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Bioaccumulation efficiency and biochemical responses of *Tradescantia spathacea* under lead, cadmium, chromium and copper exposure

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Abstract
The study was conducted on a tropical sculptural herbaceous perennial plant *Tradescantia spathacea* under the greenhouse at ambient temperature. This ornamental plant was subjected to different concentrations of heavy metal 40, 80 and 100 mg/kg, to evaluate biochemical and metal accumulation response of *Tradescantia spathacea* under enriched mixed treatments of a heavy metal solution containing metals *viz*. lead, cadmium, chromium and copper. The exposure time of heavy metals on plants was 30 days. Its biochemical analysis was performed and after harvesting its heavy metal analysis from shoot and root was performed, it was found that treatments were highly significant ($p < 0.05$) under pot trials. Exposure to heavy metals substantially reduced the chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, and protein at 100 mg/kg. The result showed an increment in the proline, phenol, catalase, peroxidase pertaining to 100 mg/kg. Shoot metal uptake potential of Pb (85.10 mg/kg), Cd (53.94 mg/kg), Cr (52.42 mg/kg) and Cu (59.74 mg/kg) and Root metal potential uptake of Pb (307.5 mg/kg) Cd (92.42 mg/kg), Cr (66.74 mg/kg) and Cu (77.59 mg/kg) was observed. The result of this study indicates that *Tradescantia spathacea* has a bioconcentration factor >1 for Pb, Cd, Cr and Cu and Translocation Factor <1. Hence it can be used for phytostabilization of Cr, Cd, Cu, and Pb. It could be used to remediate the heavy metal contaminated soil upto 100 mg/kg.

Keywords: heavy metal; *Tradescantia spathacea;* biochemical; phytostabilization; translocation factor; bioconcentration factor

Introduction

Globally, there are more than 5 million sites contaminated by different metalloids or heavy metals, covering at least 500 million ha of land, where concentrations are higher than monitoring levels (Liu *et al*., 2018) [1]. Metals occur naturally in the soil, their concentration increases owing to various anthropogenic activities, and exposure to heavy metals even causes harm to plants and animals. Activities like sewage, agriculture runoff, mining, fossil fuel combustion, and fertilizers are the foremost anthropogenic cause of surface soil pollution (Chibuike and Obiora, 2014) [2]. Due to tremendous growth in the industrial and agriculture sector, heavy metal contamination has become a global environmental matter, posing a consequential warning for food security and the environment (Sarwar *et al*., 2016) [3]. Most of the plants have the ability to concentrate both essential and non-essential heavy metals from the soil (Maharia *et al*., 2010) [4], but few of them can concentrate on specific parts favorably. The term "Hyper-accumulator plants" can be distinguished from the "non-hyper-accumulator" as they can accumulate extraordinarily high amounts (50-100 times than non-accumulator) of metals in the shoot without any visible phytotoxic effects (Rascio and Navari-Izzo, 2011) [5]. To remediate the contaminated soil several methods have been used such as physical, chemical and biological. Various methods such as encapsulation, solidification, stabilization, soil washing, vitrification, and electrokinetics consume a lot of economy and energy (Marques *et al*., 2009) [6]. Phytoremediation is a plant-based and sustainable alternative where plants are capable of uptake, sequestering and/or detoxifying heavy metals along with other pollutants naturally (Raskin, 1996) [7]. Phytoextraction uses the ability of plants to uptake pollutants from the soil by plant roots and facilitates translocation from roots to other harvestable parts (Bhargava *et al*., 2012) [8]. In phytostabilization process roots of the plant play a vital role by reducing the mobility of the contaminants by accumulating them in the roots or immobilizing them within the rhizosphere (Bolan *et al*., 2011) [9]. In Phytovolatilization heavy metals are absorbed by the plants and converted into the least toxic volatile forms and afterward released into the atmosphere (Kong and Glick, 2017) [10]. Phytotransformation or Phytodegradation is the breakdown of organic contaminants that occur within the rhizosphere of the plant and toxic substances are metabolized into the least toxic substances (Paz-Alberto and Sigua, 2013) [11]. Phytoremediation is a good strategy to remove metal toxicity from the contaminated environment and it's a time taking process to accomplish its function (Zhu *et al*., 2019) [12]. United Nations Environment Programme also stated that phytoremediation plants efficiently remove, detoxify, and immobilize environmental contaminants from the environment (UNEP, 2019) [13]. There are versatile mechanisms such as absorption of pollutants through roots, storage in plant tissues, or transforming and decomposing contaminants into low toxic levels. Worldwide Several techniques of phytoremediation are used (Yadav *et al*., 2018; Favas *et al*., 2018) [14, 15]. *Tradescantia spathacea* is also known as Rhoeo discolor and its common name is Moses of the cradle. Plant is short

stemmed tender foliage small in size, dense and spreading clumps. Its leaves are six to eight-inch long and sword-shaped and mainly green and purplish below. *Tradescantia spathacea* is also known to have antioxidant and chemoprotective antimutagen (Arriaga *et al*., 2011) [16] antimicrobial properties (Tan *et al*., 2015) [17].

Therefore, the present study was to explore the efficiency of *Tradescantia spathacea* for metal uptake and biochemical response under metal stress conditions. Secondly, to investigate its phytoremediation potential.

Methods and Material

Characterization of the experimental soil

The soil used in this experiment was collected from the Nursery of Anand Agriculture University, Anand, Gujarat. The soil was dried in the air, sieved, and stored for its physico-chemical and heavy metal analysis.

Physico-chemical analysis

Physico-chemical properties such as bulk density, pore space and, moisture content were analyzed using standard methods. The pH of the soil was measured by pH meter by dissolving 20 g of soil in 100 ml double-distilled water and then after homogenization on the mechanical shaker for about 1 hour, pH was checked using a pH meter and electrical conductivity by a conductivity meter (Maiti, 2003) [18]. The organic carbon and organic matter were determined by using the chromic acid wet oxidation method (Walkley and Black, 1934) [19]. The Potassium content from the soil was measured by using a flame photometer (Maiti, 2003) [18].

Experimental design

Tradescantia spathacea was grown in greenhouse at ambient temperature to assess their suitability for phytoremediation application. Plants were grown in the soil brought from the Nursery in 5 kg polyethylene pots for 60 days. Plants were given treatments of a heavy metal solution containing four heavy metals for 30 days. To make the desired quantity of metal solution, different quantity of salts was used and then applied to the plants. Pb, Cd, Cr, and Cu, lead sulfate, cadmium sulphate, potassium dichromate, copper sulphate salts of Merck (AR grade) were used. The soil was spiked with mixed heavy metal solutions of Pb, Cd, Cr and Cu at different concentrations of 40 mg/kg, 80 mg/kg, and, 100 mg/kg. The plant's pots were divided into four lots. Plants grown in garden soil served as control (only distilled water was added to the pot) and the other three pots were given a mixed metal supply.

After metal treatment, on the 15th day, biochemical parameters were analysed by using standard methods. Chlorophyll estimation by (Arnon, 1949) [20]. Carotenoids were determined by following (Duxbury and Yentsch, 1956) [21]. Protein estimation was performed by (Lowry *et al*., 1951) [22]. Proline measurement by (Bates *et al*., 1973) [23] method. Phenol measurement by (Bray and Thorpe, 1954) [24]. Catalase by (Kar and Mishra, 1976) [25]. Peroxidase by (Britton and Mehley, 1955) [26].

After treatment, soils from each pot were collected and dried at 70^{o} C in the oven for 48 hours. Dried samples were grinded into a fine powder and stored for further analysis. 1 mg of soil sample was taken in a 250 ml of a glass beaker and digested using 5 ml of Nitric acid and 3 ml of Hydrogen peroxide and diluted to 25 ml with milli-Q water. For elemental analysis (Pb, Cd, Cr and Cu), filtrate samples were analysed by an Inductively Coupled Plasma, Perkin Elmer Corporation.

Plant samples were harvested after 90 days of plant growth for heavy metal analysis. Plants from each pot were cut and cleaned, then samples were washed under tap water and rinsed using double-distilled water. Plants parts (root, leaves and stem) were separated and dried in the oven at 70^{o} for 48 hours and then grounded using mortar and pestle. To analyze the metal concentration (Pb, Cd, Cr, and Cu) of plant parts samples were digested with 5 ml of HNO_3 and 3 ml of H_2O_2 and then diluted to 25 ml using milli-Q water. Heavy metal testing was done by (ICP-OES), at SICART, Anand, Gujarat.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using MS Excel and a value of $p < 0.05$ was considered. Data were means of three replications.

Results

The physico-chemical properties of soil used for the present study is represented in (table 1). The parameters such as moisture content, pore space, and bulk density were found to be 32.92%, 52.45%, and 1.39 gm/cm^3 respectively. Whereas, pH of the soil was (6.9), electrical conductivity (230 µs/m), organic carbon (1.0%), organic matter (1.7%), and potassium was 160 mg/kg respectively. The metal contents of the soil were noted to be 0.003, 0.905, 0.105, and 0.038 mg/kg before treatment of Pb, Cd and Cr and Cu respectively (shown in figure 3).

Exposure to heavy metal has also affected the biochemical attributes of *Tradescantia spathacea* as shown in (figure 1). The total chlorophyll contents in *Tradescantia spathacea* grown under heavy metal treatment at different concentrations were decreased from 4.059 to 0.610 mg/g with an increase in the concentration of heavy metal. Among the three concentrations used, 100 mg/kg exposure showed a decrement in carotenoid content from 1.044 to 0.336 mg/g. The protein content of the *Tradescantia spathacea* also showed the same trend of decrement in its content. The content of phenol and proline was increased after metal treatment. It was observed that enzymatic activity of the plant was also increased along with increment in the treatment levels from 40 mg/kg to 100 mg/kg (depicted in figure 2).

In the present study maximum uptake of heavy metal was recorded for 100 mg/kg of treatment level. Total accumulation of Pb was 392.6 mg/kg, followed by 23.24 mg/kg in leaves, 61.86 mg/kg in stem and 307.5 mg/kg in roots. Overall accumulation of Cd was 146.4 mg/kg, its concentration in leaves, stems, and roots were 22.40, 31.54, 92.42 mg/kg respectively. Net accumulation of Cr was 119.2 mg/kg where, uptake in leaves was 19.32, in stem 33.10 and roots uptake was 66.74 mg/kg. The total accumulation of Cu was 137.3 mg/kg, 19.16, 40.58, and 77.59 mg/kg in leaves, stems and roots respectively (shown in fig.4, 5, 6).

Translocation factor was calculated for 40 mg/kg was Pb > Cu > Cd > Cr, for 80 mg/kg of treatment TF was Cu > Cr > Pb > Cd and for 100 mg/kg Cr > Cu > Cd > Pb shown in table 2. Bioconcentration factor when 40 mg/kg of treatment was given following pattern was observed Cu > Cr > Cd > Pb for 40 mg/kg, Pb > Cd > Cu > Cr for 80 mg/kg and Pb > Cd > Cr > Cu for 100 mg/kg of treatment shown in table 3.

Discussion

In the present investigation biochemical and phytoremedial potential of *Tradescantia spathacea* was estimated under the green house at ambient temperature to evaluate its metal tolerance and heavy metal accumulation efficiency. Zhang *et al*., (2017) [27] stated that heavy metal toxicity leads to the production of ROS, affects fundamental processes like respiration, photosynthesis, stomatal functional, cell disintegration, etc. and in the end, leads to the death of the plants. Hence, in this study reduction of Chl-a, Chl-b, and total Chl were observed. Carotenoid content was also reduced along with the increase in heavy metal treatment levels from 1.044 to 0.336 mg/g. According to Aggarwal *et al*., (2012) [28], a decrease in chlorophyll content in most of the plants has been observed due to metals like mercury, copper, chromium, cadmium, and zinc. Vital Indicators of photosynthetic efficiency are chlorophylls and carotenoids which under heavy metal stress act as ROS quenchers (Sidhu *et al*., 2017c) [29]. According to this study protein content decreased in comparison to control (1.606 to 0.337 mg/g) in leaf after mixed metal supply. Proteins are the prime targets of heavy metals. Proteins are vital components of the cell and they are primarily targeted by heavy metals depending on the bioavailability and environmental exposures. The thiol (-SH) group containing peptides and amino acids is primarily involved in the binding with heavy metals (Tamas *et al*., 2014) [30].

Here as per the study, the data indicated that phenol content increased from 0.523 to 1.040 mg/g with the increase in heavy metal concentration. Similarly, in the study conducted by Ullah *et al*., (2019) [31], phenolic content increased in the roots of the *Parthenium* plant followed by *Euphorbia, Cannabus, and Rumex*. A high accumulation of phenols was found in the leaves of both *Parthenium* and *Euphorbia* in comparison with *Cannabis* and *Rumex* plants. Michalak, (2006) [32] phenolic compounds can act as metal chelators during heavy metal stress and on the other hand, phenolics can directly scavenge reactive oxygen species. Our findings are relatable with the results of different investigators who stated that heavy metal (Cd, Pb, Zn, and Cu) stress induces a high accumulation of proline (Roy and Bera, 2002) [33]. Stress caused by the heavy metal leads to proline accumulation in plants and prevents oxidative injury (Hare and Cress, 1997) [34]. The present study depicts the increase in proline content from 0.789 to 1.919 µg/ml.

The defense mechanism of plants consists of enzymatic components against ROS including dismutase, peroxidase, catalase, and metabolites like glutathione, ascorbic acid, carotenoids, a-tocopherol (Sairam *et al*., 2000) [35]. Increased enzymatic activity causes modulation of gene expression or certain enzymatic inhibitors are blocked (Seregin and Ivanov, 2001) [36]. Catalase and peroxidase concentration in the present investigation were increased along with the increase in the treatment levels. Jesitha and Harikumar, (2018) [37] conducted a study on *Tradescantia spathacea* and exhibited that it can accumulate 47% lead, 45.3% cadmium and, nano-phytoremediation treatment can accumulate 84.4% lead and 64.8% Cadmium. According to Enot and Mahinay, (2017) [38] *Tradescantia spathacea* Sw. accumulated lead 215.6 mg/g in roots then accumulation in the stem was 93.89 mg/g and 6.096 mg/g was translocated to the leaves. This study supports our current findings where heavy metal accumulation in roots was more than in the shoot and *Tradescantia spathacea* accumulated a higher amount of Pb (307.5 mg/kg) and Cd (92.42 mg/kg) in their roots. In shoot maximum uptake of Pb (85.10 mg/kg) and Cu (59.74 mg/kg) was observed. Casparian strips obstruct the metal transportation from the root to other parts of the plant and act as barriers found in the root's endodermis (Sharma and Dubey, 2005) [39]. Metal cations are less mobile in plants than the other nutrients, mostly after absorption they tend to accumulate in the tissues of the root (Marschner, 2011) [40]. Roots initially come into contact with metals first and the exposure time is a main concerning factor for final concentration. Root exudates typically include metabolites such as amino acids, phenolics, carboxylic acids, organic acids, sugars, etc., they speed up the heavy metal uptake by the root's hairs (Walker *et al*., 2003) [41]. The translocation factor is also called as shoot-root quotient, it depicts the plant's ability to translocate heavy metal from root to shoot. If the value of TF is less than 1 plant is considered for phytostabilization (Hou *et al*., 2020) [42]. Restricting translocation of metal in plants species from the root to shoot parts can be categorized as beneficial phytostabilizers (Khan *et al*., 2013) [43]. It supports the present finding where the TF value was found to be less than one and the BCF value was greater than 1 in *Tradescantia spathacea*.

Conclusion

In the present investigation, the response of *Tradescantia spathacea* grown under greenhouse at ambient temperature showed maximum BCF of Pb, Cd, Cr and Cu. Overall findings concluded that the plant accumulated a higher amount of Pb (392.6 mg/kg) and Cd (146.4 mg/kg) in the root parts. The findings are also helpful to study plant metal interaction and biochemical behavior. In India, this type of cost-effective technique is needed to remediate soil contaminated with heavy metals. Further research is needed to study the antioxidant behavior, anatomical changes and, role of chelating agents to enhance the capability of metal transport from root to shoot. This study could be useful as precursor baseline data for trace elements for future monitoring and assessment.

Acknowledgment

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

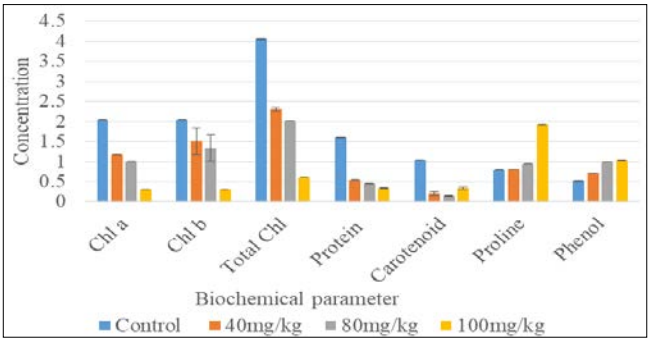

Fig 1: Effect of mixed metal exposure on biochemical parameter of *Tradescantia spathacea*: Photosynthetic pigment (mg/g), Carotenoid (mg/g), Protein (mg/g), Phenol (mg/g) and Proline (µg/ml)

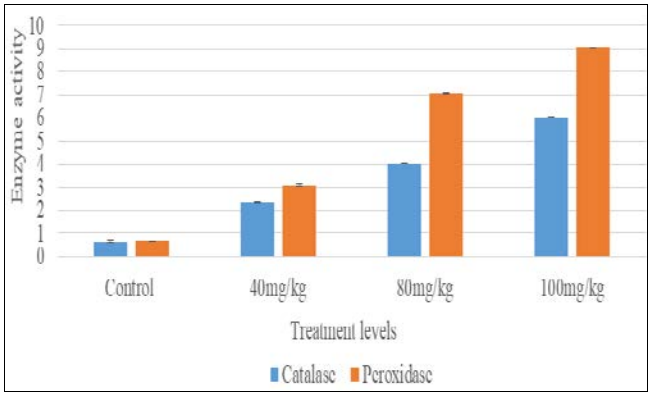

Fig 2: Effect of mixed metal exposure on enzymatic activity of *Tradescantia spathacea:* Catalase (µM H_2O_2 decomposed min^{-1} g^{-1} fresh leaf) and Peroxidase (µM purpurogallin formed min^{-1} g^{-1} fresh leaf). Values are mean ±SD (n=3). (p≤ 0.05).

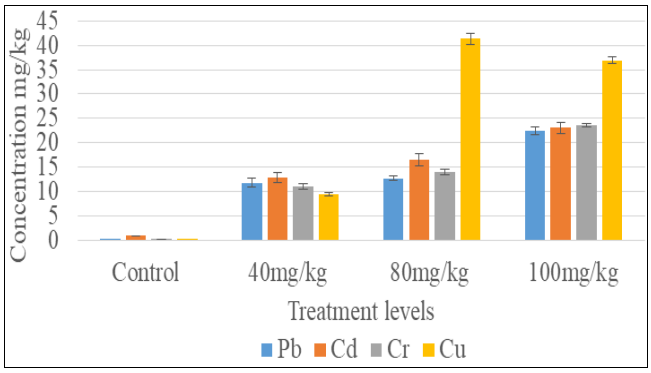

Fig 3: Heavy metal concentration in soil before and after the experiment±SD (n=3). (p≤ 0.05).

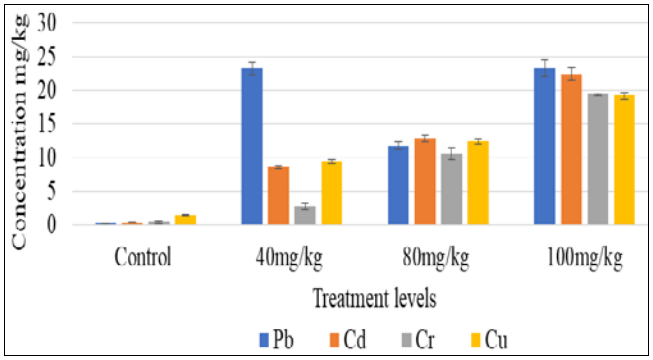

Fig 4: Heavy metal accumulation in the leaves of *Tradescantia spathacea*. Values are mean±SD (n=3). (p≤ 0.05).

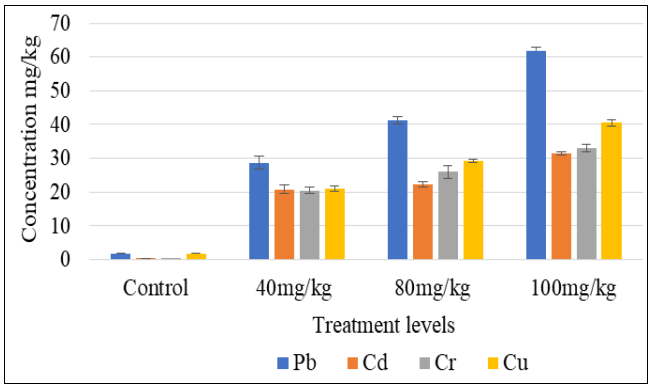

Fig 5: Heavy metal accumulation in the stem of *Tradescantia spathacea*. Values are mean±SD (n=3). (p≤ 0.05).

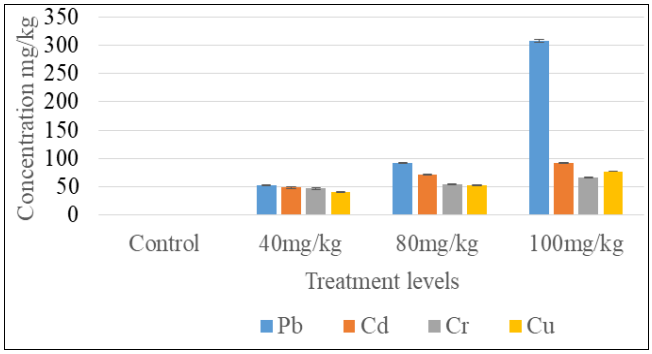

Fig 6: Heavy metal accumulation in the roots of *Tradescantia spathacea*. Values are mean±SD (n=3). (p≤ 0.05).

Table 1: Physico-chemical characterization of the experimental soil

Parameter	Unit	Result
Physical		
Bulk density	gm/cm^3	1.39
Moisture content	%	32.92
Pore space	%	52.45
Chemical		
pH	-	6.9
Electrical conductivity	µs/m	230
Organic carbon	%	1.0
Organic matter	%	1.7
Potassium	mg/kg	160

Values are mean-SD (n=3)

Table 2: Translocation factor of *Tradescantia spathacea*

Heavy metal	40 mg/kg	80 mg/kg	100 mg/kg
Pb	0.982	0.578	0.277
Cd	0.605	0.485	0.584
Cr	0.489	0.673	0.785
Cu	0.748	0.801	0.770

Table 3: Bioconcentration factor of *Tradescantia spathacea*

Heavy metal	40 mg/kg	80 mg/kg	100 mg/kg
Pb	6.065	6.502	6.343
Cd	6.401	6.461	5.036
Cr	7.573	2.274	3.719
Cu	7.939	3.896	3.404

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