytoremediation, which has subsequently attracted additional interest. It is economical, powered by solar energy, attractive, and environmentally beneficial (Chai et al., 2012; Ullah et al., 2015). According to many publications and evaluations by Lone et al., (2008) along with Xia et al., (2019), over 400 plants may be potential to remove heavy metals through phytoremediation.

In order to avoid reaching into the food chain, plants chosen for phytoremediation ought to possess fast growth rates, substantial biomass generation, hyperaccumulation of heavy metals, wide distribution, translocation process between stem to shoot, leniency of hazardous heavy metal repercussions, resistance to pests and pathogens, appropriate to changing environmental conditions, ease in development and harvest time, and no enticement to herbivores (Shabani and Sayadi, 2012; Ali et al., 2013). In light of the foregoing, the weed *Parthenium hysterophorus* L., among the members of the family Asteraceae, has been selected in present study. This is a widespread alien plant which colonises every region, especially grasslands, agriculture, and roadway vegetation in countries such as India, Pakistan, Australia and certain regions of Africa (Dhawan & Dhawan, 1996). In the past, the capacity of the aforementioned plant to remove lead (Pb) using the phytoextraction process was studied, and the findings were favourable (Hadi and Bano, 2009). Thus, the purpose of the present investigation sought to assess the impact of various levels of cadmium chloride on growth, biomass, and phytoremediation efficiency of *Parthenium hysterophorus* L.

II. MATERIALS AND METHODS

2.1. Plant material and experimental conditions:

The soil was obtained within the botanical conservatory (1-30 cm depth) at Sri Venkateswara University in Tirupati. The finely crushed soil then sterilised by autoclave allowed to dry for seven days, then pulverised using a mortar and pestle before being put over 2 mm tubes of sieving. The maximal water retention ability of the soil ($300 \text{ ml water/kg soil} \pm 3$) was determined (Keen, 1931). Considering the research of Ramesh and Damodharam, (2017) the fundamental characteristics about the soil was determined. Clay (54.2%), silt (10.6%), soil (33.9%), pH (6.6), electrical conductivity ($0.39 \text{ } \mu\text{Mhos/cm}$), organic matter (6.2 g/kg), available phosphorus (59.26 mg/kg), available potassium (97.45 mg/kg), total nitrogen (55.13 mg/kg), total copper (11 mg/kg), and soil Cd (0.16 mg/kg) had been its physiochemical attributes.

Throughout this completely randomised designed (CRD) experiment, plastic pots ($16 \times 11 \text{ cm}$) have been utilised, while each pot contained 1 kilogramme of soil. *Parthenium hysterophorus* seeds have been collected from plants thriving in uncontaminated locations; there were a minimum of 10 seeds within each pot. Because of its adaptable nature, *P.hysterophorus* cannot require an excessive amount of water for development, therefore tap water is supplied twice per week. One seedling (*P.hysterophorus* plantlet) was placed into every pot after being chosen for its size being uniform. Thirty pots all together were put to use with five of them allocated to each treatment.

When the seeds were sown, the ambient temperature had been $34/22^\circ\text{C}$ (day/night) and $28/19^\circ\text{C}$ when the crop had been harvested. For the experiment of the control, water has been provided everyday to keep the soil moistened at a level between 75 - 85%. In contrast to the treatment condition, soil had been experimentally supplied with various CdCl_2 concentrations (5, 10, 15, and 20 mg/kg). Throughout the course of the study, the soil received no extra fertiliser applications.

2.2. Determination of plant growth parameters:

In order to compare the parameters of plant growth responses of being treated and untreated seedlings subjected to varying amounts of CdCl_2 stress, seedlings of *P.hysterophorus* that were 30, 60, and 90 days old were collected. Plants were harvested and then washed with tap water and each plant separated into three parts (roots, stems, and leaves).

The roots were further washed with a solution containing 5 mM Tris-HCl pH 6.0 and 5 mM EDTA, and they were then rinsed with distilled water in order to remove the surface-bound metal ions (Genrich et al., 2000). Plant height and root length were measured with a centimeter ruler from the root and shoot joint to the apices. The fresh of each plant (roots, stems, and leaves) was measured using an analytical balance.

2.3. Determination of phytoremediation indices:

2.3.1. Quantification of cadmium levels in plant and soil:

According to mentioned earlier in Usman et al., (2019), cadmium quantification within the soil as well as the plant (*P.hysterophorus* seedlings of 30, 60, and 90 days old exposed to the concentrations of 5, 10, 15, and 20 mg/kg CdCl_2) shoot along with root has been carried out. In brief, employing an extensive volume digesting apparatus at alternate temperatures, samples of around 0.5 g (plants) and 0.25 g (soil) were subjected to digestion with a solution of nitric acid (HNO_3) along with solution of hydrogen peroxide (H_2O_2) or hydrogen fluoride (HF) as long as solutions became transparent. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used to analyse digested materials. Cd levels in each sample have been measured using five separate samples along with standard reference materials.

2.3.2. Computations of phytoremediation indices:

The bio-concentration factor (BCF), which measures the ratio of metal concentration in plant roots to soil concentration, the translocation factor (TF), which measures the ratio of metal concentration in the shoot to metal concentration in the root, and a bio-absorption coefficient (BAC) were all assessed as that of earlier reported in Raj et al., (2020).

2.4. Statistical Analysis:

The results were expressed as the mean \pm standard error of the five determinations.

III. RESULTS

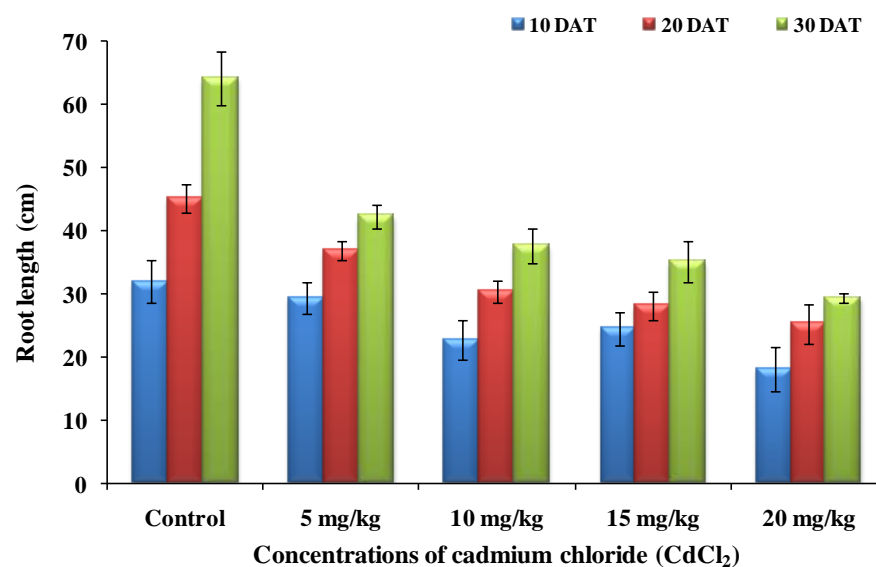
In assessing the lengths of both shoots and roots either with or without using the inclusion of an intended heavy metal, response of *P. hysterophorus* to Cd has been established. Tables 1, 2, and 3 and Figures 1, 2, and 3 demonstrate the manner in which the growth of plants responded to various exposure levels of 5, 10, 15, and 20 mg/kg CdCl₂ for 30, 60, and 90 days, correspondingly. These results reveal that stress caused by Cd reduced root length, shoot length, and biomass. The overall length of the roots found in *P. hysterophorus* young plants declined gradually along with an upsurge in the level of Cd on 30, 60, and 90 days following treatment, but there was only a slight difference across the 5 mg/kg of CdCl₂ treatment, with the comparable measurements in the control group being 29.3 \pm 2.5, 36.8 \pm 1.5, and 42.3 \pm 1.9cm. The total length of the root of the plants ultimately dwindled as the concentration of Cd soared from 5 mg/kg to 20 mg/kg (Table 1 and Fig 1), being substantially shorter by 18.1 \pm 3.4, 25.3 \pm 3.1, and 29.3 \pm 0.8cm accordingly, through the concentration of 20 mg/kg CdCl₂, compared to those observed in the untreated plants at 31.9 \pm 3.4, 45.1 \pm 2.3, and 64.1 \pm 4.2.

Table 1. Effect of cadmium chloride on root length (cm) of *P. hysterophorus* L.

Concentration	30 DAT	60 DAT	90 DAT
Control	31.9 \pm 3.4	45.1 \pm 2.3	64.1 \pm 4.2
5 mg/kg	29.3 \pm 2.5	36.8 \pm 1.5	42.3 \pm 1.9
10 mg/kg	22.7 \pm 3.1	30.4 \pm 1.7	37.6 \pm 2.8
15 mg/kg	24.5 \pm 2.6	28.1 \pm 2.3	35.1 \pm 3.3
20 mg/kg	18.1 \pm 3.4	25.3 \pm 3.1	29.3 \pm 0.8

DAT: Days After Treatment; Values are mean \pm standard error of five replications

Fig 1. Effect of cadmium chloride on root length (cm) of *Parthenium hysterophorus* L.

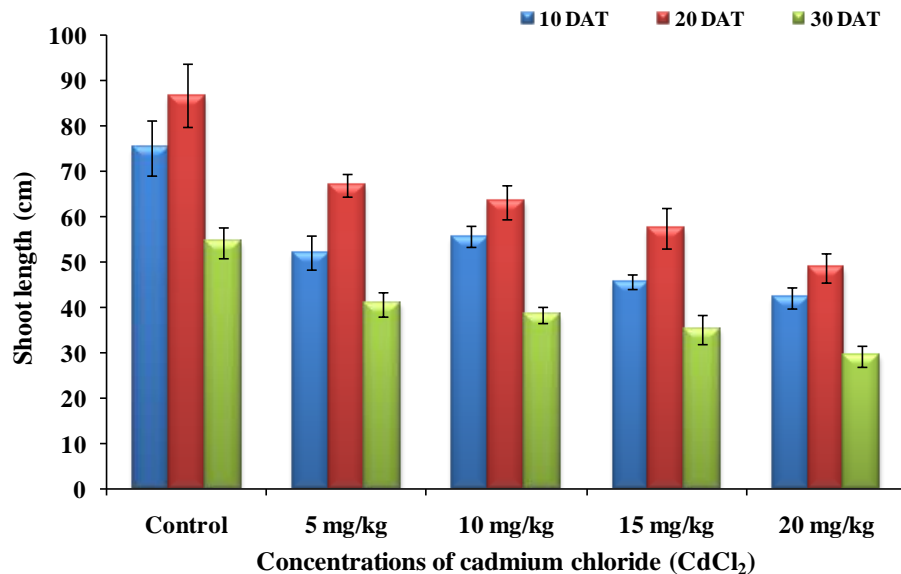


The identical findings were found for the levels of Cd (5, 10, 15, and 20 mg/kg) regarding shoot length among *P. hysterophorus* young plants, through the level of 20 mg/kg of CdCl₂ possessing substantially decreased (42.1 \pm 2.3, 48.6 \pm 3.2, and 29.3 \pm 2.4cm) on 30, 60, and 90 days subsequent to the course of treatment, accordingly. The shoot lengths remained shorter at intermediary Cd doses (10 mg/kg and 15 mg/kg) compared with the untreated group (75.3 \pm 6.1, 86.7 \pm 6.9, and 54.3 \pm 3.5cm) throughout the 30th, 60th, and 90th days following treatment with Cd (55.6 \pm 2.4, 63.3 \pm 3.7, 38.4 \pm 1.7 and 45.6 \pm 1.7, 57.4 \pm 4.4, and 35.1 \pm 3.3cm). Most of the treatments throughout the research produced shorter shoot lengths in comparison to the untreated, particularly the maximum values being 52.1 \pm 3.7, 67.1 \pm 2.5, and 40.7 \pm 2.6cm with plants that were exposed to 5mg/kg CdCl₂ for 30, 60, and 90 days, amongst the remaining concentrations (Table 2 & Fig 2).

Table 2. Effect of cadmium chloride on shoot length (cm) of *P. hysterophorus* L.

Concentration	30 DAT	60 DAT	90 DAT
Control	75.3±6.1	86.7±6.9	54.3±3.5
5 mg/kg	52.1±3.7	67.1±2.5	40.7±2.6
10 mg/kg	55.6±2.4	63.3±3.7	38.4±1.7
15 mg/kg	45.6±1.7	57.4±4.4	35.1±3.3
20 mg/kg	42.1±2.3	48.6±3.2	29.3±2.4

DAT: Days After Treatment; Values are mean ± standard error of five replications

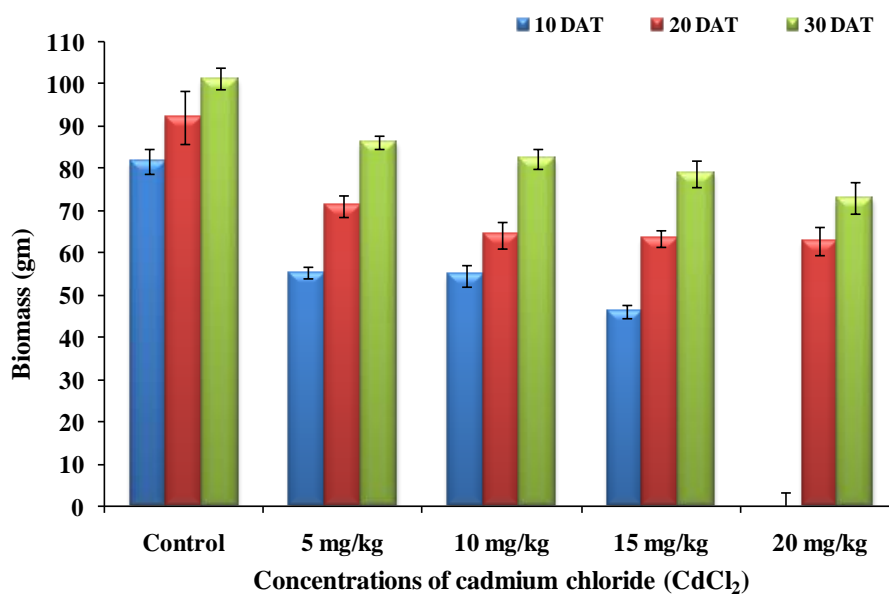
Fig 2. Effect of cadmium chloride on shoot length (cm) of *Parthenium hysterophorus* L

The total amount of biomass associated with *P. hysterophorus* fluctuated similarly, exhibited decreased patterns for all of the collected days (30, 60, and 90) following treatment as Cd contents soared. During 30, 60, and 90 days following the course of treatment, accordingly, the amount of biomass produced was substantially reduced with the 20 mg/kg exposure to Cd stress (45.4±3.1; 62.8±3.2 and 73.0±3.8gms) compared the untreated control levels (81.7±2.9, 92.2±6.4 and 101.3±2.5gm) (Table 3 and Fig 3). The biomass content of *P. hysterophorus* young plants heightened within 15, 10, and 5 mg/kg cadmium stress (46.1±1.6, 63.4±2.1 and 78.7±3.1cm, 54.7±2.5, 64.3±3.1, and 82.3±2.2cm, and 55.3±1.4, 71.1±2.6, and 86.1±1.6cm, respectively) together with a decrease in CdCl₂ levels on their harvest days, displaying a pattern opposite from that seen with respect to the untreated control group. *P. hysterophorus* that received 5, 10, or 15 mg/kg of Cd stress had reduced BAC, BCF, and TF values across each the freshly collected plants at 30, 60, and 90 days compared to *P. hysterophorus* that was exposed to 20 mg/kg of CdCl₂ (Tables 4, 5, and 6). The aforementioned plants can clearly absorb and withstand an overabundance of cadmium in the tissues that is evidenced by the rise in BAC, BCF, and TF values in tandem with rising metal level. *P. hysterophorus* showed BAC & TF levels between 5 and 20 mg Cd kg⁻¹, as well as BCF values greater than 1 on all study-related harvested days. The BCF regarding stress of 5 to 20 mg Cd kg⁻¹ on 30, 60, and 90 days following harvest varied from 1.02-2.65, whereas the BAC for cadmium metal throughout the *P. hysterophorus* ranged from 0.16 to 0.37. In contrast, the TF of cadmium metal varied between 0.10 and 0.20.

Table 3. Effect of cadmium chloride on biomass (gm) of *P. hysterophorus* L.

Concentration	30 DAT	60 DAT	90 DAT
Control	81.7±2.9	92.2±6.4	101.3±2.5
5 mg/kg	55.3±1.4	71.1±2.6	86.1±1.6
10 mg/kg	54.7±2.5	64.3±3.1	82.3±2.2
15 mg/kg	46.1±1.6	63.4±2.1	78.7±3.1
20 mg/kg	45.4±3.1	62.8±3.2	73.0±3.8

DAT: Days After Treatment; Values are mean ± standard error of five replications

Fig 3. Effect of cadmium chloride on biomass (gm) of *Parthenium hysterophorus* L.**Table 4. Effect of cadmium chloride on bioabsorption coefficient of *P. hysterophorus* L.**

Concentration	30 DAT	60 DAT	90 DAT
5 mg/kg	0.16	0.22	0.31
10 mg/kg	0.18	0.23	0.32
15 mg/kg	0.19	0.25	0.35
20 mg/kg	0.20	0.28	0.37

DAT: Days After Treatment

Table 5. Effect of cadmium chloride on bioconcentration factor of *P. hysterophorus* L.

Concentration	30 DAT	60 DAT	90 DAT
5 mg/kg	1.15	1.46	1.77
10 mg/kg	1.17	1.51	1.80
15 mg/kg	1.02	1.62	1.83
20 mg/kg	1.71	2.33	2.65

DAT: Days After Treatment

Table 6. Effect of cadmium chloride on translocation factor of *P. hysterophorus* L.

Concentration	30 DAT	60 DAT	90 DAT
5 mg/kg	0.10	0.11	0.13
10 mg/kg	0.16	0.14	0.17
15 mg/kg	0.15	0.16	0.18
20 mg/kg	0.18	0.18	0.20

DAT: Days After Treatment

IV. DISCUSSION

An extensive environmental concern, Cd soil pollution calls for long-lasting remedies. The Earth's crust may contain this hazardous substance naturally or it may have been created artificially. In soil, Cd may over time persist and relocate. As a result, there is a very high possibility that it will make its way into the food chain, therefore the repercussions on the living things throughout the food chain remain quite concerning (Bouida et al., 2022). According to recent claims (Nikalje and Suprasanna, 2018; Ejaz et al., 2022), plants are better appropriate for using and cleaning up metal-contaminated soil. But this information is still far from being attained, in part because the mechanisms and tactics influencing hazardous metal tolerance and absorption in plants are not well understood. As a means to aid in avoiding heavy metals being introduced into the food chain, plants chosen for the phytoremediation concerning heavy metal contaminants must also have a rapid growth rate, be stress resistant, and be bland to herbivores (Fazal et al., 2014). As a result, the goal of the present investigation attempted to assess how CdCl₂ affected the growth, biomass, and phytoremediation indicators of *P. hysterothorus* in the context of a pot experiment.

The majority of the key factors influencing the development of plants are biomass, shoot length, as well as the root length of plants. Stress from heavy metals has been shown to have a significant impact on the growth of plants and biomass (Arun et al., 2005; Hadi and Bano, 2009; John et al., 2009; Hadi et al., 2010). Accordingly, the presented research likewise showed a significant decrease in parameters related to growth (root length, shoot length, and biomass) of *P. hysterothorus* seedlings that were exposed to cadmium concentrations of 5, 10, 15, and 20 mg/kg with an increasing trend on 30, 60, and 90 days after the plants were harvested. According to Houshmandfar and Moraghebi, (2011), the detrimental impact of heavy metals on meristematic proliferation and cell growth and diversification may be specifically accountable for the diminution in plant height (root length, shoot length) and also biomass. The impact associated with metals upon the components of cell walls and their underlying structures could be a contributor to the causes of inhibition of cell elongation (Poschenrieder et al., 1989). The reduced in growth and biomass may additionally be related to changes in the functionality of several of the significant enzymes that regulate the metabolism (John et al., 2009) or the reduction of chlorophyll synthesis (Padmaja et al., 1990). Both Zheng et al., (2010) and Khatamipour et al., (2011) observed comparable reductions with stress from Cd in the *Glycyrrhiza uralensis* plant. Additionally, according to current research, the plant *P. hysterothorus* that received Cd toxins showed decreased root and shoot development in addition to biomass along enhanced metal absorption when given a combination of ethylenediaminetetraacetic and gibberellic acid as treatments (Nasir and Fazal, 2015; Ejaz et al., 2022). The ion transporters within the membranes of plant cells which are intended for transferring nutritional ions are used by plant for absorption of cadmium that limits the usual metabolic processes of the plants and hinders their development and biomass (Noman and Aqeel, 2017; Khalid et al., 2019).

Through describing the accumulative attributes and translocation patterns of metals within plants, multiple phytoremediation indicators, such as the bioaccumulation coefficient (BAC), bioconcentration factor (BCF), and translocation factor (TF), may be employed to determine if plants are appropriate for phytoremediation (i.e., phytoextraction or phytostabilization). As stated by Fitz and Wenzel, (2002), plants having BCF values below 1 are not appropriate for the phytoextraction whereas those having BAC values over 1 are regarded as prospective phytoextractors that are suitable for phytoextraction. Additionally, a plant qualifies as a hyperaccumulator if its BCF value is greater than 1, but an excluder is indicated by a number lower than 1. According to Usman et al., (2020), TF value impacts how well plants move heavy metals through the root towards the shoot. Whenever TF is more than 1, a plant is said to be effective in transporting metal from its root to the shoot; this is because the metal transportation network is effective. The fact that such plants acquire metals more frequently in the rhizomes as well as roots compared to the shoots or leaves is supported by TF values below one, which imply poor metal transport (Yoon et al., 2006). Accordingly, taking into account the foregoing, the BAC, BCF, and TF values were computed as well within this research in order to assess the capacity of plants to acquire metals into the root system from the soil and to transfer them to apical tissues (leaves). *P. hysterothorus*, said examined plant species, showed BCF values > 1, BAC and TF values < 1 for all cadmium treatments (5, 10, 15, 20 mg/kg) during 30, 60, and 90 days after the harvest, showing that the plant species may potentially be described as a phytoextraction/ phytostabilizer of cadmium. The arrival at the discovery of plant-mediated detoxification pathways depending upon the sequestration of ions within its vacuole through adhesion with ligands (proteins, organic acids, and peptides) enables for the transfer of these heavy metals, which ended in elevated translocation values (Sharma et al., 2021; Durante-Yáñez et al., 2018). The following may be attributed to the relatively small amounts of these elements within the soil, of which serve as an impediment for accumulation. The significant BAC, BCF, and TF values presented for cadmium particularly elucidate why this metal seems more easily transported and hence bioconcentrates with greater ease in plant roots. Regarding *Clidemia sericea* and *Miconia zamorensis*, respectively, Durante-Yáñez et al., (2022), Marrugo-Negrete et al., (2016), and Chamba et al., (2016) reported comparable results for mercury. The phytoremediation ability of *Cyamopsis tetragonoloba*, *Sesamum indicum*, *Tetraena qataranse*, *Calotropis procera*, *Salsola imbricata*, *Phragmites australis* and *Typha augustifolia* have been investigated by several other researchers (Parveen et al., 2023; Usman et al., 2020; Hira et al., 2018) Gibberellic acid and chelator application boosted the levels of phytoremediation indices, while sorghum and alfalfa were additionally found for the extraction and transportation of heavy metals to the upper regions of plants (Ejaz et al., 2022 and Nasir and Fazal, 2015).

According to this research, this can be inferred that *P. hysterothorus* is appropriate for phytoextraction, phytostabilization, and identification as metal hyperaccumulators due to its rapid development, biomass, bioaccumulation coefficient, bioconcentration factor, and translocation factor about cadmium, as well as its unappetizing nature to herbivores which may avoid entry from metal into the food web. Understanding the processes underlying absorption of metals and tolerance by plant requires more research.

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