

## Evaluation of *Atriplex lindleyi* Moq. for morpho-physiological and metal accumulation responses under Cd, Ni, and Zn stress

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### Abstract

Several halophytic *Atriplex* species have been explored in the past for their possible use in reclamation of salt-affected soils. The aim of this research is to evaluate the phytoremedial potential of *Atriplex lindleyi* for Cd, Ni, and Zn along with its defense techniques and physio-morphological changes as it can interact, sequester and detoxify heavy metals. The results showed greater accumulation of Ni and Zn in plant tissues as compared to Cd. Defense responses through photosynthetic pigments and proline were also found to be varied in heavy metal treated plants. It was observed that most of the heavy metals absorbed by the plant were retained in the root i.e., 4.97, 29.31, and 597.2 mg kg<sup>-1</sup> in comparison to the metal transported to above-ground parts of the plant i.e., 2.92, 23.25, and 182.74 mg kg<sup>-1</sup> for Cd, Ni and Zn, respectively establishing this plant as a phytostabilizer. In conclusion, *Atriplex lindleyi* can be recommended as a potential candidate for the phytoremediation of Ni and Zn.

**Keywords:** *Atriplex lindleyi*, saltbush, phytoremediation, heavy metals

### Introduction

Heavy metal contamination of soil and wastewater is a significant environmental problem that has a detrimental effect on human health and agriculture and is harmful or toxic even at low levels (Lenntech Water Treatment and Air Purification, 2004) <sup>[1]</sup>. Several heavy metals such as Cd, Zn, Cu, Pb, Ni, Cr, As are common pollutants present in wastewater which can dispersed in the environment for a long time and become a serious threat to different levels of food chains (Singh *et al.*, 2010) <sup>[2]</sup>. There are multiple sources of heavy metals in the environment, such as natural sources, agricultural sources, domestic effluents, and industrial sources. However, in recent decades anthropogenic activities have been increasingly associated with the accumulation of heavy metals in the environment. Industrial activities such as smelting, mining, dumping of municipal sewage sludge, burning of fossil fuels, and agricultural practices related to long-term use of excessive pesticides, fungicides, and fertilizers have led to a dramatic increase in heavy metal pollution in different areas (Ernst and Nelissen, 2000; D'Amore *et al.*, 2005) <sup>[3,4]</sup>.

Heavy metals can also minimize the photosynthetic pigments, disturb the pigment-protein apparatus, or obstruct the light-driven photosynthetic electron transport within the chloroplast (Stepien and Klobus, 2006) <sup>[5]</sup>. In coping with the heavy metal stress, root tissue is the first to be exposed to the associated toxins, and its cell wall has a mechanism of exchange that fixes the metal ions, thereby limiting the transfer of toxins to other plant tissues (Branquinho *et al.*, 1997) <sup>[6]</sup>. To alleviate the heavy metals toxicities, chemical application and agronomical crop management practices have been used in past with a little bit of success. In related activities, the exogenous application of osmolytes such as

proline has emerged as an alternative technique to induce plant potential to effectively overcome the harmful situation of heavy metals toxicity.

Several species belonging to the genus *Atriplex* are well adapted to harsh environmental conditions and is characterized by its great diversity with more than 400 species (Le Houérou, 1992) <sup>[7]</sup>. In the present study, experiments were carried out on the species *Atriplex lindleyi* because of its excellent drought and salinity tolerance and the well-developed growth profile. It is commonly known as "saltbush," which belongs to the subfamily Chenopodioideae of the family Amaranthaceae. *A. lindleyi* is a halophyte and has been suggested to be ideally suited for environmental susceptibility, particularly heavy metal stress. *Atriplex* is of specific importance in this context as these species are found naturally in an area characterized by an abundance of toxic ions, primarily Na<sup>+</sup> and Cl<sup>-</sup> which favors the species survival in disturbed habitats. These species produce a high amount of oxalic acid, which may have a beneficial effect on resistant mechanisms towards toxic metals (Sayer and Gadd, 2001) <sup>[8]</sup>.

The present investigation was carried out to evaluate the phytoremedial potential of *A. lindleyi* for uptake of heavy metals (Cd, Ni, and Zn) and to study the changes in the morphological (such as root length, shoot length, biomass) and biochemical parameters (chlorophyll, proline) of the plant under HM stress.

### Materials and Methods

Procurement of certified seeds of *Atriplex lindleyi* was done from the United States Department of Agriculture – Agriculture Research Service (USDA-ARS), Washington State University, USA and Arid Forest Research Institute

(AFRI), Jodhpur.  $\text{HgCl}_2$  (0.1%) was used for the sterilization of seeds and then seeds were kept in Petri plates containing distilled water for at least 3 h. After this seeds were placed away from the sunlight till the start of germination. Collection of experimental soil was done from the botanical garden of Department of Botany, St. John's College, Agra (27.18°N 78.02°E), India, and a greenhouse experiment was designed. General properties of soil were first analysed (sandy loam-sand 65-80%, silt 15-25%, clay 7-14% with pH  $7.2 \pm 0.51$ , Electrical conductivity (EC)  $1.07 \pm 0.26$  dS/m, Moisture content  $7.92 \pm 0.45$  % and Organic carbon  $1.15 \pm 0.2$  %). Ion content of the soil was  $725 \pm 4$ ,  $4.2 \pm 0.3$ , and  $22 \pm 5$  meq  $\text{l}^{-1}$  for sodium (Na), calcium-Magnesium (Ca+Mg), and potassium (K) respectively. The soil was allowed to air-dry after the process of autoclave. Each pot was filled with 4 kg soil and then mixed with their respective treatments. Cd, Ni, and Zn were applied to the pots in the form of an aqueous solution of  $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ ,  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ , and  $\text{ZnCl}_2 \cdot 3\text{H}_2\text{O}$  salts, respectively. Apart from treatment pots, control pots were also maintained without any metal treatment. After this one seedling per pot was transplanted and was observed for various growth and physicochemical parameters (root length, shoot length, biomass, chlorophyll, and proline content) throughout the experiment. Any signs of metal toxicity exhibited by plants were visually observed during the entire experimental period. Plant samples were taken for metal analysis 90 days after treatment. The treatments selected were as follows: 1. Control (0 ppm HM), 2.  $\text{Cd}_{25}$  ppm, 3.  $\text{Cd}_{50}$  ppm, 4.  $\text{Cd}_{150}$  ppm, 5.  $\text{Ni}_{50}$  ppm, 6.  $\text{Ni}_{100}$  ppm, 7.  $\text{Ni}_{150}$  ppm, 8.  $\text{Zn}_{200}$  ppm, 9.  $\text{Zn}_{400}$  ppm, and 10.  $\text{Zn}_{600}$  ppm

## Biochemical Analysis

### Chlorophyll

Chlorophyll extraction was done in 10 mL acetone (80 % v/v) according to the method given by Lichtenthaler (1987)<sup>[9]</sup>. Leaf (0.5g) and acetone mixture was kept for incubation in dark for at least 12 h followed by centrifugation at 4000 rpm for 3-5 minutes or till it becomes fully clear. The optical density of the supernatant was measured at 663, 645 nm. The chlorophyll concentration of the sample was expressed in mg/g FW (fresh weight).

## Proline

Proline content of the leaves was measured as a stress marker as per the method given by Bates *et al.*, (1973)<sup>[10]</sup>. Fresh leaves (500 mg) were grounded in 3% sulphosalicylic acid using a mortar and pestle. The supernatant of the homogenized mixture was separated by centrifugation at 2000 rpm for 15-20 min. Ninhydrin (2 ml) and glacial acetic acid (2 ml) were added to the supernatant (2 ml) and allowed to boil in a water bath for 30 min. The reaction was stopped by cooling down the temperature of the solution in an ice bath. At last 4 ml, toluene was added followed by thorough shaking of the tubes. Absorbance (520 nm) of the upper layer was measured using toluene as control. Proline content was expressed in  $\mu\text{mol g}^{-1}$  FW.

## Heavy Metal analysis

Wet digestion of soil and plant material was carried out for heavy metal analysis.  $\text{HNO}_3$  (69%, Merck) and HCl (30%, Merck) were used for soil, and  $\text{HNO}_3$  (69%, Merck) and  $\text{H}_2\text{O}_2$  (30%, m/v; Merck) were used for plant material. Sample analysis for each metal (mg/kg) was done by flame atomic absorption spectrophotometer.

## Heavy metal accumulation characteristics of plants

Phytoremediation capability of plants is additionally influenced by the mobility and availability of contaminants in soil and plants. In order to examine the phytoaccumulation capacity or the potential of plants, some factors have been employed based on simple ratios of contaminant concentration in plant parts and growth matrix. These were calculated as follows:

1. Bioabsorption Coefficient [BAC] =  $\frac{\text{metal concentration in shoot}}{\text{metal concentration in soil}}$
2. Bioconcentration Factor [BCF] =  $\frac{\text{metal concentration in root}}{\text{metal concentration in soil}}$
3. Translocation Factor [TF] =  $\frac{\text{metal concentration in shoot}}{\text{metal concentration in root}}$

## Results

### Metal uptake in *Atriplex lindleyi*

**Table 1:** Metal uptake ( $\text{mg kg}^{-1}$ ), root length, shoot length and metal accumulation characteristics in *A. lindleyi* at final day of study.

Treatments	Root ( $\text{mg kg}^{-1}$ )	Shoot ( $\text{mg kg}^{-1}$ )	Root length (cm)	Shoot length (cm)	BAC	BCF	TF
Control	N.D.	N.D.	$17.8 \pm 0.44$	$46.6 \pm 0.69$	N.D.	N.D.	N.D.
$\text{Cd}_{25}$ ppm	$4.83 \pm 0.41$	$2.14 \pm 0.39$	$12.6 \pm 0.51$	$38.1 \pm 1.03$	0.09	0.20	0.44
$\text{Cd}_{50}$ ppm	$4.97 \pm 0.36$	$2.92 \pm 0.24$	$10.5 \pm 0.70$	$34.3 \pm 0.48$	0.06	0.10	0.59
$\text{Cd}_{100}$ ppm	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$\text{Ni}_{50}$ ppm	$21.37 \pm 0.28$	$15.79 \pm 0.38$	$13.9 \pm 0.46$	$42.6 \pm 0.68$	0.32	0.44	0.74
$\text{Ni}_{100}$ ppm	$25.33 \pm 0.49$	$19.33 \pm 0.29$	$12.2 \pm 0.58$	$39.3 \pm 0.87$	0.20	0.26	0.76
$\text{Ni}_{150}$ ppm	$29.31 \pm 0.23$	$23.25 \pm 0.23$	$11.2 \pm 0.35$	$35.2 \pm 1.0$	0.16	0.20	0.79
$\text{Zn}_{200}$ ppm	$159.43 \pm 0.51$	$41.52 \pm 0.51$	$16.3 \pm 0.69$	$45.8 \pm 0.53$	0.21	0.81	0.26
$\text{Zn}_{400}$ ppm	$413.94 \pm 0.57$	$115.46 \pm 0.35$	$12.5 \pm 0.51$	$39.1 \pm 1.1$	0.29	1.04	0.28
$\text{Zn}_{600}$ ppm	$597.21 \pm 0.31$	$182.74 \pm 0.24$	$11.4 \pm 0.62$	$36.5 \pm 0.62$	0.31	1.00	0.31

BAC= Bioabsorption Coefficient; BCF= Bioconcentration Factor; TF= Translocation Factor

Treatments with metals (Cd, Ni, and Zn) significantly affected the plant growth parameters like root length, shoot length, and biomass in positive correlation with metal concentration (Table 1). Plants treated with Cd experienced more toxicity (low shoot and root length) as compared to higher doses of Ni and Zn. In the case of Cd, higher uptake

values of  $4.97 \text{ mg kg}^{-1}$  (roots) and  $2.92 \text{ mg kg}^{-1}$  (shoots) were recorded at  $\text{Cd}_{50}$  ppm. It was not able to tolerate the higher concentrations of  $\text{Cd}_{100}$  ppm in the growing medium, showing toxicity symptoms such as chlorosis, necrosis, drooping, and eventually death.

A gradual increase in Ni accumulation in both roots and shoots with the increase in Ni concentration was observed. The Ni uptake trend in the treatments was observed in the following order  $Ni_{150\text{ ppm}} > Ni_{100\text{ ppm}} > Ni_{50\text{ ppm}}$ . The data shows that at low concentration, i.e.,  $Ni_{50\text{ ppm}}$ , the minimum uptake of  $21.37\text{ mg kg}^{-1}$  (roots) and  $15.79\text{ mg kg}^{-1}$  (shoots) reached to  $29.31\text{ mg kg}^{-1}$  (roots) and  $23.25\text{ mg kg}^{-1}$  (shoots) at the higher dose of  $Ni_{150\text{ ppm}}$ .

Similarly, on the final day of study, i.e., 90 DAT, the accumulation of Zn in roots and shoots exhibited the trend  $Zn_{600\text{ ppm}} > Zn_{400\text{ ppm}} > Zn_{200\text{ ppm}}$ . In treatment  $Zn_{200\text{ ppm}}$ , maximum uptake of Zn was  $159.43\text{ mg kg}^{-1}$  (in roots) and  $41.52\text{ mg kg}^{-1}$  (in shoots) which went up to  $597.2\text{ mg kg}^{-1}$  (in roots) and  $182.74\text{ mg kg}^{-1}$  (in shoots) at the higher dose of  $Zn_{600\text{ ppm}}$ . Accumulation of Zn in *Atriplex lindleyi* was almost two times higher in root as compared to the shoot across all treatments. Further, no sign of toxicity symptoms were observed in Zn treated plants.

Translocation of metal from root to shoot increased with increasing metal concentration and exposure period (Table 1). TF is low for *A. lindleyi* in all the Cd treatments as the roots of the plants showed highest heavy metal accumulation as compared to the aerial parts of the plant while for Ni and Zn there was greater metal translocation as TF values were higher than their respective BCF values. Among all the tested metals, treatments of Ni i.e.  $Ni_{50}$  (0.74),  $Ni_{100}$  (0.80), and  $Ni_{150\text{ ppm}}$  (0.79) showed highest TF values.

#### Effect of heavy metals on root-shoot length, and biomass

A significant difference in growth was recorded among all the treatments. Cd notably affected the growth of the plants and showed more profound effects as compared to Ni and Zn which indicates the specific adaptability and preference of the plant towards heavy metals. Plants treated with  $Cd_{100\text{ ppm}}$  showed a maximum reduction in morphological characters (Fig 1). Though all the metals affected the growth of the plants, Zn was the least affecting metal followed by Ni. No significant difference was found in root and shoot length for Ni and Zn treated plants as both the metals showed almost similar impact on root and shoot length (Table 1). Growth parameters were found to decrease with increasing concentration of all the tested metals.

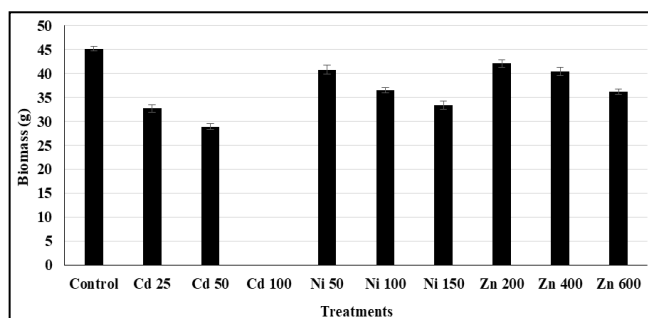


Fig 1: Total biomass of *A. lindleyi* at final day of study.

#### Effect of heavy metals on chlorophyll content

Contrary to other growth parameters, chlorophyll content of the plants did not show a reduction in any of the treatments except for Cd treated plants (Fig 2). In *A. lindleyi*, patches of chlorosis followed by necrosis were observed before the plant's death for  $Cd_{100\text{ ppm}}$ . Plants treated with  $Ni_{50\text{ ppm}}$  and  $Ni_{100\text{ ppm}}$  showed maximum increase in chlorophyll even in

the presence of heavy metals as compared to control ( $3.98$  and  $3.89\text{ mg FW}^{-1}$ , respectively). It was observed that in the case of Ni and Zn treated plants after an initial shock, plants grew even better as compared to the control plants and chlorophyll contents were near to normal when estimated after 60 days showing that plants had acclimatized by that time.

#### Effect of heavy metals on Proline content

Proline content of the leaves was found to increase with increasing concentrations of heavy metals. Plants treated with Cd and Ni showed maximum proline accumulation while plants treated with Zn showed least difference in proline as compared to control. For Zn treatments, higher values were obtained till 30 DAT and after that plant started to stabilize as a significant decline in proline concentration was observed till the end of the experiment (Fig 3). After 90 DAT, the values in Zn treatments were more similar to control. While for  $Cd_{100\text{ ppm}}$  *A. lindleyi* did not survive after few days the heavy metal was added to the rhizosphere, indicating the vulnerability of the species to Cd contamination.

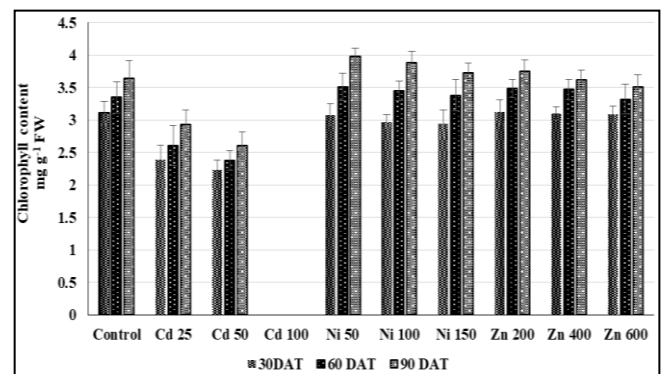


Fig 2: Chlorophyll content in *A. lindleyi* at successive days of study.

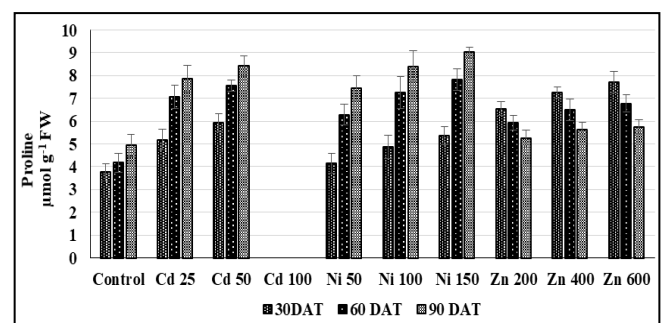


Fig 3: Proline content in *A. lindleyi* at successive days of study.

#### Discussion

In the present investigation, the phytoremediation potential of *A. lindleyi* was evaluated using greenhouse pot experiments. Pot experiments are frequently used for direct identification concerning their metal tolerance and ability to accumulate them. The overall uptake and accumulation trend of different metals in *A. lindleyi* was found in the following order  $Zn > Ni > Cd$ . Maximum values for Zn and Ni accumulation were  $597.2\text{ mg kg}^{-1}$  and  $29.31\text{ mg kg}^{-1}$  respectively while the highest uptake for Cd was  $4.97\text{ mg kg}^{-1}$  (roots). It was observed that increase in the metal concentration increases the metal uptake and translocation except for  $Cd_{100\text{ ppm}}$  where the plant died due to Cd toxicity.

*A. lindleyi* exhibited the lowest values for root length, shoot length, and biomass under Cd stress, whereas under Ni and Zn treated soils, plants showed better growth even at high concentration of heavy metals because of its high tolerance. Similar results were obtained by Lutts *et al.*, (2004) [11] they showed that *Atriplex halimus* is the potential candidate for the removal of Cd and Zn from a metal-contaminated site. The uptake of metals by the plants depends mostly on the bioavailable form of the element in the soil. Among all the heavy metals studied, Ni was the most mobile heavy metal in terms of translocation within plant tissues followed by Zn and Cd. Neil, (2007) [12] also reported, Zn as the most phytoavailable metal as compared to the less mobile metals like Cd, Cu, Pb, Cr, and Ni which are found in smaller quantities in non-accumulator plants.

Further, *A. lindleyi* showed a greater accumulation of Cd, Ni, and Zn in the roots than the above-ground shoot, this could be attributed to the increased metal adsorption on the root surface, being facilitated by relatively low mobility of metals in the root zone (Hasan *et al.*, 2007) [13]. The results revealed that the metal accumulation was metal-specific, which may be due to several factors, including environmental conditions, metal species, and their available forms.

Similarly, Truong and Baker, (1998) [14] reported that the levels of Pb up to 800 mg kg<sup>-1</sup> did not affect the growth of *Vetiver*. Besides, they also reported the tolerance of *Vetiver* grass to other heavy metals such as Cd (toxic level 20–60 mg kg<sup>-1</sup>), Cu (50–100 mg kg<sup>-1</sup>), and Cr (50 mg kg<sup>-1</sup>). *A. lindleyi* was found to tolerate nickel and zinc concentrations up to 150 and 600 ppm thus confirming its ability to grow well in Ni/Zn polluted soil. These results demonstrated that the growth and performance of the test plant was not affected by exposure to these metals in the rhizosphere (except for Cd).

Among the tested metals, the least accumulation was shown in the case of Cd, which showed lack of specificity of *A. lindleyi* for Cd, a common phenomenon in non-accumulators. A significant decrease in the root length, shoot length, and biomass was also noted with the increase in metal concentrations (at the initial days of study, i.e., 30 DAT), but as the study progressed the growth of plants in higher concentrations of metals becomes comparable to the control at 90 DAT showing the adaptability of the plants in the metal-contaminated soil environment. However, *A. lindleyi* showed the symptoms of Cd toxicity such as, reduction in the biomass, reduced leaf area, wilting, chlorosis, and necrotic lesions at higher concentrations (Cd<sub>100 ppm</sub>) which are in concurrence with many investigators (Barton *et al.*, 2000) [15]. *A. lindleyi* was found to survive only at the low concentration, i.e., up to Cd<sub>50 ppm</sub>. These physiological disturbances might have primarily influenced the phytoextraction/phytostabilisation efficiency of this plant.

The rise in heavy metal concentration causes a decrease in photosynthesis and inhibits chlorophyll synthesis; both of these observed changes may be taken as biomarkers of heavy metal toxicity on the plant. In *A. lindleyi*, the total chlorophyll level was seen to decrease till 90 DAT with the increasing metal concentration. Ni was found to be the least toxic. *A. lindleyi* exhibited no disturbance in chlorophyll content when treated with Ni and Zn. However, showed low photosynthetic pigment content at higher Cd concentrations. *A. lindleyi* was most impervious to Cd toxicity, survived

only at low concentrations (Cd<sub>25</sub> and Cd<sub>50 ppm</sub>), and then succumbed to higher doses. Wide bands of chlorosis followed by necrosis were observed in treatment (Cd<sub>100 ppm</sub>), before the plant's death.

The presence of heavy metals mostly harms plant health. Heavy metals have been shown to interfere and inhibit various biological processes of the plant such as plant-water interactions, chlorophyll biosynthesis, transpiration rates, enzymatic activity, nutrient uptake, root elongation, plant biomass, and growth (Cheng, 2003) [16]. The decrease in photosynthesis may be due to the inhibition of chlorophyll synthesis because of the blocking of Mg, Mn, and Fe ions (Gopal and Rizvi, 2008) [17], or by the destruction of chloroplasts (Sharma and Dubey, 2005) [18].

Accumulation of proline in response to different heavy metal treatments was seen to vary among test plants. Plants have been shown to accumulate proline under environmental stress (Ahmad and Jhon, 2005; Ahmad *et al.*, 2008) [19, 20]. A high level of proline content can be considered beneficial to stressed plants (Hyun *et al.*, 2003) [21]. In the present study, highest proline content was found in the case of Cd followed by Ni and Zn which indicated that Zn was the least toxic metal followed by Ni and Cd. It is to be noted that at low metal doses, the accumulation of proline in the plant was also low, showing a positive relation in the amount of proline accumulation and metal concentration applied. Thus, the data indicated that increase in proline content is related to a rise in metal stress. Increased proline level may be either attributed to *de novo* synthesis or decreased degradation (Kasai *et al.*, 1998) [22]. The significant correlation between enhanced tolerance and proline accumulation in plants under heavy metal stress has been reported by Ashraf and Foolad (2007) [23]. Proline accumulation is an adaptation strategy to various abiotic stresses, and it plays a significant role in the detoxification of reactive oxygen species, stabilization of proteins, and protein complexes (Slama *et al.*, 2015) [24].

The biomass of the test plant in control (45.2 g) was significantly higher than the metal treatment. It indicates that a high level of metal in the soil inhibited the growth of the test plant species. Cd was found to be responsible for a major reduction in biomass as compared to Ni and Zn. More than 36% reduction in the biomass of *A. lindleyi* was observed due to Cd contamination (28.9 g). Whereas, *A. lindleyi* exhibits moderate Ni uptake and accumulation in roots and shoots. Zn at low concentrations (Zn<sub>200 ppm</sub>) did not affect the growth and biomass in the test plant. As Zn is required in small amounts by plants as a micronutrient, it did not affect growth too much; although, at higher concentrations, it appears to be toxic. The order in which the metals affected the growth and biomass of the test plants is Cd > Ni > Zn. Phetsombat *et al.*, (2006) [25] reported a significant reduction in the relative growth and biomass of *Salvinia cullata* supplemented with Cd and Pb, with an increase in concentration and exposure time.

## Conclusion

*Atriplex* species have excellent tolerance to HM, salinity, and drought. These plants could be used to remediate contaminated soils with heavy metals. This study indicated that *Atriplex lindleyi* plants can accumulate metals. Significant differences in heavy metal (Cd, Ni, and Zn) concentration and their transport from the roots to shoots were found in *Atriplex lindleyi* exhibiting its potentiality as

a suitable candidate. More studies are needed in this field exploring other amendments for metal uptake enhancement, especially in the Indian context. The results of this greenhouse pot experiment showed that *A. lindleyi* can be satisfactorily grown in heavy metal contaminated soil though some growth parameters were slightly reduced in metal contaminated soil as compared with those grown in control soil but the low levels of toxic metals in the above-ground tissues indicate a promising sign for the profitable cultivation of *A. lindleyi* in heavy metal contaminated sites as a suitable feed for cattles.

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