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Vetiver Morphological Changes Under the Influence of Phytoremediation and Absorption of Heavy Metals

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Abstract: Today, human activities such as pesticides, fertilizers, industrial and nuclear wastes, and Nano pollutants, which contain heavy metals, affect the performance and flexibility of soil ecosystems, microorganisms, and plants. It also pollutes surface water, groundwater and the food chain. The aim of this study was to investigate the phytoremediation potential of heavy metals from soil by *Vetiver* and the effect of these metals on plant morphological changes in greenhouse conditions. Soil contamination with heavy metals has become a worldwide concern. This research was conducted as a factorial design with four different heavy metals (lead, cadmium, manganese and nickel) with three varying levels and also three replications for each treatment. The results of analysis of variance, Duncan test, showed that the effect of applied treatments on lead uptake in roots and shoots increased significantly ($P \leq 0.05$) with increasing levels of treatments. The biological concentration factor was more than one, and the transfer factor was close to one. Therefore, it can be used as a phytostablization plant. Also, with different levels of heavy metal treatments, the highest average height of total plants was related to lead, manganese, nickel and cadmium treatments with values of 167.54, 166.66, 165.85 and 163.88cm, respectively. The highest average root roots of plants were related to lead, manganese, nickel and cadmium treatments with values of 42.27, 42.36, 41.85 and 41.61 Cm, respectively. The highest average root roots of plants were related to the treatments of lead, manganese, nickel and cadmium with values of 125.28, 124.29, 124.02 and 121.68 Cm, respectively. Therefore, among all treatments, plants treated with cadmium had the lowest growth. The results showed that *Vetiver* can be considered as a refining plant due to its vegetative characteristics, cost-effectiveness and high adaptation to environmental conditions.

Keywords: Phytoremedation, *Vetiver* Grass, Heavy Metals, Morphology

1. Introduction

One of environmental pollution is soil contamination as a feature of land corruption caused by the effects of xenobiotic (human-made) synthetic substances or other change in the regular soil condition. As soil is the basic natural medium, which is liable to various poisons because of various human exercises [24, 51]. As a part of biosphere, soil plays an important role in food production and environmental sustainability [24]. Soil heavy metal pollution is among the most important environmental challenges through the world. Heavy metals are not biodegradable and tend to be accumulated in living organisms. The effects on human health and ecosystems associated with heavy metal (HMs) pollution have been proven [22, 71]. Become even more worrying given their tendency to accumulate and magnify along trophic levels [22, 2].

For example, due to their bioaccumulation they can have toxic effects on living organisms when they exceed a certain concentration. Representing a risk to human health when transferred thought the food chain [22, 37]. Therefore soil contamination is a serious worldwide concern; therefore effective remediation approaches are necessary.

Several in-situ and ex-situ physical, chemical, and biological methods have been used for soil pollution remediation [26]. Non-mobilization and extraction methods by physiochemical techniques are expensive and only useful for small size areas. Besides, soil leaching causes several negative effects on soil properties. Phytoremediation removes pollutants from soil and environment and conserves biological and physical nature of the soil with reasonable cost, is of great benefit [55, 13].

Phytoremediation is a technology that transfers pollutants from soils and sediments to the plant tissues without soil structure degradation and soil productivity decrease. Heavy metal uptake by plants is dependent to soil metal concentration and is also affected by plant physiology [18].

Phytoremediation is an energy-efficient, cost-effective and aesthetically pleasing alternative to remediation sites with low to moderate levels of pollution [23, 33, 43, 61]. In the phytoremediation method, plants are used for the elimination of contamination. The most ideal plant for phytoremediation is a plant with high biomass, high growth rate, and higher ability to accumulate metals [30, 31, 33]. Phytoremediation is the utilization of plant to remove and accumulate contaminants from environment, including the use of plants to mitigate, transfer, stabilize or degrade pollutants in soil, sediments and water [21, 33, 50].

Numerous plants have being studied over the years, with reports suggesting *Vetiver* grass, *Vetiveriazizanioides* (Linn.) Nash to be one of the most promising plants, with a fast growth rate, and the ability to adapt to many environmental conditions and stress, in addition to being able to tolerate a wide range of extreme HM contamination in soils, The *Vetiver* plant has been considered for phytoremediation due to its special characteristics [68, 69]. *Vetiver* grass is belongs to the Poaceae family and it is native to south and south-east [19]. *Vetiver*, a medicinally important perennial plant, known to control soil erosion, tolerates wide range of pH and elevated levels of toxic metals [21, 25, 72]. *Vetiver* is hydrophilic terrestrial plant which has physiological characteristics like the ability to absorb dissolved nutrients such as Nitrogen and Phosphorus, reduce BOD, COD, TSS, oil spill, HMs, Absorption of contaminants in batik production wastewater, tofu production waste water, and high tolerance to herbicides and pesticides [19, 56, 66, 72]. Recent studies by Singh et al [62] have solely focused on the phytoassessment of a single metal accumulation. However, there is a growing concern on mixed (Cd–Pb-Mn-Ni) metal contamination with *Vetiver* urgent clarification [47]. Although some phytoremediation studies have been carried out using *Vetiver* grass [3, 62, 53].

The objective of the present research was to study the capability of *Vetiver* grass in refining and reducing pollution of Pb, Cd, Mn and Ni from contaminated soils, and the effect of these metals on plant morphological changes in greenhouse conditions.

In this research, *Vetiver* grass (*Vetiveriazizanioides*) will be investigated in terms of its potential application in phytoremediation of soils contaminated with heavy metals (cadmium, lead, manganese, nickel). Different indicators such as biological aggregation index, and transfer factor were calculated. Previous studies have focused on the uptake of one element in by the plant, but in this study, four elements were investigated. Also, transfer and bioaccumulation factors have been studied. Also, in this study, in addition to investigating the interactions of heavy metals in adsorption, their effects on plant growth and morphology were also studied. Due to the high pollution associated with heavy metals and the high cost of physical and chemical methods, especially in developing countries, it is necessary to move to new and green methods. This study describes the application, research experience and future prospects in relation to applying phytoremediation of Vetiver grass as a suitable natural tool for promoting a sustainable environment.

2. Materials and Methods

The research method is descriptive-analytical and applied in terms of purpose. Data collection is collected through library, field, laboratory and database studies. This study was conducted for 5 months from April to September 2021 in the greenhouse. The research was implemented in greenhouse in a factorial design including three replications from four heavy metal types (Pb, Cd, Mn and Ni) in three replications (Table 1). The greenhouse temperature was between 20-25°C. After soil preparation and determination of its physical and chemical properties, matched *Vetiver* seedlings were planted. After the growth period, root and shoot samples Were harvested to determine the amount of absorption of heavy metals, and transferred to the laboratory, to determine the amount of absorption of heavy metals, using the Perkin-Elmer PinAAcle 900TT Atomic Absorption spectrophotometer (AAS), the amount Absorption was determined by plant organs (ASTM D3335 - 85a. ASTM E1835-14. ASTM D3831 – 12 [7- 9].

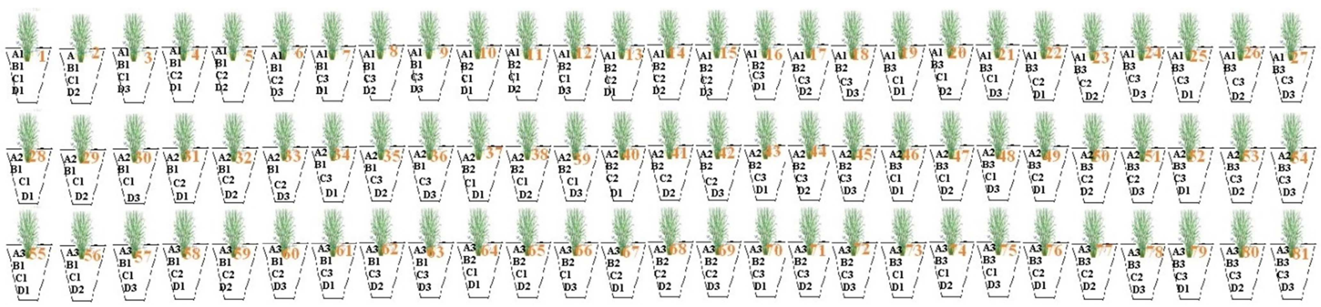

Figure 1. *Schematic implementation of factorial design for 4 treatments.*

Table 1. *Heavy metal Levels used.*

Levels Pb	Unite mg/kg^{-1}	Levels Cd	Unite mg/kg^{-1}	Levels Mn	Unite mg/kg^{-1}	Levels Ni	Unite mg/kg^{-1}
Level 1	0	Level 1	0	Level 1	0	Level 1	0
Level 2	300	Level 2	600	Level 2	100	Level 2	100
Level 3	700	Level 3	800	Level 3	200	Level 3	200

Table 2. *Physico-chemical properties of growth media soil.*

Parameter (unit)	Mean
Soil texture	
Sand (%)	65.58
Silt (%)	19.48
Clay (%)	14.94
Temperature (°C)	30.3 ± 4.5
pH	6.5-7.4
ECedS.m $^{-1}$	1.6
Water content (%)	5.72 ± 2.03
Field capacity (%)	40.93 ± 6.3
Saturation level (%)	13.97
Bulk density (g/cm^3)	1.62 ± 0.78
Porosity (%)	38.87 ± 4.39
Available P (ppm)	12.1
Metal contents (mg/kg)	
Pbmg kg^{-1}	13.05 ± 4.01
Cd mg kg^{-1}	3.86 ± 1.76
Mn mg kg^{-1}	23.48 ± 3.
Ni mg kg^{-1}	14.97 ±3.26
Available K (ppm)	215
Total Nitrogen (%)	0.225
Organic Compounds (%)	2.09

Ability for Metal Translocation and Accumulation: The ability for metal translocation and accumulation were evaluated by the biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF), Alloway. Ali et al. Ng et al. [6, 4, 46, 48]. as follows:

$$BCF = \text{HMs concentration in roots - shoots / HMs concentration in soil} \quad (1)$$

$$BAC = \text{HMs concentration in shoots / HMs concentration in soil} \quad (2)$$

$$TF = \text{HMs concentration in shoots / HMs concentration in roots} \quad (3)$$

3. Results

Cadmium as the dependent variable in roots soil, it was shown that the effect of Cadmium levels on its absorption, there is a significant difference in the level of 99%. In evaluating the effect of lead treatment levels on Cadmium absorption, there is a significant difference in the level of 99%. In evaluating the effect of Manganese treatment levels on Cadmium absorption, there is a significant difference in the level of 99%. And in the effect of nickel levels on Cadmium absorption there is a significant difference in the level of 99%.

Analysing the interaction of two HMs treatments in the roots soil, lead and cadmium, cadmium and manganese, cadmium and nickel, lead and manganese, lead and nickel as well as manganese and nickel, they showed in absorbing Cadmium through the roots, there is a significant difference in the level of 99% Analysing the interaction of three treatments of HMs in the roots soil, lead, cadmium and manganese, the interaction of lead, cadmium and nickel, the in traction of lead, manganese and nickel, as well as the interaction of cadmium, manganese and nickel treatments, in the absorption of Cadmium by the roots, there is a significant difference in the level of 99%. Also, analysing the interaction of four HMs treatments in the roots soil, namely lead, cadmium, manganese and nickel, in the absorption of cadmium by the roots, there is a significant difference in the level of 99%.

Table 3. *Single and combined effect, different treatments and their interaction with cadmium metal absorption by the roots of the Vetiver plant.*

Sources of changes	MS ± S (Mean Square ± Standard Deviation)	Fs (F Calculated)
Effect of Lead	1530526.1**± 1237.1	206616/780
Effect of Cadmium	158/479 ** ± 12/58	21/394
Effect of Manganese	45/171** ± 0/88	6.105
Effect of Nickel	44/844** ± 4/76	6/060
Effect of Lead/Cadmium Interaction	435.72** ± 87.20	58/822
Effect of Lead/Manganese Interaction	68/1** ± 2/858	9.18

Sources of changes	MS ± S (Mean Square ± Standard Deviation)	Fs (F Calculated)
Effect of Lead/Nickel Interaction	57/379** ± 60/3	7/754
Effect of Cadmium/Manganese Interaction	50/305** ± 30/5	6/798
Effect of Cadmium/Nickel Interaction	50/675** ± 4/59	6/848
Effect of Manganese/Nickel Interaction	47.31** ± 6.87	6/388
Effect of Lead/Cadmium/Manganese Interaction	62/863** ± 5/77	8/495
Effect of Lead/Manganese/Nickel Interaction	36.89** ± 6.07	4/981
Effect of Lead/Cadmium/Nickel Interaction	55/965** ± 4/35	7/560
Effect of Cadmium/Manganese/Nickel Interaction	34.12** ± 5.84	4/607
Effect of Lead/Cadmium/Manganese/Nickel Interaction	52/766** ± 5/54	153/7
Error Sum Correction	29.6** ± 5.4	

**Significance at 99% confidence level
*Significance at 95%confidence level
ns: No significant difference.

According to Table 4, in data analysis of variance table with lead as the dependent variable in shoots, that, the effect of Cadmium levels on its absorption, there is a significant difference in the level of 99%. in evaluating the effect of lead treatment levels on Cadmium absorption in the shoots, there is a significant difference in the level of 99%, and the effect of nickel levels on Cadmium absorption, there is a significant difference in the level of 99%, and the effect of manganese levels on Cadmium absorption in shoots, there is a significant difference in the level of 99%.

Analysing the interaction of two HMs treatments in shoots, lead and cadmium, lead and manganese lead and nickel, cadmium and manganese, of cadmium and nickel as well as manganese and nickel, in absorbing Cadmium in the shoots there is a significant difference in the level of 99%.

Analysing the interaction of three treatments of HMs in the soil, lead, cadmium and manganese, the interaction of lead, cadmium and nickel, the interaction of lead, manganese and nickel, as well as the interaction of cadmium, manganese and nickel treatments, in the absorption of cadmium by the shoots, there is a significant difference in the level of 99%. Also, analysing the interaction of four treatments of HMs in the soil, namely lead, cadmium, manganese and nickel, in the absorption of cadmium by the shoots, there is a significant difference in the level of 99%.

Table 4. *Single and combined effect, different treatments and their interaction with cadmium metal absorption by the shoots of the Vetiver plant.*

Sources of changes	MS ± S (Mean Square ± Standard Deviation)	Fs (F Calculated)
Effect of Lead	7530/274** ± 86/77	105/060
Effect of Cadmium	812147/105** ± 901/19	11330/854
Effect of Manganese	580/822** ± 24/10	8/103
Effect of Nickel	773/742** ± 27/81	10/795
Effect of Lead/Cadmium Interaction	8509/259** ± 9/22	118/719
Effect of Lead/Manganese Interaction	454/764** ± 21/32	6/345
Effect of Lead/Nickel Interaction	358/198** ± 18/96	4/997
Effect of Cadmium/Manganese Interaction	256/983** ± 16/94	3/585
Effect of Cadmium/Nickel Interaction	2946/801** ± 54/28	41/113
Effect of Manganese/Nickel Interaction	363/131**± 19/05	5/066
Effect of Lead/Cadmium/Manganese Interaction	524/469**± 22/90	7/317
Effect of Lead/Manganese/Nickel Interaction	153/827** ± 12/40	2/146
Effect of Lead/Cadmium/Nickel Interaction	234/304** ± 15/30	3/269
Effect of Cadmium/Manganese/Nickel Interaction	227/775** ± 9/18	3/176
Effect of Lead/Cadmium/Manganese/Nickel Interaction	110/743** ± 10/52	1/545
Error Sum Correction	71/676**± 71/67	

**Significance at 99% confidence level
*Significance at 95%confidence level
ns: No significant difference.

According to Table 4 in data analysis of variance table with lead as the dependent variable in shoots, that, the effect of Cadmium levels on its absorption, there is a significant difference in the level of 99%. In evaluating the effect of lead treatment levels on Cadmium absorption in the shoots, there is a significant difference in the level of 99%, and the effect of nickel levels on Cadmium absorption, there is a significant difference in the level of 99%, and the effect of manganese levels on Cadmium absorption in shoots, there is a significant difference in the level of 99%.

Analysing the interaction of two HMs treatments in shoots, lead and cadmium, lead and manganese lead and nickel, cadmium and manganese, of cadmium and nickel as well as manganese and nickel, in absorbing Cadmium in the shoots there is a significant difference in the level of 99%.

Analysing the interaction of three treatments of HMs in the soil, lead, cadmium and manganese, the interaction of lead, cadmium and nickel, the interaction of lead, manganese and nickel, as well as the interaction of cadmium, manganese and nickel treatments, in the absorption of lead by the shoots, there is a significant difference in the level of 99%. Also, analysing the interaction of four treatments of HMs in the soil, namely lead, cadmium, manganese and nickel, in the absorption of lead by the shoots, there is a significant difference in the level of 99%.

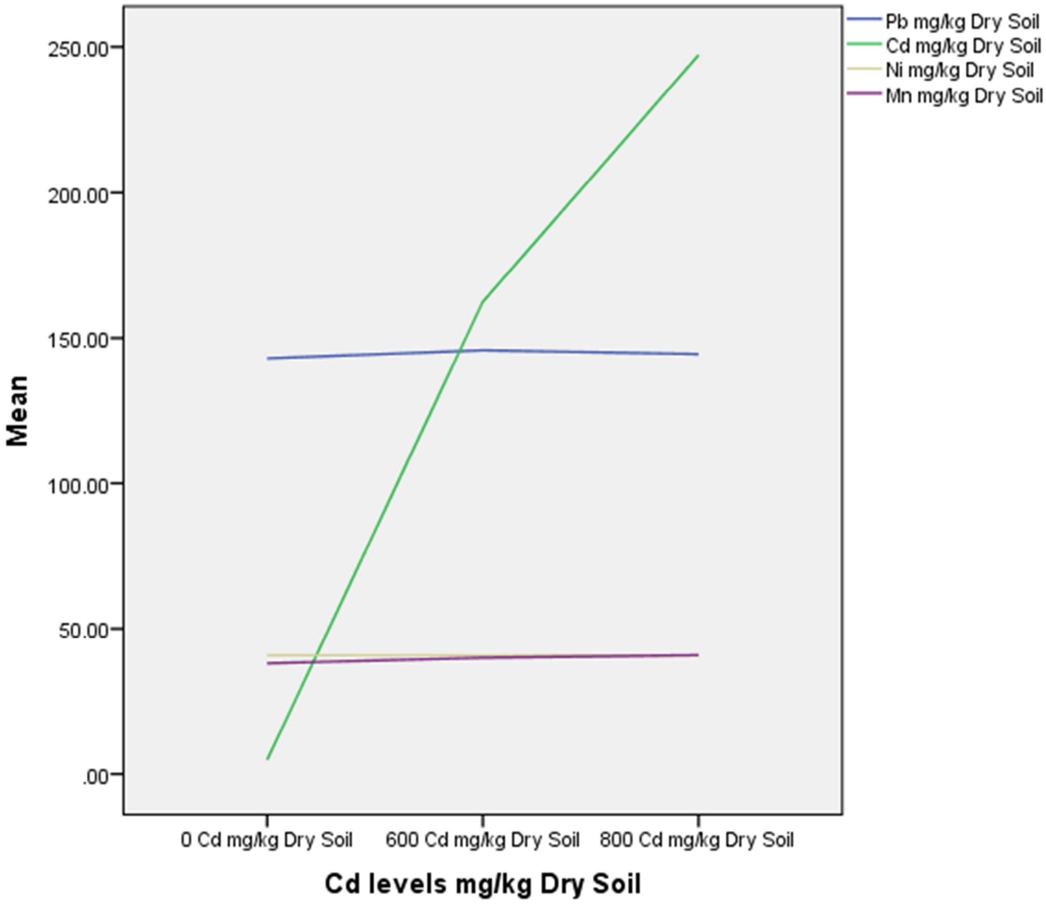

Figure 2. *Line chart of the effects of heavy metals treatment on cadmium uptake in roots.*

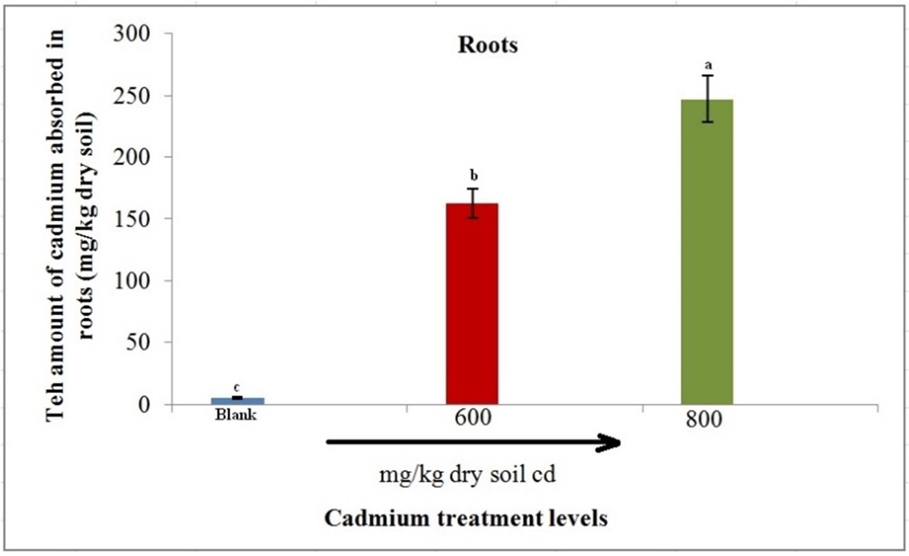

Figure 3. *Effect of lead treatment levels, on cadmium uptake in roots.*

* Different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in cadmium absorption.

* Each number in the graph is average of three repetitions.

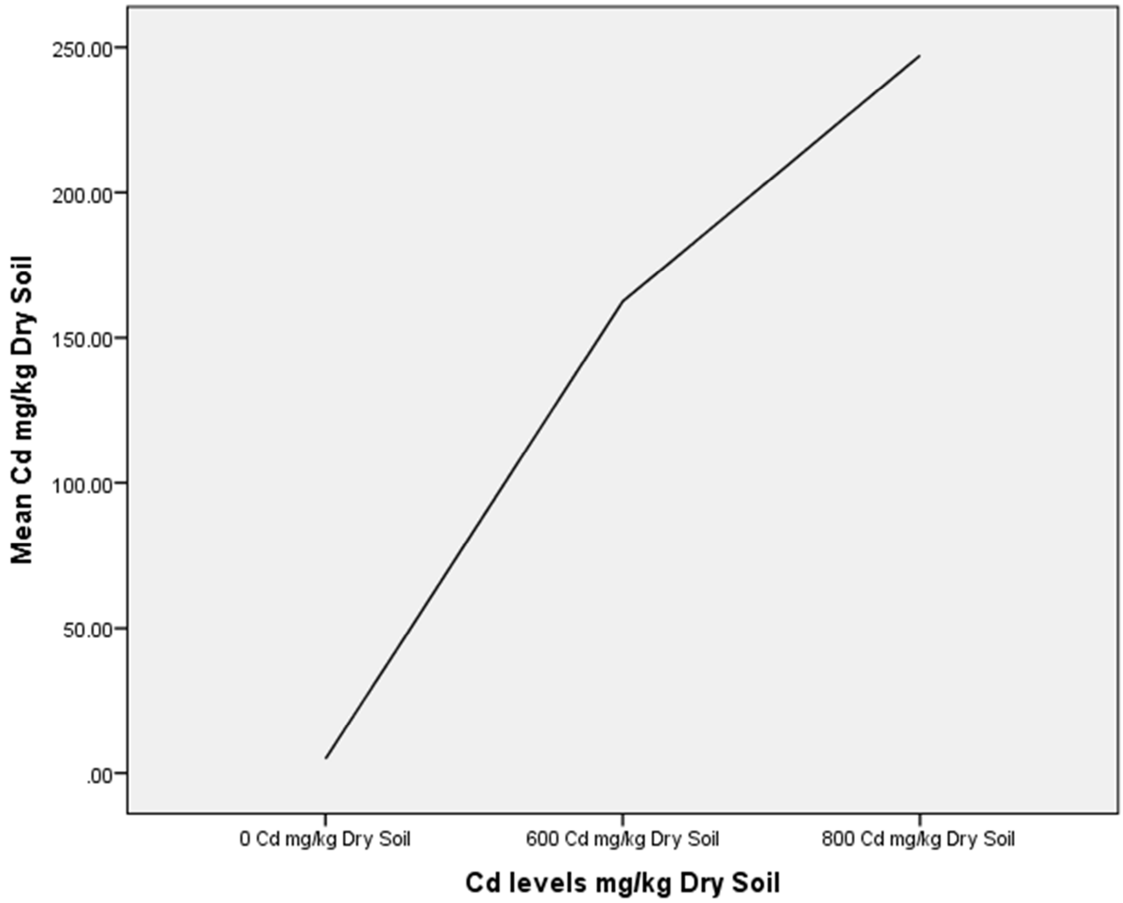

Figure 4. *Line chart of the effects of cadmium treatment on cadmium uptake in roots.*

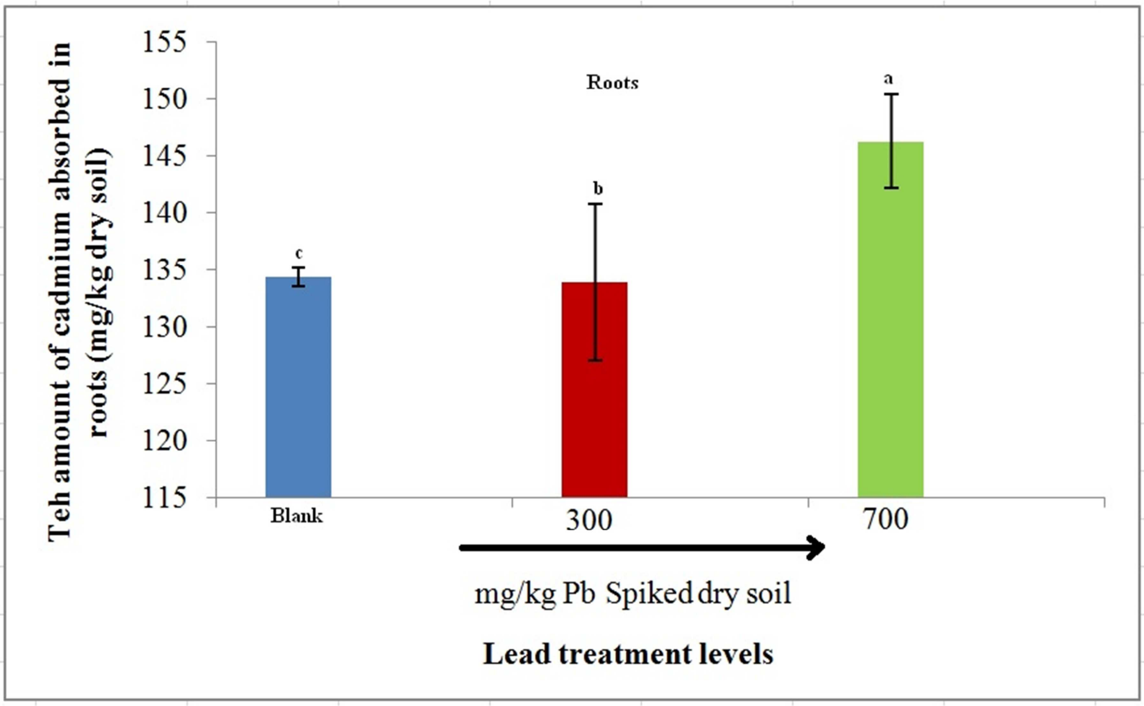

Figure 5. *Effect of cadmium treatment levels, on cadmium uptake in roots.*

* Different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in cadmium absorption.

* Each nu**m**ber in the graph is average of three repetitions.

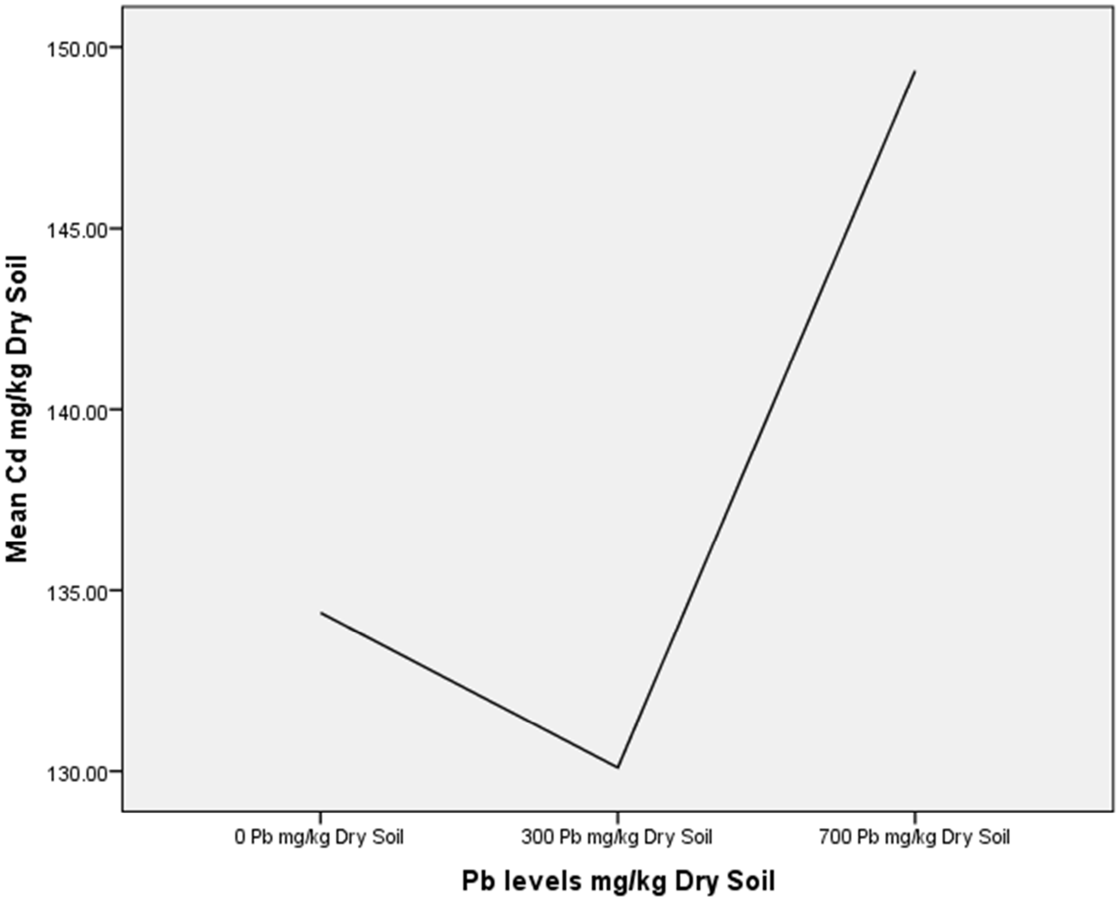

Figure 6. *Line chart of the effects of lead treatment on cadmium uptake in roots.*

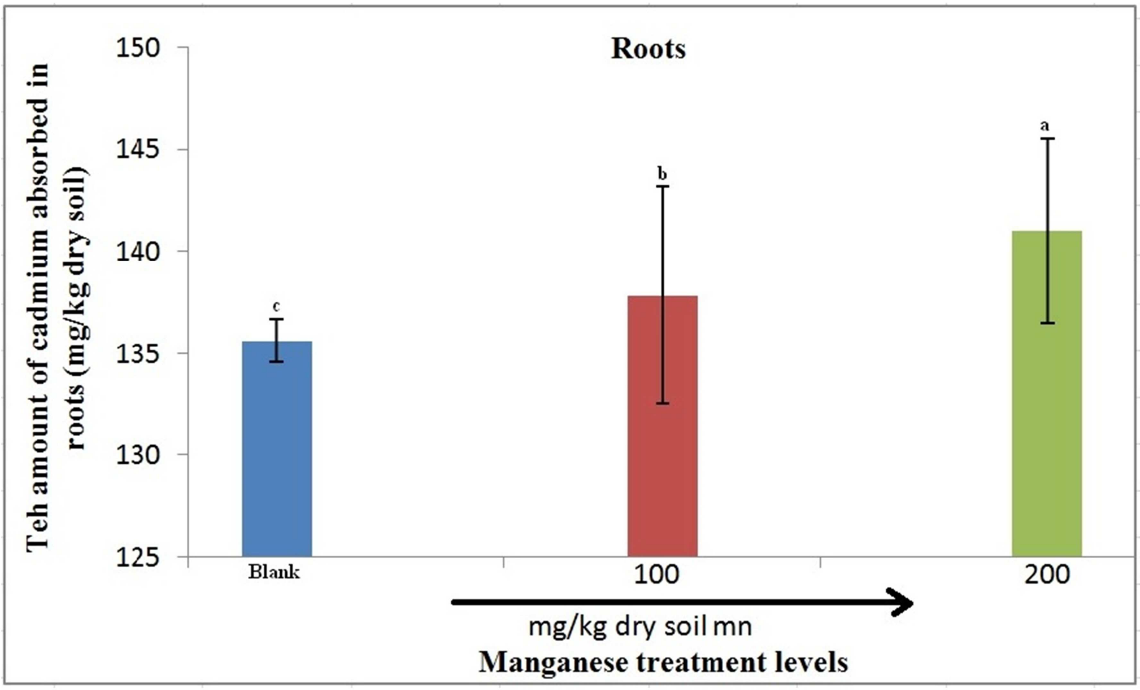

Figure 7. *Effect of manganese treatment levels, on cadmium uptake in roots.*

* Different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in cadmium absorption.
* Each number in the graph is average of three repetitions.

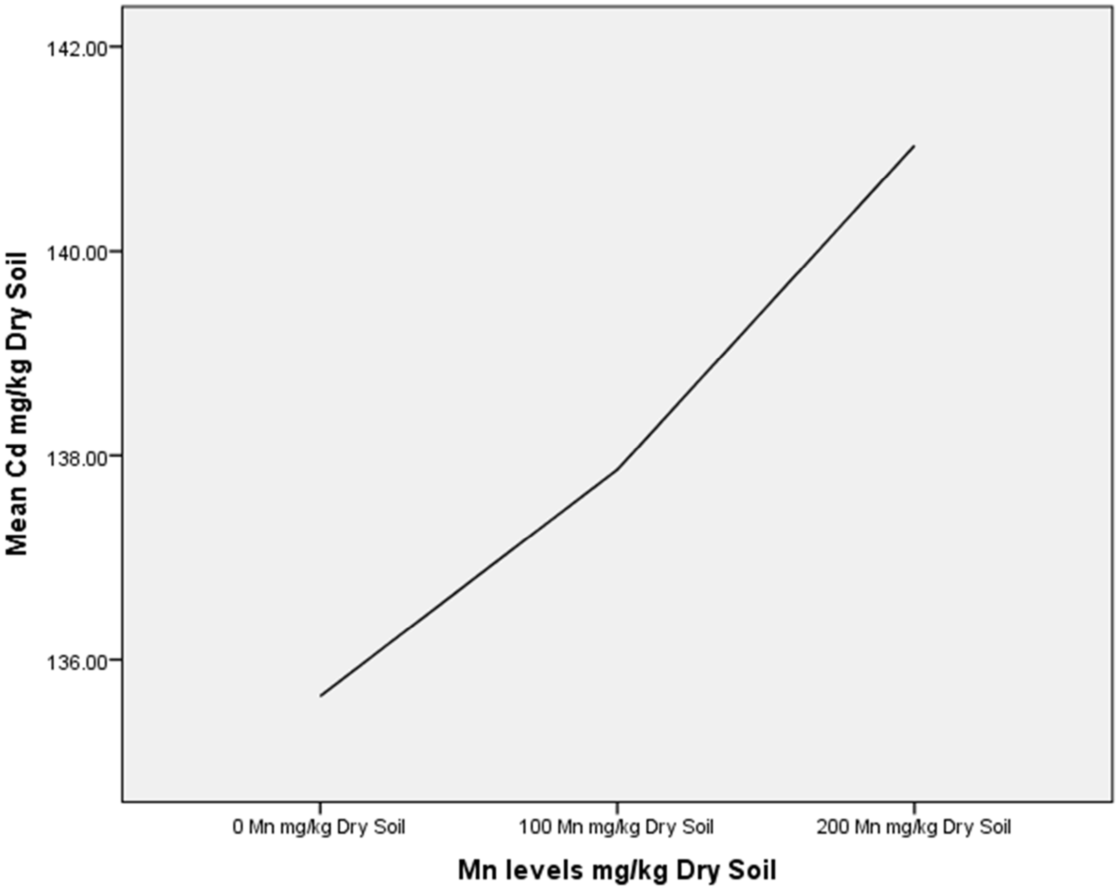

Figure 8. *Line chart of the effects of manganese treatment on cadmium uptake in roots.*

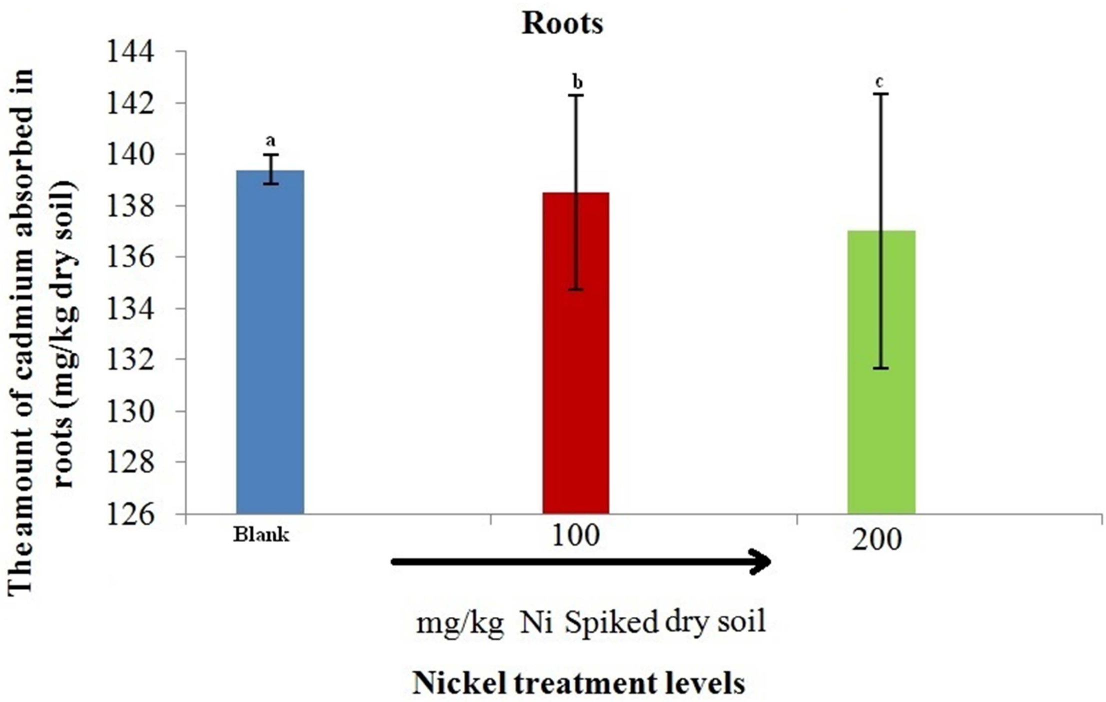

Figure 9. *Effect of nickel treatment levels, on cadmium uptake in roots.*

* Different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in cadmium absorption.

* Each number in the graph is average of three repetitions

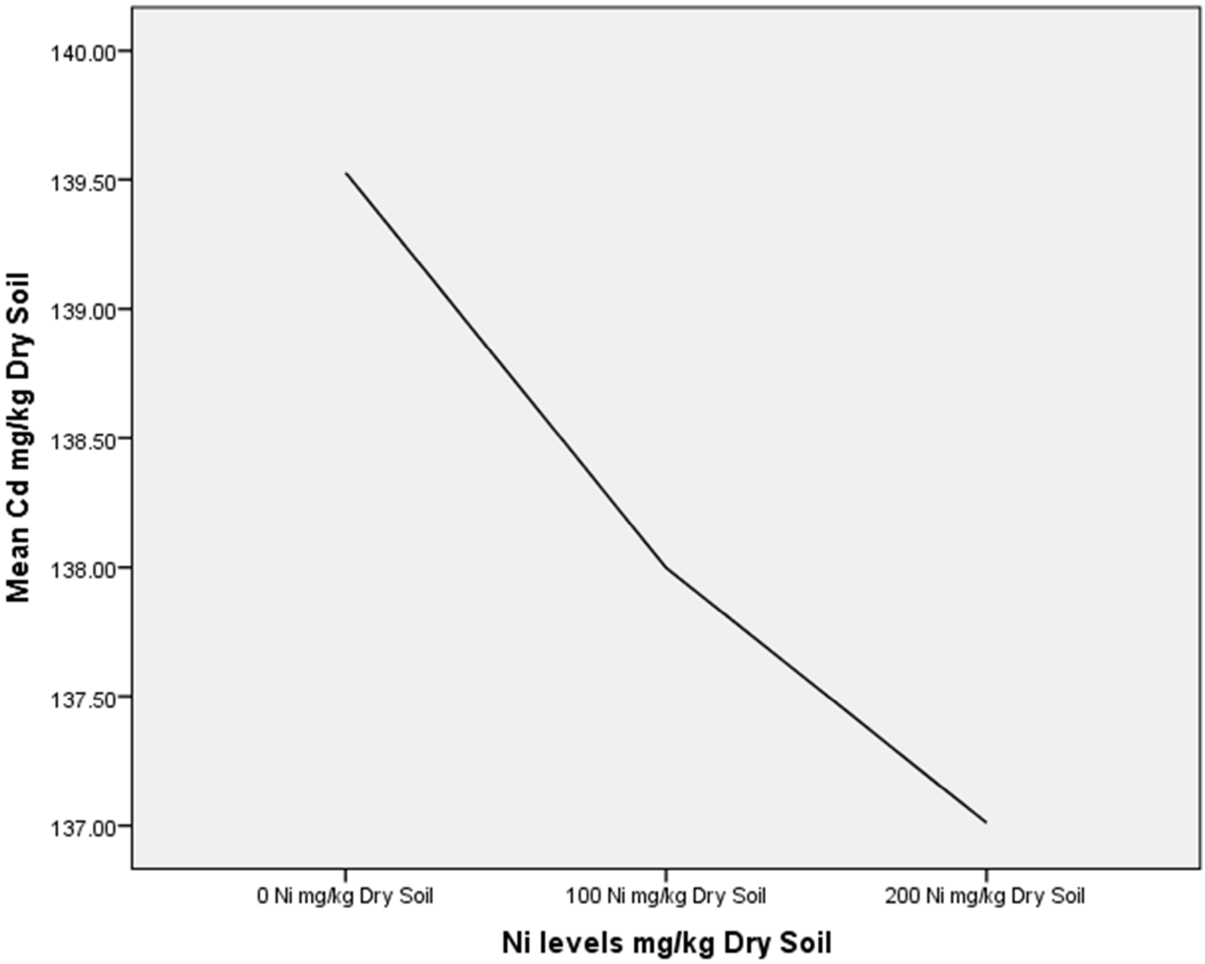

Figure 10. *Line chart of the effects of nickel treatment on cadmium uptake in roots.*

According to Figures 3 and 4, in the evaluation of Duncan Multi-Range Test regarding the effect of changes in different amounts of cadmium accumulated in the roots (cadmium treatment levels), it was evident that with the increase of cadmium values, the amount of accumulation and absorption of cadmium in roots, showed a significant difference in the level of 95%. Which imply cadmium absorption in the roots increases with the increase of its treatment levels, which is consistent with the analysis of variance results for the cadmium independent variable (Table 3).

According to Figures 5 and 6, in the evaluation of Duncan Multi-Range Test regarding the effect of changes in different amounts of lead added to the soil in pots (lead treatment levels) on the absorption rate of cadmium by the plant roots, it was determined that with the increase of lead values (lead treatment levels), in the accumulation and lead absorption by *Vetiver* plant roots, showed a significant difference in the level of 95%, that showing a decreasing trend in the absorption of cadmium in the roots with the increase of lead treatment levels, which is consistent with the results of analysis of variance for cadmium dependent variable in the presence of lead and its effect on cadmium absorption in roots (Table 3).

Considering the result shown in Figures 7 and 8, in evaluating Duncan Multi-Range Test regarding the effect of changes in different amounts of manganese added to soil pots (manganese treatment levels), on the adsorption rate of cadmium by the plant roots, it was determined that with the increase of manganese values (manganese treatment levels), there is no significant difference in the accumulation and cadmium absorption by *Vetiver* plant roots, and with the increase of manganese treatment levels, not much effect was observed in the absorption of cadmium, which is consistent with the results of analysis of variance for cadmium dependent variable in the presence of manganese and its effect on cadmium absorption (Table 3).

Based on data depicted in Figures 9 and 10, in evaluating Duncan Multi-Range Test regarding the effect of changes in different amounts of nickel added to soil pots (nickel treatment levels), on the adsorption rate of cadmium by the plant roots, it was determined that with the increase of nickel values (nickel treatment levels), in the accumulation and cadmium absorption by *Vetiver* plant roots, showed a significant difference in the level of 95%, which shows a slight decrease in the absorption of cadmium with the increase of nickel treatment levels, thus it had negative effects on the absorption of cadmium, which is consistent with the results of analysis of variance for cadmium dependent variable in the presence of nickel and its effect on cadmium absorption in the roots (Table 3).

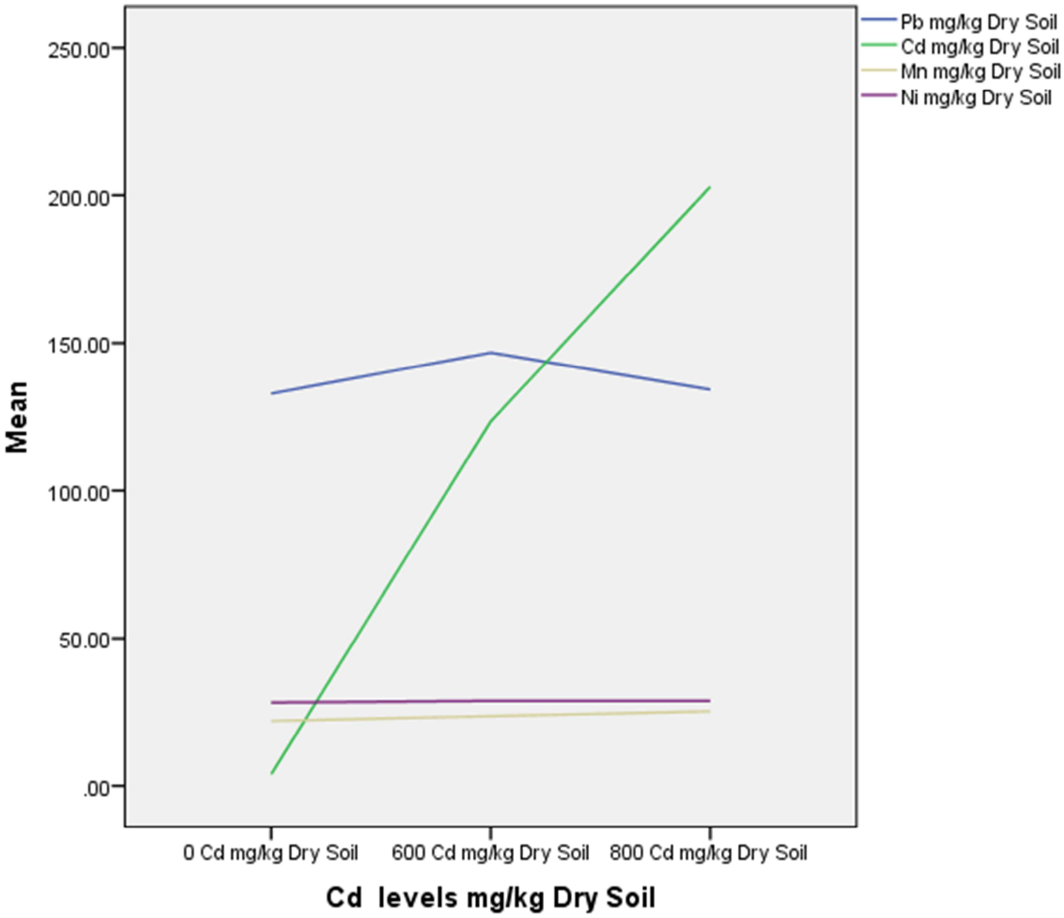

Figure 11. *Line chart of the effects of heavy metals treatment on cadmium uptake in shoots.*

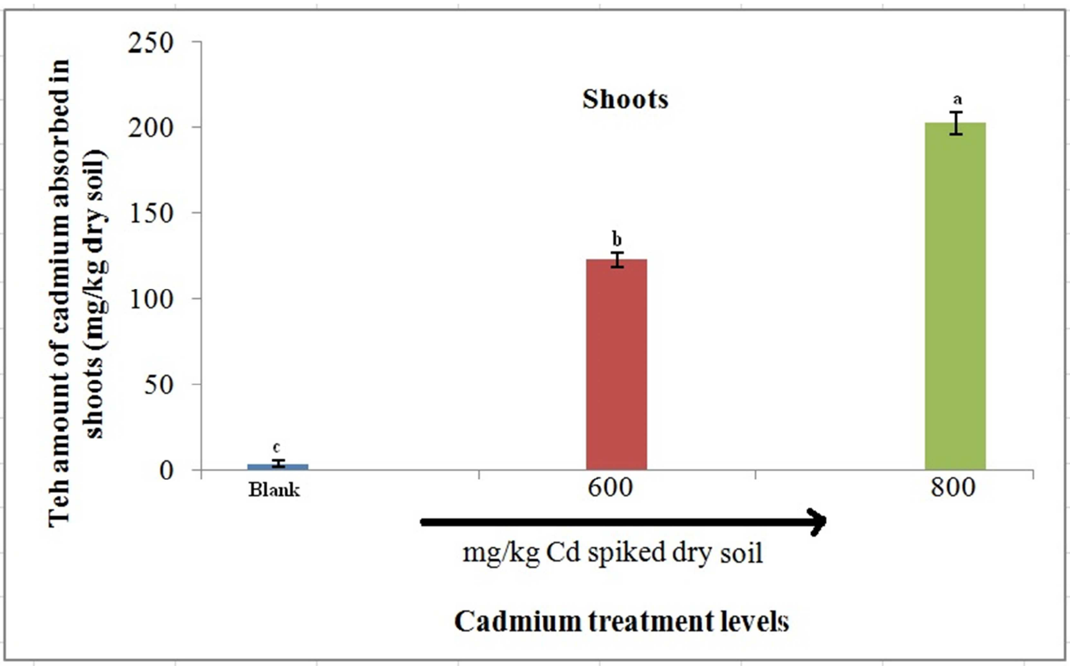

Figure 12. *Effect of cadmium treatment levels, on cadmium uptake in shoots.*

* Different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in cadmium absorption.

* Each number in the graph is average of three repetitions.

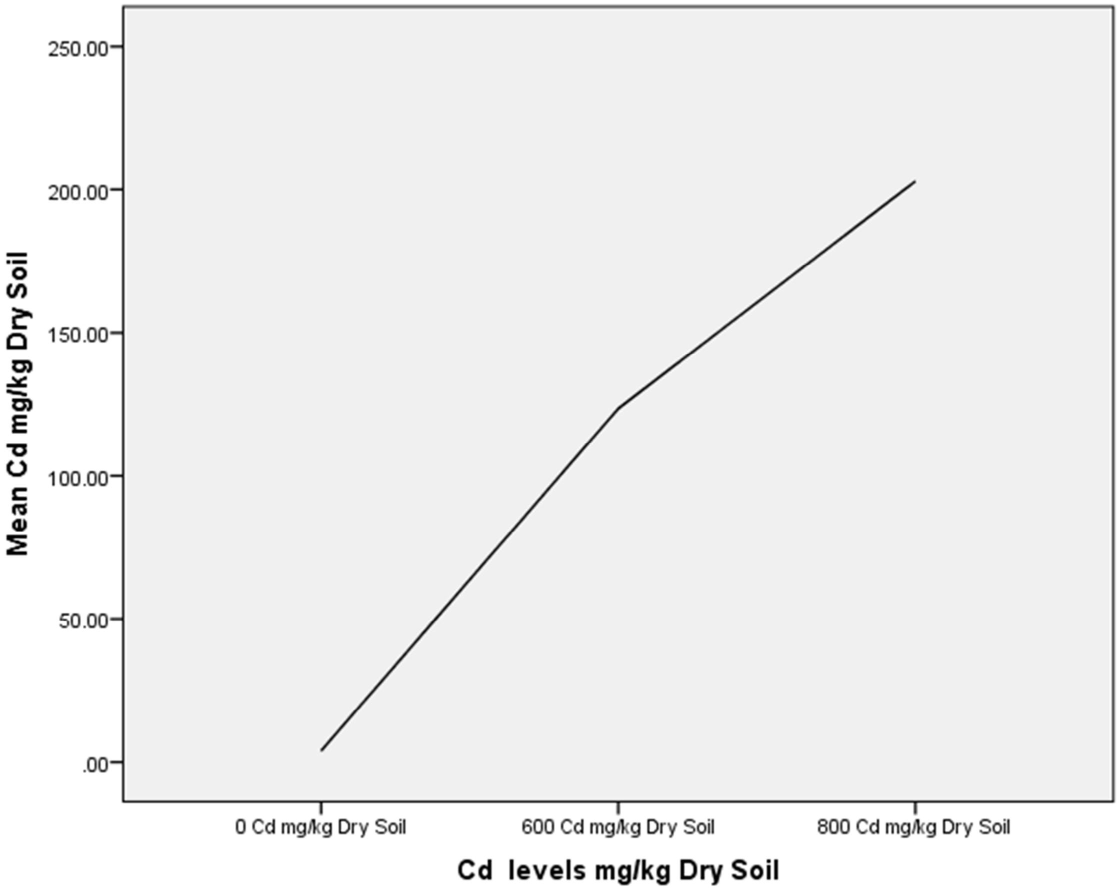

Figure 13. *Line chart of the effects of cadmium treatment on cadmium uptake in shoots.*

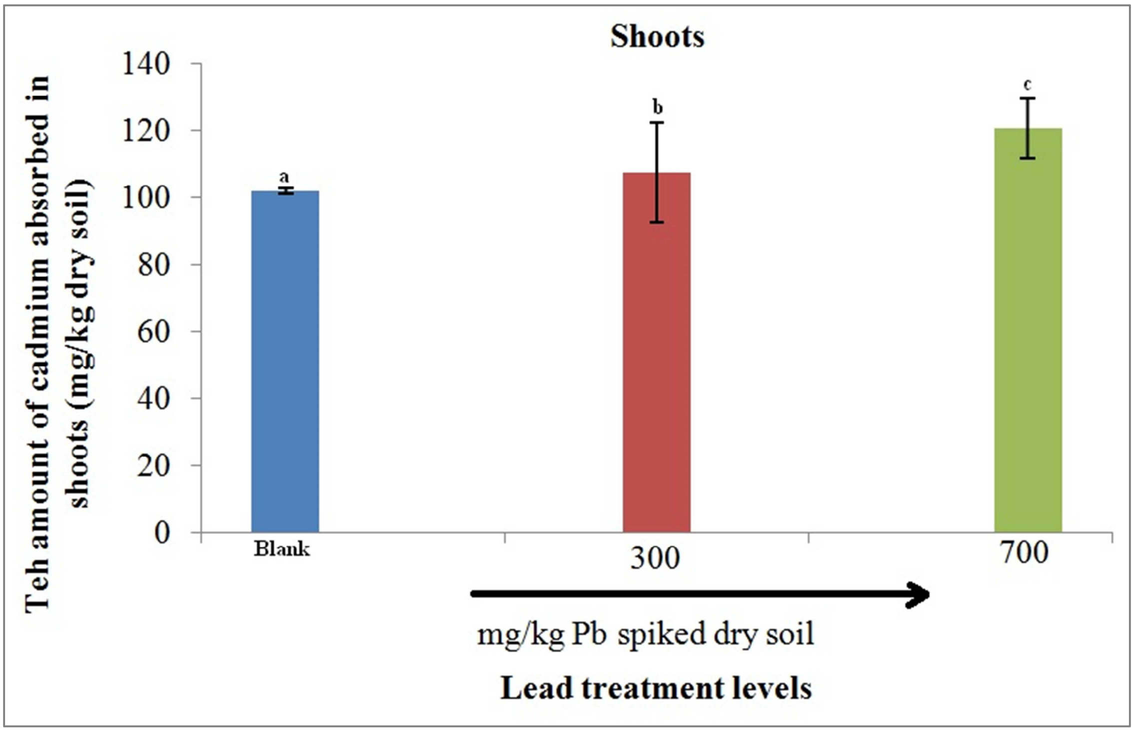

Figure 14. *Effect of lead treatment levels, on cadmium uptake in shoots.*

* Different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in cadmium absorption.
* Each number in the graph is average of three repetitions.

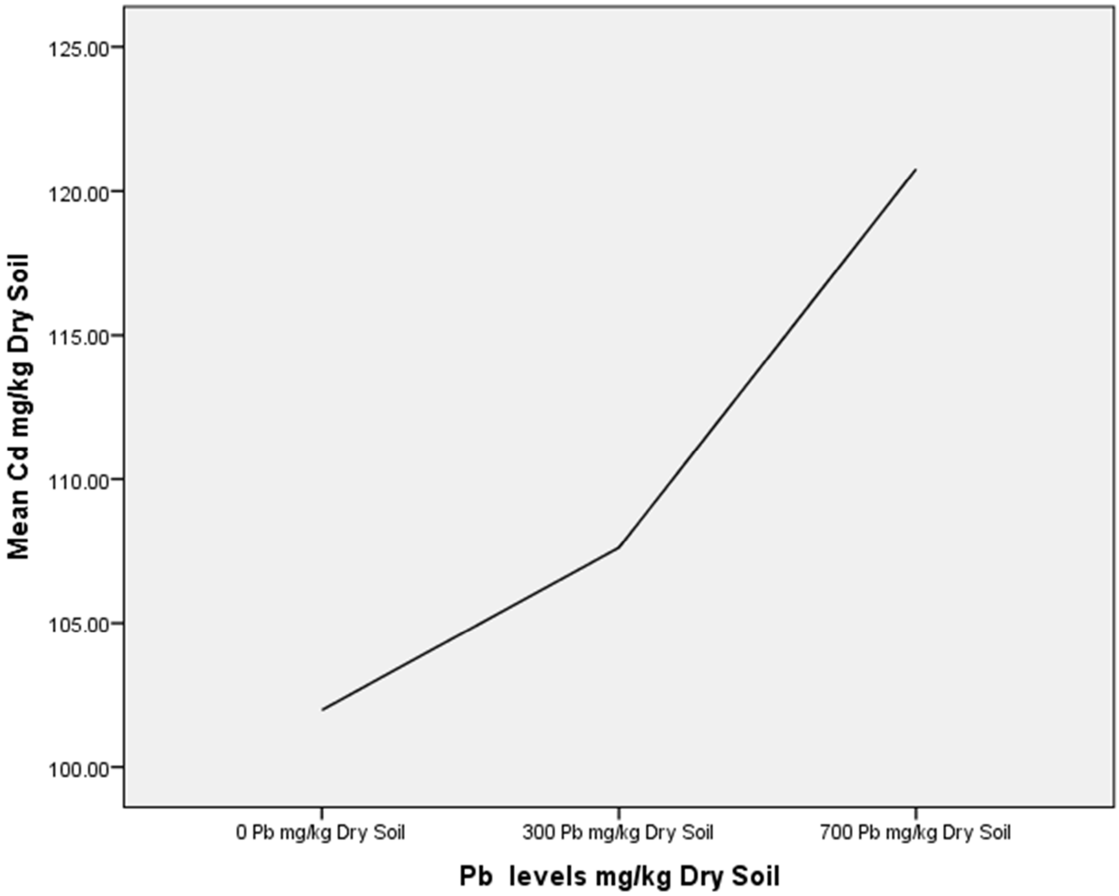

Figure 15. *Line chart of the effects of lead treatment on cadmium uptake in shoots.*

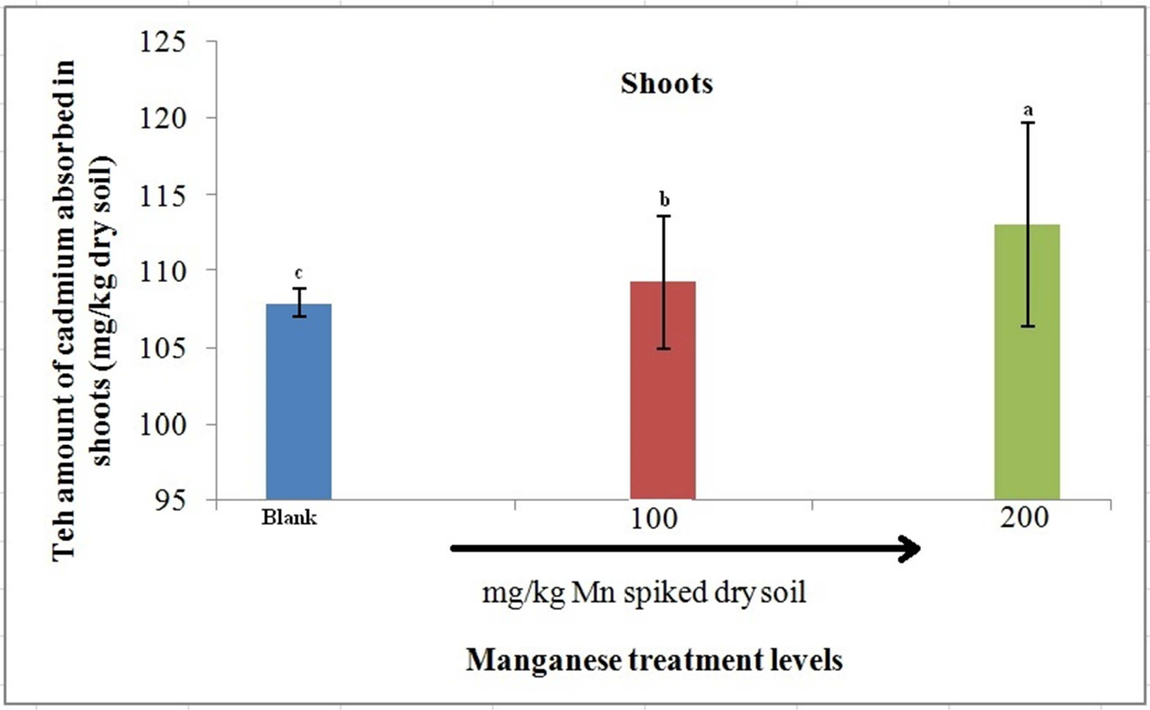

Figure 16. *Effect of manganese treatment levels on cadmium uptake in shoots.*

* Different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in cadmium absorption.

* Each number in the graph is average of three repetitions.

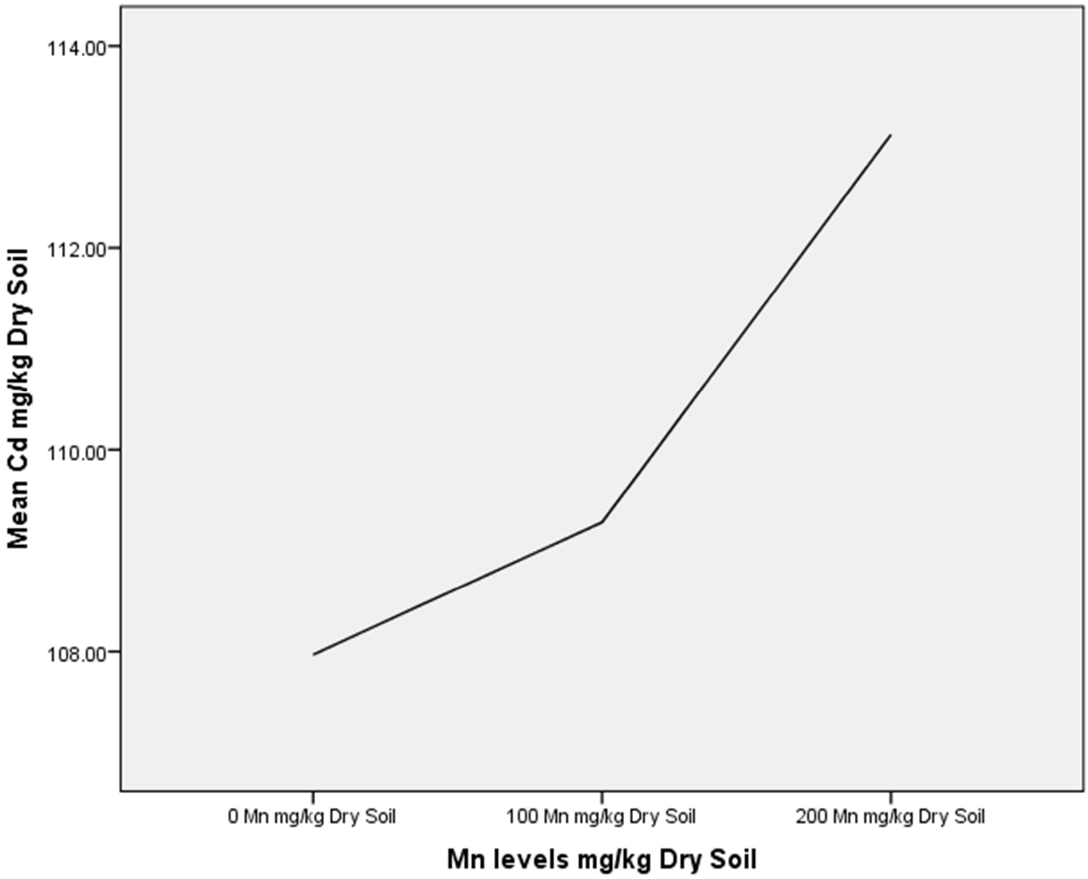

Figure 17. *Line chart of the effects of manganese treatment on cadmium uptake in shoots.*

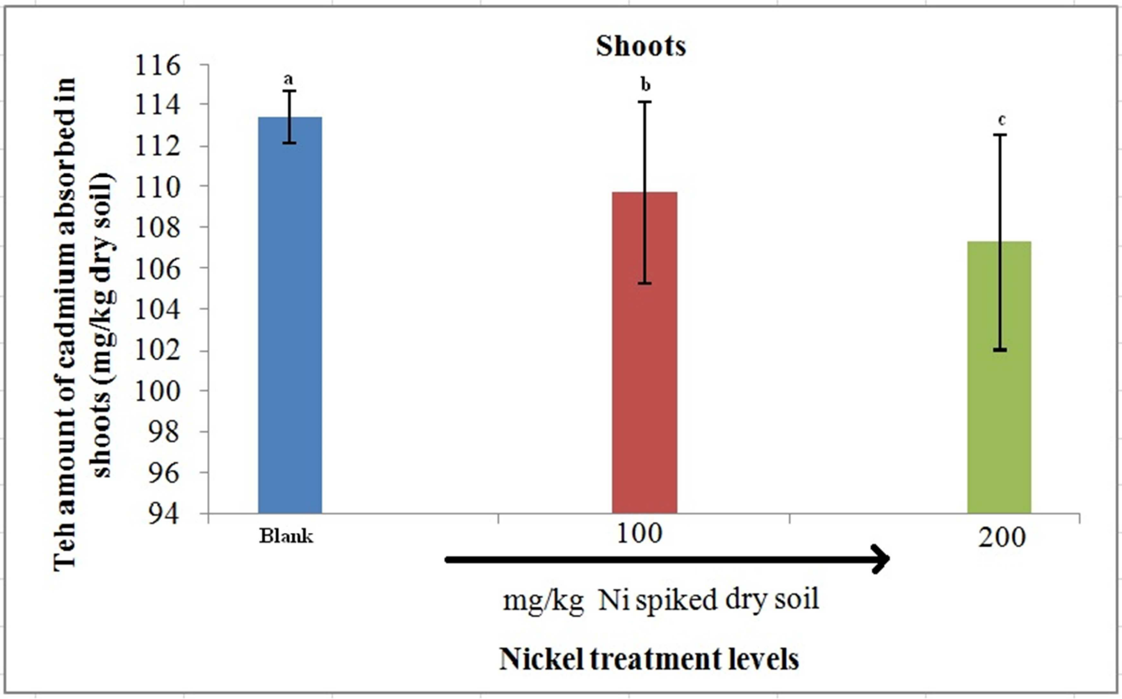

Figure 18. *Effect of nickel treatment levels, on cadmium uptake in shoots.*

* Different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in cadmium absorption.
* Each number in the graph is average of three repetitions.

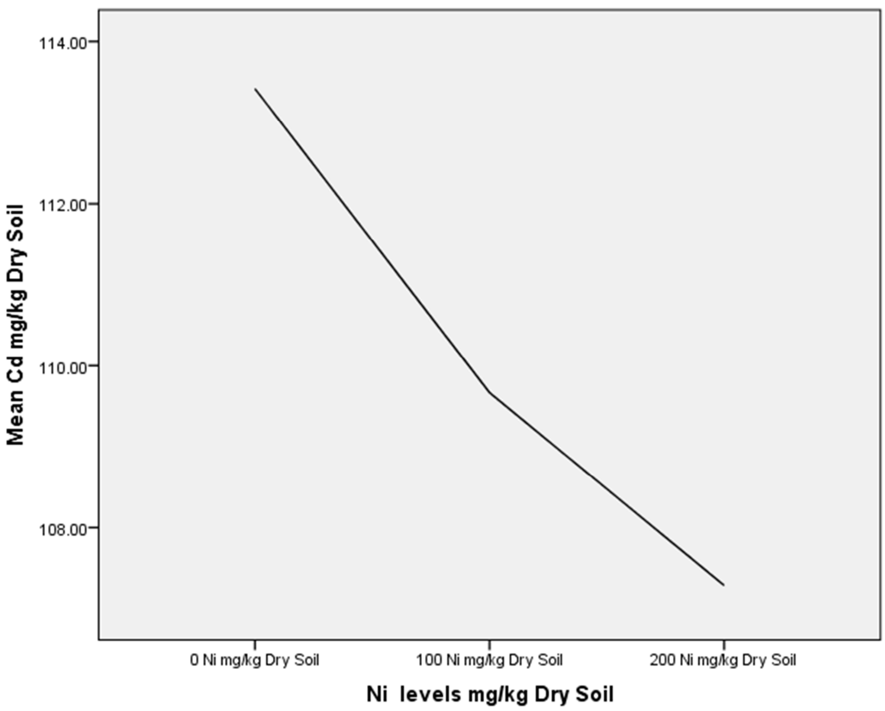

Figure 19. *Line chart of the effects of nickel treatment on cadmium uptake in shoots.*

According to Figures 12 and 13, in the evaluation of Duncan Multi-Range Test regarding the effect of changes in different amounts of cadmium accumulated in the shoots (cadmium treatment levels), it was evident that with the increase of cadmium values, the amount of accumulation and absorption of cadmium in shoots, showed a significant difference in the level of 95%, which indicates that cadmium absorption in shoots increases with the increase of its treatment levels, which is consistent with the analysis of variance results for the lead independent variable (Table 4) as well as Duncan's test for the roots (Figure 3).

According to Figures 14 and 15, in the evaluation of Duncan Multi-Range Test regarding the effect of changes in different amounts of lead added to the soil in pots (leadtreatment levels) on the absorption rate of cadmium by the shoots, it was determined that with the increase of leadvalues (leadtreatment levels), in the accumulation and cadmium absorption by *Vetiver* plant roots, showed a significant difference in the level of 95%, showing a decreasing trend in the absorption of cadmium in the shoots with the increase of leadtreatment levels, which is consistent with the results of analysis of variance for cadmium dependent variable in the presence of lead and its effect on lead absorption in the shoots (Table 4).

Based on data depicted in Figures 16 and 17 in evaluating Duncan Multi-Range Test regarding the effect of changes in different amounts of manganese added to soil pots (manganese treatment levels), on the adsorption rate of cadmium by the shoots, it was determined that with the increase of manganese values (manganese treatment levels), in the accumulation and cadmium absorption by *Vetiver* shoots with, showed a significant difference in the level of 95%. This shows the increase of cadmium absorption in the shoots as a result of increase in manganese treatment levels.

Based on data depicted in Figures 18 and 19, in evaluating Duncan Multi-Range Test regarding the effect of changes in different amounts of nickel added to soil pots (nickel treatment levels), on the adsorption rate of cadmium by the shoots, it was determined that with the increase of nickel values (nickel treatment levels), in the accumulation and cadmium absorption by *Vetiver* plant shoots, showed a significant difference in the level of 95%, which shows an decrease in the absorption of cadmium in shoots as a result of increasing nickel treatment levels, and contrary to Duncan's test for the roots regarding the effect of nickel treatment levels on roots absorption which had an increasing trend (Figure 9), an decreasing trend was observed for the shoots.

Table 5. *Total average of adsorption and the effect of various heavy metals treatments on biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF) and transfer efficiency factor.*

Treatment	BCF (total-roots/shoots)	BAC	TF
	Total Bio Concentration Factor	**Bio Accumulation Factor**	**Translocation Factor**
Lead	5.02	2.45	0.95
Cadmium	1.13	0.5	0.796
Manganese	1.67	0.0625	0.597
Nickel	2.16	0.89	0.7

Table 6. *Mean root and shoot length in the presence of lead treatment levels.*

Lead levels (mg/kg Dry Soil)	Total plant height	Root length	Shoot length
	Standard deviation ± mean	**Standard deviation ± mean**	**Standard deviation ± mean**
Blank	5.96 ± 173.86	2.94 ±44.35	3.23 ±129.53
Level 2 treatments, 300 mg / kg dry soil lead nitrate	3.38 ± 167.10	1.54 ±42.33	1.89±124.77
Level 2 treatments, 700 mg / kg dry soil lead nitrate	4.91 ±161.67	2.01 ±40.14	3.05 ±121.55

Table 7. *Mean root and shoot length in the presence of cadmium treatment levels.*

Cadmium levels (mg/kg Dry Soil)	Total plant height	Root length	Shoot length
	Standard deviation ± mean	**Standard deviation ± mean**	**Standard deviation ± mean**
Blank	5.84±171.3	2.87± 43.84	3.14 ± 127.46
Level 2 treatments, 600 mg / kg dry soil cadmium nitrate	5.69 ± 161.58	2.81±41.43	3.00 ±120.17
Level 2 treatments, 800 mg / kg dry soil Cadmium nitrate	6.01 ± 158.78	2.65±39.56	3.55±117.24

Table 8. *Mean root and shoot length in the presence of manganese treatment levels.*

manganese levels (mg/kg Dry Soil)	Total plant height	Root length	shoot length
	Standard deviation ± mean	**Standard deviation ± mean**	**Standard deviation ± mean**
Blank	4.67±172.68	2.76±44.52	2.10±128.16
Level 2 treatments, 100 mg / kg dry soil manganese nitrate	3.24±165.92	1.56±42.28	1.89±123.63
Level 2 treatments, 200 mg / kg dry soil manganese nitrate	405± 161.4	1.89± 40.3	2.30±121.1

Table 9. *Mean root and shoot length in the presence of nickel treatment surfaces.*

nickel levels (mg/kg Dry Soil)	Total plant height	Root length	shoot length
	Standard deviation ± mean	**Standard deviation ± mean**	**Standard deviation ± mean**
Blank	6.07±172.06	2.95 ±44.1	3.28 ±127.96
Level 2 treatments, 100 mg / kg dry soil manganese nitrate	5.84± 164.49	2.75 ±41.39	3.26 ±123.09
Level 2 treatments, 200 mg / kg dry soil manganese nitrate	5.67 ±161.08	2.63 ±40.07	3.20 ±121.01

3.1. Morphological Changes of Vetiver in the Presence of Heavy Metal Treatments During the Growing Season

In the study of plant morphology, with different levels of heavy metal treatments, the highest average height of total plants was related to lead, manganese, nickel and cadmium treatments with values of 167.54, 166.66, 165.85 and 163.88 Cm, respectively. The highest average root roots of plants were related to lead, manganese, nickel and cadmium treatments with values of 42.27, 42.36, 41.85 and 41.61cm, respectively. the highest average root roots of plants were related to the treatments of lead, manganese, nickel and cadmium with values of 125.28, 124.29, 124.02 and 121.68 Cm, respectively. Therefore, among all treatments, plants treated with cadmium had the lowest growth.

Based on the data in Tables 6 to 9, related to the average root and shoot length in the presence of the levels of treatments used (levels of lead, cadmium, manganese and nickel) was observed that with increasing levels of treatment, root and shoot length as well as total height of *Vetiver* plant, there are significant differences at 95% level. With increasing levels of treatments, limb length (roots and shoots) decreased significantly and the highest reduction in limb length was related to the third level of treatments and the lowest reduction in limb length was related to the first level or the control plant (without increasing pollution). The highest reduction in limb length was related to cadmium treatment and the lowest limb length reduction was related to lead treatment.

Table 10. *Single and combined effects, different treatments and their interaction on Vetiver plant height.*

Fs (F Calculated)	MS ± S (Mean Square ± Standard Deviation)	Sources of changes	Sig
Effect of Lead	37.48±1405.363	3177.132	0.000
Effect of Cadmium	47.81±2285.986	5158.214	0.000
Effect of Manganese	11.41±130.366	294.165	0.000
Effect of Nickel	3.46±119.780	270.279	0.000
Effect of Lead/Cadmium Interaction	8.18±66.765	150.651	0.000
Effect of Lead/Manganese Interaction	2.02±4.095	9.239	0.000
Effect of Lead/Nickel Interaction	2.65±7.041	15.888	0.000
Effect of Cadmium/Manganese Interaction	1.14±1.321	2.982	0.01
Effect of Cadmium/Nickel Interaction	0.762±0.581	1.310	0.268
Effect of Manganese/Nickel Interaction	0.910±0.829	1.870	0.118
Effect of Lead/Cadmium/Manganese Interaction	4.39±19.310	43.537	0.000
Effect of Lead/Manganese/Nickel Interaction	1.89±3.421	7.720	0.000
Effect of Lead/Cadmium/Nickel Interaction	0.57 0.321±	0.723	0.671
Effect of Cadmium/Manganese/Nickel Interaction	0.764±0.584	1.317	0.238
Effect of Lead/Cadmium/Manganese/Nickel Interaction	0.694±0.483	1.091	0.368
Error	0.665±0.443		

3.2. Single and Combined Effect of Different Treatments of Heavy Metals (Lead, Cadmium, Manganese and Nickel) and Their Interaction on the Height of Vetiver Plant

According to Table 10, analysis of variance, single and combined effects, different treatments and their interaction on the height of *Vetiver* plants. It has been shown that the effect of lead levels on plant organ height is significantly different at the 99% level. Also, in the Investigation of analysis of variance, the effect of the levels of cadmium, manganese and nickel on the height of plant limbs is significantly different at the level of 99%. In the analysis of variance analysis table, the interaction effects of the presence of two heavy metal treatments, the interaction of lead and cadmium, lead and manganese, lead and nickel on the height of plant organs, there is a significant difference at the level of 99%. In the analysis of variance analysis table, the interaction effects of the presence of two heavy metal treatments, the interaction of cadmium and manganese on the height of plant organs, there is a significant difference at the level of 95%. In the analysis of variance analysis table, the interaction effects of the presence of two heavy metal treatments of the interaction of cadmium and nickel, manganese and nickel on the height of plant organs are not significantly different. In the analysis of analysis of variance, the interaction effects of the presence of three treatments of heavy metal, the interaction of lead, cadmium and manganese, lead and manganese and nickel, the height of plant limbs there is a significant difference at the level of 99%. In the analysis of variance analysis table, the interaction effects of the presence of three heavy metal treatments, the interaction of lead, cadmium, manganese and nickel, on the height of plant organs, there is no significant difference. In the analysis of variance analysis table, the interaction effects of the presence of three heavy metal treatments of the interaction of lead, cadmium, manganese and nickel, on the height of plant limbs, there is no significant difference.

4. Discussion

4.1. Removal of Heavy Metals (Pb, Cd, Mn, and Ni) from Con-taminated Soil

The plant was uptakes heavy metals and accumulate in its different parts such as root, stem and leaves. Therefore, different parts of the selected pants were analyzed for these metals. After assessment of tests and collection, it was seen that Pb, Cd, Mn, and Ni are accessible in all of them in any case, its aggregate varies in plant parts and besides. Pb, Cd, Mn, and Ni are available in all of them regardless of its all-out changes in plant parts Regular appraisals of HMs contents in different models uncovered that the plant showed beneficial take-up of HMs and moved it in various plant tissues. The order of HMs accumulation in plant body parts was in order fol lowed, Root > shoot. All outcomes by then were differentiated, and the previous works the metal storing up in various parts were starting late focused by various investigation scientists as Li DZ, et al. Lin, et al. Turner, et al. [5, 16, 34, 70]. The Shin, et al. [59] examined accumulations of metals in plant roots of the Alnus nepalensis. The watched decline in dry issue crea-tion because of metal weight is in concurrence with that revealed prior in plants other than Brassica júncea plant [52, 65]. Cd contents in different models uncovered that the plant appeared to be productive take-up of Cd and move it in various tissues of the plant. Accumulation of Cd in plant parts were ordered in followed Root>Shoot.

4.2. Accumulation, Translocation, Bioaccumulation Factors of Pb Cd, Mn, and Ni in Plants Tissues

The plant was observed and thus suggest that *Vetiver* plant could prove useful for Phyto- stabilization for Pb as well for Cd, Mn, and Ni As can be seen by the bioconcentration factor (BCF) values of more than one in the shoot and root of the plant as well as the transfer factor (TF) of less than one. Details are given in (Table 5).

Translocation factor is of particular importance in the remediation technique for plants since shoots are harvested through this technique. Translocation factor in plant species and cultivars must be higher than 1 for the remediation technique. In other words, concentration of heavy metals in shoots must be higher than in roots. In this study, it was found that the transfer factor was less than one and close to one in all heavy metal treatments (Table 5).

4.3. The Effect of Heavy Metal Treatments on Plant Growth

Treatments of the soils with Pb, Cd, Mn, and Ni in this study resulted in a reduction in root length, and root and shoot. Also, In the presence of these metals, their concentrations in roots and shoots increased. Heavy metal stress including that Pb, Cd, Mn, and Ni are one the most important limiting factors for root growth which is related to the reduction in cell division and elongation [29, 42, 44].

Heavy metals stop plant growth in various ways. On the one hand, heavy metals reduce cell division and control its growth by reducing cell turgescence [11, 44] On the other hand through accumulation on cell walls and entering cytoplasm and then disturbing normal metabolism in cells, they reduce the growth in them [29, 42, 45, 78].

High levels of Pb, Cd, Mn, and Ni reduced the growth in plant shoots. It is likely that heavy metals limit the growth in plant roots and stems directly by controlling cell division or elongation or a combination of both [73]. Reduced growth of roots and shoots under influence of Pb, Cd, Mn, and Ni is already reported by other researchers like Mohammadzaeh et al [40]. As a result of the reduction in root growth, the uptake of water and mineral ions is reduced with a consequence of reduction in the plants' general growth [44, 63, 78].

Plants absorb Pb and accumulate in plant tissues because they are not metabolized [38] which will affect metabolic activity. The main effect of lead poisoning on plants is inhibition of root growth, because it inhibits cell division that occurs at the root tip. This shows that Pb inhibits cell division at the roots of several plant species, including Triticuma estivum [27, 32, 39, 75], decrease in root length and dry weight due to lead toxicity. Decreased photosynthetic pigment content because lead inhibits chlorophyll synthesis by interfering with the absorption of important photosynthetic pigment elements, such as Mg and Fe [54], Photosynthetic organs are also damaged because chlorophyllase activity is limited under abundance of lead, also causes an increase in chlorophyll destruction in this condition chlorophyll a which is more influential than chlorophyll b Photosynthetic organs are also damaged because chlorophyllase activity is limited under abundance of lead, also causes an increase in chlorophyll destruction in this condition chlorophyll a which is more influential than chlorophyll b [15].

Lead accumulation in roots is higher than leaves in *Vetiver* plant plants with TF <1. Lead is very low in solubility, and has low translocation power from roots to other plant organs [10]. Lead acts as mobility in the process of absorption of metals from the roots of plants to the leaves to form complex compounds [67]. Following the flow of transpiration to the upper part of the plant through the tissues mainly through the xylem vessels and subsequently carried throughout plant parts by phloem where metals are stored in vacuoles. So that it will be carried to the plant tissue.

A heavy metal, nickel plays an important role in plants. While it has no toxic effect on plants at low concentrations, nickel is poisonous for plants at high concentrations [14, 44, 78]. Nickel causes chlorosis and necrosis in cereals with white strips in leaves [44, 57, 78]. Excessive nickel may disturb electron transport chain during photosynthesis and prevent electron establishment and stomatal transactions [17]. In plants under nickel stress, uptake of minerals, root growth, cell metabolism, photosynthesis, and respiration are heavily disturbed [35, 78]. Reduced biomass is also reported in plants such as wheat and Jatropha curcas under high concentrations of nickel [74, 44]. There are many studies suggesting that plants can redeem nickel from the environment and accumulate it in their roots and shoots [1].

Manganese is an essential trace element in plants' nutrition which because of the possibility of interchanges in its oxidation forms plays an important role in redox (oxidation and reduction). It is toxic for plants at high concentrations and in some soils such as acid and volcanic soils, excessive reduction of this element results in manganese toxicity in many agricultural and rangeland soils [64]. Symptoms of manganese toxicity are first observed in leaves and in many plants such as barley, green pea, sunflower, and beans, these symptoms include dark brown spots on old leaves [41]. Excessive concentration of manganese is reported to reduce yield, yield components, and photosynthesis in plants.

Considering all the sources of cadmium contamination in soils, the main sources of pollution are atmospheric precipitation and phosphate fertilizers. Application of phosphate fertilizers increases the concentration of cadmium in the soil solution. However, the increase in cadmium may be due to its release from soil tissue [29, 49, 78].

Although cadmium is not essential for plant growth, it is readily absorbed through the root bark, and enters the wood tissue through the membrane or intra-membrane wire or apoplasty. Studies have shown that cadmium affects cell division and growth, overall plant growth, cell division in the meristem area, and regulates plant growth [29, 49, 76, 78]. Decreased levels of total chlorophyll, a and b, carotenoids in plants and disorders of carbohydrate metabolism. The most important cause of the destructive effect of cadmium is that it produces reactive oxygen radicals such as superoxide (-2O) hydroxide (-OH) and hydrogen peroxide free radicals. These radicals are produced in organisms with aerobic metabolism through electron-transmitting organs such as mitochondria, chloroplasts, and plasma membranes. These radicals react rapidly with DNA, fats and proteins, and cause cell destruction. Plants use enzymatic antioxidants (such as superoxide dismutase, catalase, peroxidases, etc.) and non-enzymatic (such as glutathione, ascorbate, volatile carotenoids, and proline) to counteract these free radicals [29, 49, 58, 60]. Among non-enzymatic antioxidants, proline is of particular importance. Proline is an antioxidant that scavenges free radicals and prevents toxicity by binding to cadmium and forming a cadmium-proline complex. In general, cadmium reduces tolerance to water stresses and reduces cell wall electricity. Cell degradation in woody tissues due to reduced water transfers. According to Tables 6 to 9 cadmium has reduced growth in roots and shoots in Vetiver plants.

4.4. Plant Defense Mechanism Against the Effects of Heavy Metals

The mechanism of uptake of metals from the roots is such that plants that are highly absorbent of metals release protons around their rhizosphere, which acidify the soil, increase the mobility of metal ions, and make it available to the roots. they give. But charged ions can pass through the cell's lipid membrane and enter the cell through transport proteins that attach to metal ions and transport them from the extracellular space to the cell. Natural chelates also bind metal ions to unload them, one of which is EDTA. The high accumulation of metal ions and quasi-metals in the plant occurs in coordination with several processes. These processes include intensifying the adsorption of metal ions to the plant, effective transfer of metals from the root to the stem and effective detoxification of these metals in the leaves [29, 77].

Root secretions have a variety of roles, chelating metals that may increase the absorption of certain metals. Apoplast is the first site of metal uptake in the root [29, 49]. Some of the metals adsorbed to the apoplast attach to cell wall compounds. In the cell wall, pectins such as polygalacturonic acid and its negatively charged carboxyl groups act as cation exchangers. The other part of the adsorbed metals is transferred to the hydroponic part of the apoplast and some of them are transferred to the cytoplasm through the plasma membrane. Plants have different mechanisms for absorbing heavy metals [29, 49]. Among amino acids, proline is more sensitive to environmental stresses. Increasing proline causes the cell to adapt more to the stress conditions and protects cytosolic enzymes and cell structures. Proline has several cellular roles, stabilizing proteins, protecting against cold, and regulating redox potential. Proline accumulates mainly in the cytoplasm to balance the osmotic potential of the vacuole, such as pH adjustment. Many studies have been done on the pathways of biosynthesis and catabolism of proline. Vetiver plant, like other plants, seems to be one of the ways to increase the amount of free proline in order to defend against the stress caused by the heavy metal cadmium.

Investigation of Vetiver morphological changes in different concentrations of applied treatments:

In general, changes in root morphology due to increasing concentrations of heavy metals lead, cadmium, manganese and nickel and changes in root structure reduce nutrient uptake and lead to reduced growth [24]. Reduction of sub-branches of parsley root due to increase in nickel concentration, change in root colour and decrease in root diameter are among the effects of nickel on Petroselinumcrispum, which has been confirmed in other plants [41]. Toxic concentrations of nickel, cadmium and manganese have had a negative effect on physiological processes such as transpiration, respiration, photosynthesis and ultimately reduced plant growth by changing the membrane structure of root cells and reducing water absorption levels [24]. Heavy metals have a negative effect on root structure and functions and reduce water and salt uptake levels, reducing water uptake and creating secondary drought stress in plants [12]. A study on Petroselinumcrispum found that high concentrations of nickel had a significant effect on root length, so that with increasing nickel concentration, root length decreased [28]. In a study of barley and rice, Yang et al. Reported that the root length of these plants decreased with increasing nickel concentration [74]. Papazoglu et al. In a study on Arundodonax stated that with increasing nickel and cadmium, root length decreases. Due to the effect of copper and cadmium on Pinus and Pinuspinaster pineapple seedlings, Ardini et al. Noticed a decrease in root length due to the increase in the concentration of these metals [20]. In a study of Pterisvittata, Bafeigo et al. Found that with increasing heavy metals, root length decreases. Hartley et al. Studied the effect of heavy metals on Pinussylvestris seedlings and stated that root length decreases with increasing concentration of heavy metals. Lead toxicity reduces vegetative growth [56]. Lead toxicity is due to the fact that it mimics many aspects of calcium metabolic behavior and inhibits the activity of many enzymes. Also, the decrease in general plant growth due to the increase in the concentration of heavy metals has been confirmed in several studies. Peralta et al. suggested that root damage caused by heavy metals was the main cause of reduced plant growth [61]. The results of this study are consistent with the results of other researchers. In this study, the effect of heavy metal treatments (lead, cadmium, manganese and nickel) alone and their combined effects on the height of Vetiver limbs were statistically significant at the level of 1% (Tables 6 to 9). Heavy metal stress caused a significant decrease in growth traits and in general, increasing the concentration of heavy metals (lead, cadmium, manganese and nickel) caused a decrease in shoot length and root depth. And the greatest decrease in organ growth was related to cadmium treatment and then manganese, lead and nickel, respectively (Tables 6 to 9). Numerous studies have shown that when plants are exposed to high concentrations of heavy metals, their shoot and root length decreases [17]. Which was also proved in this study (Tables 6 to 9). Heavy metals cause visible damage such as chlorosis (yellowing) and necrosis (brown) in plant leaves. And reduce the length and browning of the roots [36]. Suggested that the inhibition of heavy metals on shoot and root length and leaf area could be mainly due to abnormal cell division and may also depend on the inhibition of metals by photosynthetic and respiratory processes in the stem system and root protein synthesis. Or due to reduced cell division and growth. Decreased plant growth may be due to reduced photosynthesis. Because it has been shown that exposing plants to high concentrations of heavy metals reduces photosynthesis. Damage to photosynthesis occurs mainly due to a decrease in chlorophyll and an increase in lipid peroxidation. In this study, it seems that heavy metals by affecting the photosynthesis of the plant has caused shortening of height in the plant and on the other hand Vetiver, releasing a large amount of proline has minimized this lack of growth and shortening of limbs (Tables 6 to 9).

5. Conclusion

According to the results, high adsorption of heavy metal treatments, biological concentration factor (BCF) greater than 1 and acceptable translocation factor (TF), and morphological growth of plants against high treatments of heavy metals, Vetiver can be considered as an important plant in phytoremediation due to high adsorption and Tolerance to adverse environmental conditions to be used in the treatment of pollution.

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