



Role of Phytoremediation in Reducing Cadmium and Nickle Toxicity in Soil Using Species (*Cynodon dactylon* L.)



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ABSTRACT

Background: Using plants for removing heavy metals from contaminated soils is an economical and effective strategy. Phytoremediation is one of the suggested methods that plants absorb and accumulated heavy elements in plants. The purpose of this study was to investigate the feasibility of Cadmium (Cd) phytoremediation from soil using Bermuda grass.

Methods: This experiment was conducted in a completely randomized design with four levels of (Cadmium nitrate) Cd (NO₃)₂ (0, 20, 50, 60 mg/ kg soil) and Nickle solution (NiCl₂×6 H₂O) (0, 150, 300,450 mg/ kg soil) with three replications in the Research Institute of Petroleum Industry.

Results: The obtained results of the analysis of variance showed that metal significantly affected traits such as shoot and root dry weight, plant height, stomatal resistance, chlorophyll and antioxidant enzymes. It was also observed that Cd accumulation in plant root increased with increasing cadmium concentration. Furthur, Ni accumulation in the root tissues was higher in all the treatments than aerial parts.

Conclusion: According to the findings, seedlings of *Cynodon dactylon* can be suggested for Ni and Cd remediation in polluted soils.

1. Introduction

Soil contamination with heavy metals is one of the major environmental problems which has harmful effects on plant

communities and underground water pollution through leaching. It reduces yield and crop quality and ultimately hazards to human's health. These products are very susceptible to contamination by microorganisms, which may



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quickly multiply in fish. Although heavy metals can naturally accumulate in the soil through forming minerals and rocks via the processes of weathering and natural erosion but this natural resource is not comparable to pollution caused by human activities such as the industrial manufacturers, burning fossil fuels, chemical and organic fertilizers, industrial wastewater, and sewage sludge. Metals such as Arsenic (As), Lead (Pb), Cadmium (Cd), Nickel (Ni), Mercury (Hg), Chromium (Cr), Cobalt (Co), Zinc (Zn), and Selenium (Se) are highly toxic even in minor quantity [1]. Among the heavy metals, long biological half-life of Cd which is up to 30 years, can result in a cumulative increase in the body level through aging, which is ultimately retained in the human kidney, liver, and bones. Ni as a micronutrient in low concentrations is essential for plant growth as well as the activity of some enzymes. However, this element is toxic to plants in high concentrations. Ni toxicity has been reported in various plants such as sorghum, rice, and wheat. In general, the critical toxicity level of Ni for sensitive, moderate sensitive, and resistant plants was suggested more than 10 mg/kg, 50 mg/kg, and 1000 mg/kg dry matter, respectively. Ni, which is usually absorbed only through food, may cause adverse reactions in people who are severely allergic [2]. It was also found that Cd, Ni, and Cr have higher cancer risks than other metals and suspended open soil, industrial activities, and industrial fuel and combustion are the main sources of these metals respectively [3]. Therefore, refining contaminated soils with heavy metals such as Cd and Ni is a necessary pathway.

Soil contamination with heavy metals can be cleaned by various chemical, physical and biological methods; however, developed physical and chemical heavy metal remediation technologies are demanding costs which are impractical and time-consuming. Moreover, they release additional waste to the environment. Phytoremediation technology is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and/or destroy contaminants in the soil and groundwater [4]. Among the researches on the phytoremediation of some species, the results of the study by Maani *et al.* (2019) regarding the heavy metals absorption such as Cd by two species of *J. polycarpus* and *C. aronia* seedlings, is recommended to develop their cultivation level in Cd contaminated areas for phytoremediation [5]. Evaluation of toxic metal was carried out in Tehran city to investigate the density of Pb in urban polluted soils with tree species including *Pinus Eldarica*, *Cupressus arizonica*, *Robinia pseudoacacia* L., *Fraxinus rotundifolia* Mill., and *Ulmus carpinifolia*. The result of the study indicated that Pine, Cypress, and Locust tree species had the highest value of lead concentration and translocation factor [6]. Based on the results of a study by Saba *et al.* (2014) *Populus nigra* was found to be the best accumulator plant for Mn, Zn, and Cd [7]. Species and subspecies of the plant have different abilities and adaptations in the face of environmental stresses such as

heavy metals [8]. Grass species in a semi-arid region can be an important alternative for the bioremediation process. Using plants with high biomass, is able to accumulate pollutants in their tissues. Considering Iran's rich flora and the native reserve plants, endemic species resistant to environmental stresses can play a fundamental role in creating sustainable agriculture and maintaining valuable germplasm. Among warm season grass, *Cynodon dactylon* L. is the most important forage in the genus *Cynodon* in Iran. This plant reproduces sexually by producing seeds and asexually by rhizomes. These types with deep root systems can cope with the poor structure of several polluted substrates [9]. Furthermore, vegetative turfgrasses are established from plugs or sprigs (stolon and rhizome stem tissue) instead of seed and thus the stolon and rhizome are very important for turfgrass growth. Bermuda grass (*Cynodon dactylon*) was selected because they have an extensive rooting system and often are used for soil stabilization [10]. Sainger *et al.* (2011) studied the heavy metal tolerance of *C. dactylon* growing on effluent discharge from the electroplating industry and found bioaccumulation and translocation factor in order of Zn > Fe > Cu > Ni > Cr [11].

Alongside the various studied plants, *C. dactylon* is a suitable plant for clearing contaminated soils due to high heavy metals absorption and biomass. Therefore, because of the necessity of using a bio-removal method, this experiment investigated *Cynodon dactylon* (L.) estimation to clean up Cd and Ni from contaminated soils.

2. Materials and Methods

2.1. Soil sampling, plant material, and treatment

The soil sample was obtained from the topsoil (depth 0-10 cm) a Typic Haplocambids soil according to the soil taxonomic classification in Chittgar area (Tehran province), then transferred to the laboratory for drying and sieve analysis soil particles of size below 5 mm. 15 g of the soil samples were transferred to plastic jars (100 mL). Each batch of soil was treated with (nitrogen, phosphorus, potassium) based on the soil test. Next, the samples were spiked with treatment including Ni solution ($\text{NiCl}_2 \times 6 \text{H}_2\text{O}$) in three different concentrations (150, 300, 450 mg/kg soil); Cd (NO_3)₂ solution (20, 50, 60 mg/kg soil) and (zero) as control (soil without metals addition). All the soil samples were stored under FC moisture conditions for 3 weeks. Each pot was filled with 2 kg of soil. Ten seeds were sown in pots at the same time. After 10 days, 5 weak seedlings were removed. The pots were weighed and irrigated every 24 or 48 h. The plants were harvested at the end of the growth season.

2.2. Assay the morphological and phytochemical traits

After thorough washing with distilled water, aerial parts and roots were oven-dried at 70 °C for 48 h; then heated in a

triple walled relay-controlled electric oven at a temperature of 450 °C for approximately 5 h. The dry and fresh weight of shoot and root was weighted by digital balance accuracy of 0.1 g. Plant height was determined with a ruler. In addition, to estimate the amount of chlorophyll in leaves Arnon *et al.* (1949) method was used [12]. The Ascorbic peroxidase and Catalase activity were measured by the NBT method, as explained by Nakano and Asada [13]. The superoxidase activity was recorded by the Dhindsa *et al.* (1981) method [14]. The promoter device was used to measure the stomatal resistance for 5 leaves from each pot that were randomly selected.

2.3. Bioconcentration Factors and Bacterial growth

The aerial parts of the plants were cut from the soil surface by a special blade and the roots were harvested by a special sieve and alternating movements in the water. After washing in distilled water, the plant samples were transferred to the laboratory by special paper bags and dried for 4 h at 3 °C and after weighing and grinding in a special electric oven, they turned to ashes. Digestion of plant samples was done by wet digestion method and the amount of Cd and Ni in the shoots and roots of plants was measured by a Graphite-furnace atomic absorption spectrometry [15]. The bioconcentration factor (BCF) of each crop was used to assess the transfer of heavy metals from soil to plant. It can be calculated as $BCF = C_c/C_s$; where C_c and C_s are the total heavy metal concentrations in some kind of crops and corresponding soils samples, respectively. When calculating the overall BCF, C_c indicates the mean content of heavy metals in all the crops samples while C_s represents the mean content of corresponding heavy metals in all the soil samples [16]. R2A (Reasoner's 2A agar) medium supplemented with Ringer Solution was used for soil culture medium and growth conditions. 50 µL of the suspension was transferred with a sterile pipette on a slide for staining. Bacteria were counted with the conventional fluorescence microscope.

2.4. Statistical analysis

This experiment was conducted in a completely randomized block design at three replications in Iran Research Institute of Petroleum Industry. The data were subjected to analysis of variance (ANOVA) and Duncan's test was used for mean comparison at 1%. All the statistical analyses were calculated using Minitab 16 Statistical software and the graphs were drawn in Microsoft Office Excel 2013.

3. Results and Discussion

Significant differences were found between the treatment and time in plant height and shoot/root dry weight

parameter ($P \leq 0.01$) (Table 1). Growth parameters such as plant height and dry weight of shoot and root were affected by heavy metal contamination. The results revealed that Cd and Ni stress at high concentration had an inhibitory effect on plant height, dry weight of shoot and root. These traits decreased as metal concentration increased in the solution. It showed that using Cd at different concentrations decreased the plant height compared to the control after 4 months (43 cm) whereas Ni indicated significant changes as a result of (50 cm) in height. Plants growing in 60 mg/kg Cd with (9.1 g) had less shoot dry weight when (11.5 g) was observed in 150 mg/kg Ni. Similar results were obtained in root dry weight in plants treated with 60 mg/kg Cd (1.10 g) while Ni showed greater shoot dry weight (1.22 g) in fourth month (Figure. 1, 2, and 3). Cd and Ni cause toxicity in plants and can disrupt cellular functions such as processes of cell division and enlargement. Over-application of Cd leads to inhibition of biomass production and toxicity in the plant; however, low levels of Cd increase the average biomass which is consistent with previous findings that low Cd concentrations may be considered to be effective in plant growth. Metals accumulation at high concentrations leads to structural and ultrastructural changes. Morphological symptoms were similar in soil contamination and foliar application treatments including wrinkling, necrosis at the tip of young leaves and leaf area, browning of the older leaves, and apical bending of the stem (Picture 1). Leaf necrotic spots and cell death are the most common harmful heavy metal contaminations and discoloration is the most common sign of heavy metal toxicity which is due to the production of reactive oxygen species, cell destruction, photosynthesis, and accumulation of phenolic compounds. Previous studies showed that the metals accumulation such as Cd and Ni reduces mesophilic tissue and epidermal cell size [17]. The results of this study are in line with [18, 19, 20] on barley, rice, and *Crataegus aronia*, respectively.

An increase in Ascorbate peroxidase activity (APX) was recorded in plants treated with 150 mg/kg Ni with (3.7 µ mol min⁻¹ mg⁻¹ FW) during 4 months; while the rate was related to Cd treatment with an average of (2.5 µ mol min⁻¹ mg⁻¹ FW) (Figure 4). Catalase activity rate (CAT) in plants treated by 60mg/kg Cd (2.01 µ mol min⁻¹ mg⁻¹ FW); while Ni treated, was 2.22 µ mol min⁻¹ mg⁻¹ FW in the fourth month. Plants grown in 60-mg/kg Cd (3.11 µ mol min⁻¹ mg⁻¹ FW) had higher Superoxide dismutases (SOD) than other substrates. This enzyme was the highest for plants grown in Ni (4.11 µ mol min⁻¹ mg⁻¹ FW) in the fourth month and lowest for plants grown in the control group (Table 2). Superior antioxidant defenses, particularly catalase activity may play an important role in hyperaccumulator plants. Cd induced the ROS production that includes (O₂), (-OH), and (H₂O₂) which in turn affects multiple biological processes via biomolecules degradation such as membrane lipid, protein, chloroplast pigment, and enzymes.

Table 1: Analysis of Variance of traits in *Cyndon dactylon L.* treated with Cd and Ni

SOV	DF	Plant height	Shoot fresh weight	Root dry weight	APX	CAT	SOD	Chlorophyll	Stomal resistance	BFC (shoot)	BFC (root)	Bacteria
Treatment	3	0.43*	0.09*	0.29 *	0.14*	.07*	0.18 *	0.29*	0.54*	0.34*	0.33*	0.22 ^{ns}
Time	1	28.08**	1.87**	10.25**	23.20**	0.99**	16.45**	12.30*	1.56*	1.06*	1.42*	0.52 ^{ns}
Treatment ×Time	3	0.45 ^{ns}	1.13 ^{ns}	2.42 ^{ns}	1.48 ^{ns}	1.67 ^{ns}	12.13 ^{ns}	1.27 ^{ns}	2.03 ^{ns}	2.22 ^{ns}	2.32*	1.22 ^{ns}
Error	12	0.08	0.13	1.24	1.76	2.65	1.25	6.23	3.12	2.13	1.14	0.55
CV	-	11.13	18.8	32.6	2.54	7.87	11.23	9.23	12.1	11.8	3.14	11.2

*and **: Significant at the 5% and 1% probability levels, respectively; ^{ns} is not significant.

Sai Kachout1 *et al.* (2010) found that only superoxide dismutase (SOD) and probably ascorbate peroxidase (APX) were diminished by metal toxicity. However, the activities of catalase (CAT) and glutathione reductase (GR) were increased by metal stress. Hence, the plants of the three annual species or used varieties all showed an intermediate level of tolerance according to the imposed treatments [21]. The antioxidative activity seems to be fundamental for the adaptive response of *Atriplex* plants against environmental stress. Superoxide dismutase, ascorbate peroxidase, glutathione reductase, and catalase activities enhanced in all the samples of leaves, roots, and stolons in the presence of Cd [22].

The results indicated that the effects of heavy metal on the measured traits were significant for chlorophyll content (Table 1). The lowest amount of chlorophyll reduced in 60 mg/kg Cd: 0.8 mg/g F.W. Plants with Ni may contain chlorophyll concentrations as high as 1.3 mg/g F.W. in the fourth (Table 2). It seems that plants can protect themselves from environmental stress. Several mechanisms have been proposed to explain the stimulatory effect and one of the reasons is that metal ions may act as activators of the enzyme (s) in cytokinin metabolism that promote plant growth [23]. Low-dose stress can cause changes in the hormones and cytokines that regulate plant growth. It has been found that hormone treatment caused enhancement in the active cytokine that ultimately increased the chlorophyll pigments.

Cd binding formed in response to the low stress of heavy metals plays an important role in metal tolerance plants [24]. Photosynthesis, an important process of plant growth and biomass production, decreases with increasing Cd concentration in the soil. Leaf chlorosis is one of the first visible signs of heavy metal toxicity. However, there was no chlorosis in plants treated with low Cd concentrations in this experiment. It has been reported chlorophyll content increases or does not change significantly in heavy metal-tolerant species [25]. In the present study, there was less chlorophyll content in high concentration Cd treatments than in control group. Thus, it was concluded that the growth rate could be stimulated by a low and moderate dose of Cd. Liu *et al.* (2007) obtained similar results [26]. The results of this study showed that Ni treatment reduced chlorophyll synthesis. This reduction can be explained by the fact that heavy metals reduce metabolic processes by inhibiting photosynthetic enzymes. The decrease in chlorophyll content may be due to inhibiting the reductive steps in the chlorophyll enzyme biosynthetic and levulinic acid dehydratase. Ni inhibits chlorophyll synthesis by causing impaired uptake of essential elements such as Mg and Fe by plants [27]. Sharma and Gaur (1995) indicated a decrease in chlorophyll content with increasing Ni concentration [28]. Geebelen *et al.* (2003) also found that the foliar application of low concentrations of Cd and Ni in combination with nutrient solution facilitated the synthesis of chlorophyll. Their results were inconsistent with ours [29].

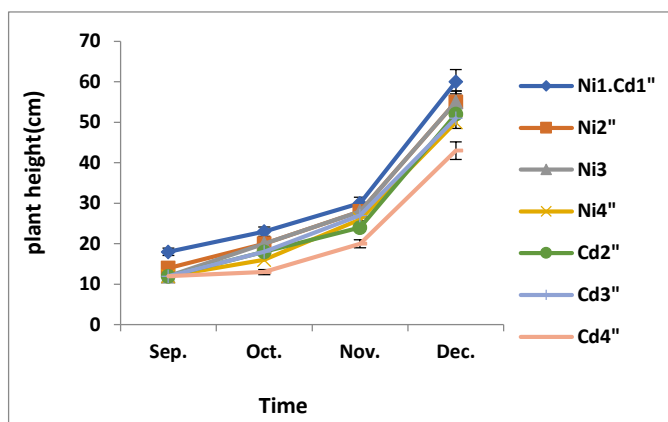


Figure 1: Changes in plant height in *Cyndon dactylon L.* treated Cd and Ni; Ni1.Cd1:0, Ni2: 150 mg/kg, Ni3: 300mg/kg; Ni4: 450 mg/kg, Cd2: 20 mg/kg, Cd3: 50mg/kg; Cd4: 60mg/kg

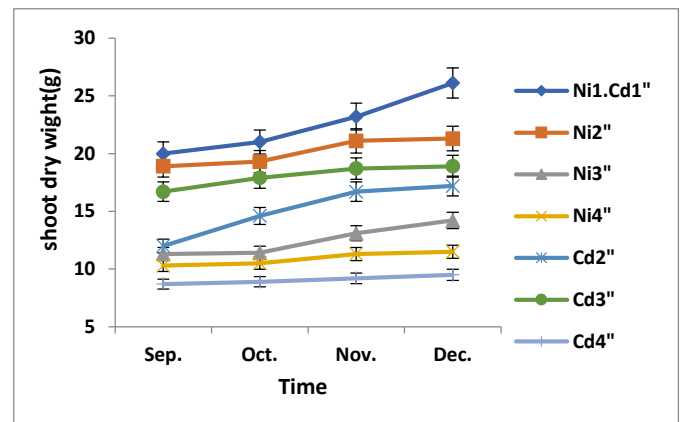


Figure 2: Changes in shoot dry weight in *Cyndon dactylon L.* treated Cd and Ni; Ni1.Cd1:0, Ni2: 150 mg/kg, Ni3: 300mg/kg; Ni4: 450 mg/kg, Cd2: 20 mg/kg, Cd3: 50mg/kg; Cd4: 60mg/kg

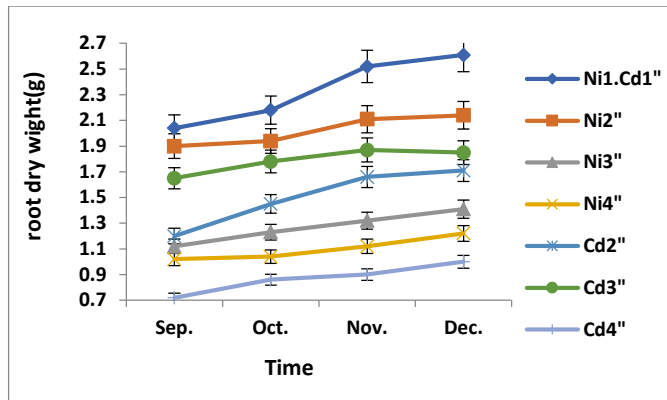


Figure 3: Changes in root dry weight in *Cyndon dactylon* L treated Cd and Ni; Ni1.Cd1:0, Ni2: 150 mg/kg, Ni3: 300mg/kg; Ni4: 450 mg/kg, Cd2: 20 mg/kg, Cd3: 50mg/kg; Cd4: 60mg/kg

Significant differences were found among treatments in stomatal resistance. 60 mg/kg Cd (3650 m/s) treatment indicated the low stomatal resistance. Similarly, a difference was observed for collected Ni (3450 m/s) in the fourth month. The lowest index was seen in the control group (Table 2). Negative effects of Ni on mineral nutrition (uptake and transport of nutrients) lead to reducing water uptake and secondary drought stress in plants [30]. It is possible that causing stress at the root and transferring it to the shoots and leaves decreases stomatal openness and consequently increases stomatal leaves resistance. Molas and Baran (2004) reported that under Ni stress the stomatal resistance of barley plants enhanced [31]. Low concentrations of Ni and Cd promoted stomatal opening, while higher concentrations inhibited this process. Our results are in consistent with [32]. They investigated that compared with control values, the stomatal perimeter, diameter, and area values were significantly reduced by all Ni and Cd treatments, as well as water stress in both main stem and first lateral branch leaves.



Picture 1: Morphological symptoms in contaminated plants with Ni and Cd

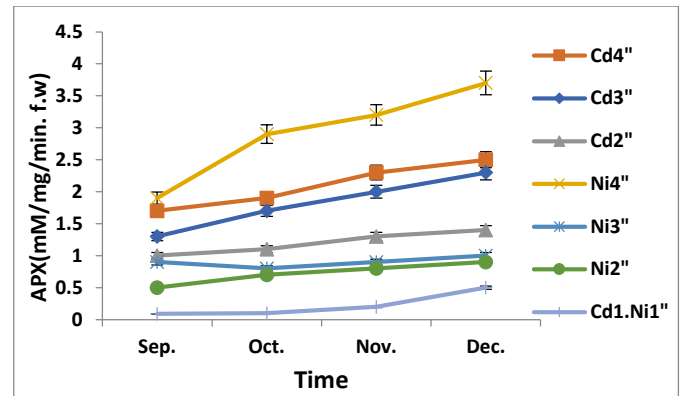


Figure 4: Changes in APX in *Cyndon dactylon* L treated Cd and Ni; Ni1.Cd1:0, Ni2: 150 mg/kg, Ni3: 300mg/kg; Ni4: 450 mg/kg, Cd2: 20 mg/kg, Cd3: 50mg/kg; Cd4: 60mg/kg

Heavy metal treatments symptoms are similar to the drought stress such as increased mesophyll thickness, upper epidermis, palisade mesophyll, the size of the upper epidermis cells, the number of stomata, and leaf stomatal resistance [17].

The treatment and time had a significant difference in BCF adsorption in shoot at 1% level. Ni level in shoot reached in maximum rate with Ni 450 mg/kg soil (3.11 $\mu\text{g/g}$) in the fourth month when the treatment belongs Cd, with an average of (2.32 $\mu\text{g/g}$). The opposite result was observed in root BFC Cd accumulation which was (3.24 $\mu\text{g/g}$) in 60 mg/kg Cd and (2.24 $\mu\text{g/g}$) Ni treatment in the fourth month (Table 2). The decrease in root and shoot growth was observed as Cd addition to soil increased. Toxic effects (both direct and indirect) lead to a decline in plant growth. The decrease in biomass production was associated with Cd accumulation in root and stem tissues. Cd content in both root and shoot parts increased significantly with increasing Cd concentration. Cd accumulation in roots was more than in shoots, which acts as an important barrier to protect plants from heavy metal [33]. These results are consistent with the findings of Yang *et al.* (2003). It has been demonstrated that roots and shoots can accumulate Ni ions from aqueous solutions [34, 35]. Increasing Ni concentration in shoot and root during the time is consistent with those of previous studies. They stated that the concentration of elements in plant tissues is affected by the concentration of heavy metals in the soil. They also showed that the plant was able to accumulate Ni up to 633 ppm. Therefore, it seems that this plant can tolerate and accumulate Ni and Cd. The high concentration of Ni in the aerial parts compared to the roots indicates the specific ability of the plant to absorb and transfer metals and to store them in aerial parts [36]. Root-to-shoot transferring heavy metal is most likely due to efficient metal transport systems such as blocking metals in vacuoles and leaf apoplasts [37]. Heavy metals uptake may decrease with time and accumulation due to their toxic effects.

Table 2: Comparison of means of traits in *Cyndon dactylon L.* treated with Cd and Ni

Treat		Catalase ($\mu\text{M}/\text{min}/\text{mg FW}$)	Superoxide desmutase ($\mu\text{M}/\text{min}/\text{mg FW}$)	Chlorophyll ($\text{mg}/\text{g FW}$)	BFC (shoot) ($\mu\text{g}/\text{g}$)	BFC (root)	Stomatal resistance(m/s)
Ni1Cd1	Sep	1.2 ^{bc}	1 ^c	1.52 ^a	0.07 ^d	0.06 ^d	2000 ^{cd}
	Oct	1.22 ^{bc}	1.21 ^c	1.72 ^{ab}	0.07 ^d	0.06 ^d	2110 ^c
	Nov	1.28 ^{bc}	1.21 ^c	1.85 ^a	0.08 ^d	0.07 ^d	2200 ^c
	Dec	1.3 ^{bc}	1.42 ^c	1.88 ^a	0.08 ^d	0.07 ^d	2370 ^{bc}
Ni2	Sep	1.41 ^b	1.71 ^c	1.82 ^a	2.11 ^{bc}	0.22 ^c	2110 ^c
	Oct	1.44 ^b	1.81 ^c	1.78 ^{ab}	2.25 ^b	0.27 ^c	2230 ^c
	Nov	1.58 ^b	1.91 ^c	1.65 ^{ab}	2.43 ^b	0.36 ^c	2380 ^c
	Dec	1.77 ^b	1.91 ^c	1.42 ^b	2.65 ^b	0.67 ^c	2520 ^{bc}
Ni3	Sep	1.45 ^b	2 ^c	1.71 ^{ab}	2.22 ^b	0.91 ^c	2200 ^{bc}
	Oct	1.53 ^b	2.22 ^{bc}	1.69 ^{ab}	2.42 ^b	1.05 ^{bc}	2460 ^{bc}
	Nov	1.67 ^b	2.43 ^{bc}	1.6 ^b	2.65 ^{ab}	1.46 ^{bc}	2630 ^b
	Dec	2.01 ^a	2.51 ^{bc}	1.39 ^c	2.98 ^{ab}	1.57 ^{bc}	2810 ^b
Ni4	Sep	1.8 ^a	2.81 ^{bc}	1.67 ^{ab}	3 ^{ab}	1.61 ^{bc}	2500 ^b
	Oct	1.9 ^a	3.83 ^b	1.63 ^{ab}	3.11 ^a	1.82 ^b	2810 ^b
	Nov	2.07 ^a	4 ^b	1.54 ^{ab}	3.24 ^a	2.05 ^b	3030 ^{bc}
	Dec	2.22 ^a	4.5 ^a	1.3 ^c	3.31 ^a	2.24 ^b	3450 ^a
Cd2	Sep	1.41 ^b	1.51 ^c	1.65 ^{ab}	1.47 ^c	2.05 ^b	2140 ^c
	Oct	1.44 ^b	1.61 ^c	1.45 ^{bc}	1.87 ^c	2.07 ^b	2320 ^c
	Nov	1.58 ^b	1.71 ^c	1.32 ^c	1.91 ^c	2.34 ^{ab}	2500 ^{bc}
	Dec	1.77 ^b	1.72 ^c	1.17 ^{cd}	2.01 ^{bc}	2.65 ^{ab}	2720 ^b
Cd3	Sep	1.32 ^{bc}	1 ^c	1.56 ^{ab}	1.87 ^c	2.35 ^{ab}	2330 ^c
	Oct	1.37 ^{bc}	1.23 ^c	1.34 ^c	2.06 ^{bc}	2.56 ^{ab}	2550 ^{bc}
	Nov	1.42 ^b	1.24 ^c	1.23 ^c	2.09 ^{bc}	2.67 ^{ab}	2700 ^b
	Dec	1.49 ^b	1.45 ^c	1.07 ^{cd}	2.2 ^b	2.89 ^{ab}	3070 ^{bc}
Cd4	Sep	1.56 ^b	2.41 ^{bc}	1.43 ^{bc}	2 ^c	3.06 ^a	2800 ^b
	Oct	1.63 ^b	2.81 ^{bc}	1.12 ^{cd}	2.13 ^{bc}	3.24 ^a	2920 ^{bc}
	Nov	1.76 ^b	3 ^b	1 ^d	2.28 ^b	3.34 ^a	3110 ^b
	Dec	2.01 ^{ab}	3.11 ^b	0.8 ^d	2.32 ^b	3.56 ^a	3650 ^a

Means within each column followed by the same letter are not different according to the Duncan's test

Ni1.Cd1:0, Ni2: 150 mg/kg, Ni3: 300mg/kg; Ni4: 450 mg/kg, Cd2: 20 mg/kg, Cd3: 50mg/kg; Cd4: 60mg/kg.

Bioaccumulation factors (BAFs,) describe the increase of contaminants such as persistent organic pollutants. Datta *et al.* (2010) found high concentrations of heavy metals in the roots compared to that in the stem which can be attributed to the direct contact of the root system with the fly ash basal medium (substratum) [38]. The relatively higher BAF in grassy species, *Typha elephantina* compared to *Phragmites karka* confirms the efficiency of the tissue system of the species in *translocating* the heavy metals from the fly ash basal medium to the shoot system. Nyitrai *et al.* (2003) stated that uptake of metals increases with increasing external metal concentration, but this is not a linear correlation [39]. Over time, the metal concentration in tissue increases which causes saturation following, and then the effective uptake decrease. Heavy metals bioaccumulation for *Lemna gibba* and *Spirodela polyrhiza* plants was dependent on and positively correlated with the external metal concentrations. Both plants were *hyperaccumulators* of Cd but not of Ni or Cu [40].

According to Table 1, no significant effects were found between treatment and time on bacterial growth.

Plant growth-promoting microbes have attracted remarkable attention because they can greatly accelerate the plants' bioremediation process by stimulating growth through various mechanisms. The cadmium-resistant bacteria could be divided into three groups—the largest group consisted of bacteria resistant to Cd by effluxing it from the cells. The bacteria of the other two groups were capable of binding Cd or detoxifying it, depending on the bacterial biomass and on the pH [41].

4. Conclusion

A more detailed seasonal picture of the bioaccumulation pattern of the heavy metals in the selected grassy species (on a long term basis) can throw light on the use of these species as potential bio-accumulators of heavy metals or as bio-indicators in the heavy metal infested zone. According to the results of the current study regarding the heavy metals absorption such as Cd and Ni by grass species of *C. dactylon* seedlings, it is recommended to develop their cultivation level in contaminated areas for phytoremediation.

Authors' Contributions

Aida Abdali Dehdezi: Data curation; Writing original drafts; Formal analysis. **Ebrahim Alaei:** Conceptualization; Project administrator; Funding acquisition; Writing-review and Editing. **Pejman Azadi:** Supervision; Methodology. **Mahmoud Shavandi:** Data curation; Visualization. **Seyed Amir Mousavi:** Software; Visualization; Validation.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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