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Evaluation of Changes in the Uptake of Heavy Metals in Leachate using Vetiver Phytoremediation

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Abstract

The aim of this study was to evaluate the potential of uptake of heavy metals from waste leachate to determine the amount of uptake of lead, cadmium, manganese and nickel by vetiver was performed in greenhouse conditions. This research it was performed Based on a completely random design in three replications with four treatments Includes leachate, 0, 30, 60 and 100%. Data analysis was performed with Spss 19 software, comparison of mean treatments with analysis of variance and Duncan test at 1 % probability level and plotting of graphs with Excel software. The results showed that the uptake of heavy metals by the plant, There is a significant difference at the 99% level. Also by increasing leachate treatment levels, Root and shoot length, There is a significant difference at the 99% level. And with increasing levels of leachate treatment, the uptake of heavy metals has increased. The highest root uptake was with an average of 200.21 mg/kg. And in the shoot, was 147.93 mg/kg in a total of four treatments. The highest rate of heavy metal uptake was related to 100% treatment with a total of 225.25 mg/kg in roots and 178.87 mg/kg in shoots for four metals lead, cadmium, manganese and nickel. Among the heavy metals absorbed in the roots and shoots, the highest levels were related to manganese, nickel, lead and cadmium, respectively. And manganese with an average of 123.88 mg, lead 91.08, nickel 79.69 and cadmium 53.49.27 mg/kg had the highest uptake by the plant. Also the biological concentration factor was more than one and the translocation factor was less than one The results showed that vetiver can be used as a Phytoestablization plant to purify contaminants. Vetiver can be considered as a refining plant due to its vegetative characteristics and cost-effectiveness.

Keywords: Phytoremediation; Vetiver; Waste Leachate; Heavy Metal

Introduction

Rapid development, an increase in population, rural-urban migration, affluence and the rate of consumption have brought about an increase in waste generation and pollution which has badly affected man and environment [1]. Landfilling is amongst the human activities that have completely changed the fate of the natural ecosystems [2-4]. Landfill leachate is generated when rainwater mixes with the waste in a landfill [5]. Landfill leachate is a potentially polluting liquid, which unless returned to the environment in a carefully controlle [6,8]. Leachate is defined as the aqueous effluent generated as a result of the filtration of rainwater through the wastes and the inherent water content of the wastes themselves deposited in landfills [7,9]. Landfill leachate is generally high in contaminants [10]. Especially heavy metals and organic and inorganic matter [8,11-14]. That can contaminate groundwater and surface water in the area near the landfill [2,8,11,14-16]. The effects on human health and ecosystems associated with heavy metal (HMs) pollution [17-19]. Become even more worrying given their tendency to accumulate and magnify along trophic levels

[20].

For example, due to their bioaccumulation they can have toxic effects on living organisms when they exceed a certain concentration [17,19,21-23]. Representing a risk to human health when transferred thought the food chain [17,18,24-28].

The leachate has a dark color, bad smell, and high organic and nitrogen loads. Leachate may carry immiscible liquids (e.g., oil), small particulates (suspended solids) and a range of organism (e.g., bacteria and viruses) [29]. Leachate may also contain a large amount of organic matter (biodegradable, but also refractory to biodegradation). Treatment for leachate is difficult because leachate contained heavy metals, humic substances, recalcitrant compounds and chlorinated organic and inorganic salt [30].

Pollution of water resources with heavy metals and other contaminants is becoming an alarming risk of global environment owing to their persistence, abundance and significant toxicity [31]. Therefore, precise management and treatment of the landfill leachate are necessary to prevent the detrimental effect that contaminants can bring into the environment before its final discharge [32].

For leachate treatment using conventional methods, it can be classified into three major groups: (a) leachate transfer: recycling and combined treatment with domestic sewage, (b) biodegradation: aerobic and anaerobic processes and (c) chemical and physical methods: chemical oxidation, adsorption, chemical precipitation, coagulation/flocculation, sedimentation/flotation and air stripping [33,34]. However, with the continuous hardening of the discharge standards in most countries and the aging of landfill sites with more and more stabilized leachates, conventional treatments (biological or physicochemical) are not sufficient anymore to reach the level of purification needed to fully reduce the negative impact of landfill leachates on the environment. In spite of being efficient, these methods are expensive, time-consuming and environmentally devastating. Moreover, these methods create soil deterioration. Therefore, developing new technologies that are low-cost and environmentally friendly is necessary [31,35]. Faced with this problem,, phytoremediation has emerged to be the green plant based cleanup solution that is able to remove, metabolize and degrade a wide range of hazardous soil HMs contaminants with minimum cost required and are non-destructive to the natural ecosystem [36,37].

Phytoremediation is an energy-efficient, cost-effective and aesthetically pleasing alternative to remediation sites with low to moderate levels of pollution [38-40]. It is a set of technologies that reduce in situ or ex situ the concentration of various compounds from biochemical processes carried out by plants and associated microorganisms [24,41-43]. In the phytoremediation method, plants and microbes 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, in phytoremediation, as a biotechnological strategy, plants are employed to extract and sequester complex pollutants from terrestrial or aquatic environments [34,44]. 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 [45,46].

Numerous plants have being studied over the years, with reports suggesting Vetiver grass, Vetiveria zizanioides (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 [47,48].

Vetiver grass is belongs to the Poaceae family and it is native to south and south-east Asia. Vetiver, a medicinally important perennial plant, known to control soil erosion, toleratesa wide range of pH and elevated levels of toxic metals [45,49]. Vetiver is hydrophilic terrestrial plant which has physiological characteristics like the ability to absorb dissolved nutrients such as N and P, reduce BOD, COD, TSS, oil spill, accumulate HMs, batik production wastewater, tofu production waste water, and high tolerance to herbicides and pesticides [50-52].

Recent studies by Singh, et al. [53] 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. Lead, cadmium, manganese and nickel elements are extremely toxic even at low concentration levels [54,55]. Although some phytoremediation studies have been carried out using Vetiver grass [53,56,57]. In this research, Vetiver grass (*Vetiveria zizanioides*) will be investigated in terms of its potential application in phytoremediation of soils contaminated with heavy metals (cadmium, lead, manganese, nickel) by leachate and, the method and rate of absorption of HMs in stems and roots of Vetiver plant has been investigated. The aim of this study was to determine the amount of adsorption of leachate HMs by the plant under study.and as well as compare the performance of different organs of the Vetiver plant (roots and shoots) in the absorption of leachate heavy metals from the soil in greenhouse conditions. Also, in order to better understand the accumulation and transfer of HMs in the plant, different indicators such as biological aggregation index, transfer factor and transfer efficiency index were calculated.

Previous studies have focused on the uptake of one element in the waste leachate by the plant, but in this study, four elements were investigated. Also, transfer and bioaccumulation factors have been studied. 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.

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. Sampling location of landfill waste leachate for conducting this project is the geographical coordinates of Zone 39S 37°05'19.20"N 49°37'50.73"E, and about 800 meters below the exit of waste leachate from the landfill area, and its flow to the leachate stream, which moves towards the Kacha River. Leachate collection was carried out exactly at a time when 3 days prior to its collection, there had been no rainfall. Also, there was no sewage or water stream in the path of the leachate stream. According to plant irrigation rate and water requirement, 20 gallons of 25 liters of leachate was collected. Then, in order to stabilize it, 5 ml of concentrated 65% nitric acid was added per each liter of leachate which preserved the compounds and elements inside the leachate for six months. After stabilization, leachate was transferred to the greenhouse and treatments of 0, 30, 60 and 100% of leachate were prepared for implementation of the experimental design and irrigation of Vetiver plants and this leachate was used for about 4 months.

Concentration of Heavy Metals in the Leachate

To determine the amount of heavy metals of leachate used for irrigation of Vetiver from the landfill of Saravan, Rasht, 3 samples were taken and after analysis of samples in the laboratory, the amount of heavy metals in leachate are presented in (Table 1) [58].

Metal	Concentration	Threshold Limit
Pb	101	4.4
Cd	45	0.1
Mn	256	1152
Ni	209	31

Table 1: Heavy metal concentrations (μg/L) of leachate of the site studied and the corresponding threshold limit values.

Plant Cultivation and Irrigation with Waste Leachate

To implement this design, two locations were considered. One was planted in the greenhouse at the location of the project implementation and, using the waste leachate collected from the location of the Saravan landfill, was irrigated based on the water requirement of plants during the growth period. This leachate was stabilized using 5ml of concentrated nitric acid (65%) per each liter of leachate. This stabilizes the leachate and preserves the compounds and elements in the leachate for 6 months [59].

Conservation

Vetivers were irrigated and maintained for about 5 months according to the same temperature conditions and water requirements and with regard to irrigation with leachate were also irrigated and maintained on the same basis, and during this time, plants were cleared of weeds.

Parameter (unit)	Mean
Soil Texture	
Sand (%)	65.58
Very coarse sand (%)	8.16
Coarse sand (%)	26.02
Medium coarse sand(%)	31.21
Fine sand (%)	13.54
Very fine sand (%)	2.07
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/cm3)	1.62 ± 0.78
Porosity (%)	38.87 ± 4.39
Metal Contents (mg/kg)	
Pbmg kg-1	16.55 ± 4.08
Cd mg kg-1	4.23 ± 1.91
Mn mg kg-1	32.94 ± 3.4
Ni mg kg-1	18.22 ±4.26
Avai. P (ppm)	12.1
Avai. K (ppm)	215
TN (%)	0.225
OC (%	2.09

Table 2: Physicochemical properties of selected soils.

In the present study, the effects of four types of leachate with different concentrations were evaluated on Vetiver plant in three replications. The Vetiver plant was cultivating in 10 kg of contaminated landfill soil. Leachate was diluted with concentrations of 0, 30, 60 and 100% compared to the original leachate. Plant height was measured weekly. Soil samples were air dried and then ground. Heavy metals were analyzed using an atomic absorption device; also, other physical and chemical properties of the soil were determined (Table 2).

Preparation of samples, Acidic digestion, HMs analysis

As accordance to Method laboratory instruction (I.R.I.DOE, 2010 Edition) After transferring the samples to the laboratory, and drying and crushing them, and determination of dry weight of plant organs (roots and shoots) (Table 3), samples were prepared for the acid digestion process using concentrated nitric acid (65%). Then using 1, 2, 3 ppm solution of each HMs from the prepared standard 1000 ppm solution, the calibration curve of the HMs solution was created. and finally, The amount of uptake by plant organs was determined using the Perkin-Elmer PinAAcle 900TT Atomic Absorption spectrophotometer (AAS) [59-62]

Dry weight of vetiver g/pot		
Leachate treatment levels	**Roots**	**Shoots**
Blank	32.12	18.61
Leachate 30%	34.54	19.52
Leachate 60%	38.01	25.11
Leachate 100%	30.01	17.21

Table 3: Dry weight of Vetiver.

Data analysis

This study was performed based on a completely randomized design in three replications with four treatments, including zero, 30, 60 and 100% leachate to determine the heavy metals lead, cadmium, manganese and nickel. Variance analysis of data was calculated with SPSS19 software, comparison of means for treatments was calculated by analysis of variance and Duncan's test with probability level of 5%, and graphs were made with Excel software.

Results

Mean heavy metal (lead, cadmium, manganese, nickel) uptake by rootand shoots. of Vetiver plant in different leachate treatments

Considering the result shown in Figures 1-8 related to the mean (HMs) uptake of lead, cadmium, manganese and nickel in different treatments of waste leachate (with different leachate concentrations of 0, 30, 60 and 100% in the roots and shoots, the mean uptake there is a significant difference in the level of 99%., and with the increase of waste leachate treatment levels, the uptake of heavy metals in roots and shoots has increased.

The results showed that the highest rate of uptake in all four treatments was related to the root with a total of 200.21 mg/kg. That is, on average, the root was able to absorb 200.21 mg / kg of heavy metals from the 4 treatments used. Among the treatments used, the highest amount of (HMs) adsorption is related to 100% treatment Among the treatments used, the highest amount of heavy metal uptake is related to 100% treatment with a total of 225.25 mg/kg for the four studied metals (lead, cadmium, manganese and nickel) in the root. Among the heavy metals absorbed in the roots, the highest levels were related to manganese, lead, nickel and cadmium, respectively. And manganese with an average of 70.70, lead 52.73, nickel 44.56 and cadmium 32.22 mg/kg had the highest uptake by the plant. In the shoots, the total amount of uptake, with an average of 147.93 mg/kg, was for four treatments. In other words, on average, the shoot was able to absorb 147.93 mg/kg of heavy metals from the 4 treatments used. Among the treatments used, the highest rate of heavy metal uptake is related to 100% treatment with a total of 178.87 mg/kg for the four metals studied (lead, cadmium, manganese and nickel) in the shoots. Among the heavy metals absorbed in the shoots, the highest amounts were related to manganese, lead, nickel and cadmium, respectively. Manganese with an average of 53.18, lead 38.35, nickel 35.13 and cadmium 21.27 mg/kg, had the highest uptake by the plant in the shoots. The results show that the plant significantly is able to absorb higher amounts of heavy metals by exposing higher concentrations of leachate. Vetiver in roots and shoots in total with exposure to four treatments used in greenhouse conditions, the highest amount of uptake was related to manganese, lead, nickel and cadmium, respectively. Of course, this amount of heavy metals absorption in the roots and shoots (maximum and minimum amount of metal absorption) is due to their amount in leachate and soil and also the concentration of leachate.

Based on Table 4, analysis of variance table with dependent variables of lead, cadmium, manganese, nickel and independent variables of leachate treatments (with different leachate concentrations of 0, 30, 60 and 100%, compared to the main leachate) shown in the root The effect of leachate treatments on the uptake of heavy metals lead, cadmium, manganese and nickel in the roots of vetiver plant has a significant difference at the level of 99%. In other words, with increasing leachate concentration in the plant soil, the uptake of heavy metals (lead, cadmium, manganese and nickel) in the plant roots increases, and the root is able to collect more heavy metals. This effect of leachate levels on the uptake of heavy metals by plant roots was also confirmed in the test (Duncan Multi-Range Test).

Source of Variation	df	Mean Square			
		Hevy metals concentration (Pb, Cd, Mn, Ni) in roots plant			
		Pb of roots	Cd of roots	Mn of roots	Ni of roots
Effects of leachate treatment	3	134.000**	89.763**	69.876**	54.110**
Error	8	0.251	0.335	0.236	0.171
C.V %	-	8.7	6.44	16.23	3.47

Table 4: Analysis of variance (mean squares) the effects of waste leachate treatments on the uptake of heavy metals (lead, cadmium, manganese and nickel) in the roots of vetiver.
** Significant at the 99% probability level; * Significant at the 95% probability level; ns: not significant

Source of Variation	df	Mean square			
		Heavy metals concentration (Pb, Cd, Mn, Ni) in roots plant			
		Pb of shoots	Cd of shoots	Mn of shoots	Ni of shoots
Effects of leachate treatment	3	182.149**	101.741**	97.400**	222.903**
Error	8	0.102	0.016	0.016	0.076
C.V %	-	5.44	4.04	10.33	4.51

Table 5: Analysis of variance (mean squares) the effects of waste leachate treatments on the uptake of heavy metals (lead, cadmium, manganese and nickel) in the shoots of vetiver.
** Significant at the 99% probability level; * Significant at the 95% probability level; ns: not significant

Based on Table 5, analysis of variance table with dependent variables of lead, cadmium, manganese, nickel and independent variables of leachate treatments (with different leachate concentrations of 0, 30, 60 and 100%, compared to the main leachate) shown in the shoots The effect of leachate treatments on the uptake of heavy metals lead, cadmium, manganese and nickel in the shoots of vetiver plant has a significant difference at the level of 99%. In other words, with increasing leachate concentration in the plant soil, the uptake of heavy metals (lead, cadmium, manganese and nickel) in the plant shoots increases, and the shoots is able to collect more heavy metals. This effect of leachate levels on the uptake of heavy metals by plant shoots was also confirmed in the test (Duncan Multi-Range Test).

Capture of Heavy Metals in Vetiver zizanioides

The concentration of the heavy metals found in *Vetiverzizanioides*, is shown in Figures 1 to 8.

Figures 1 to 8, Concentration of heavy metals in Vetiver expressed in mg/kg

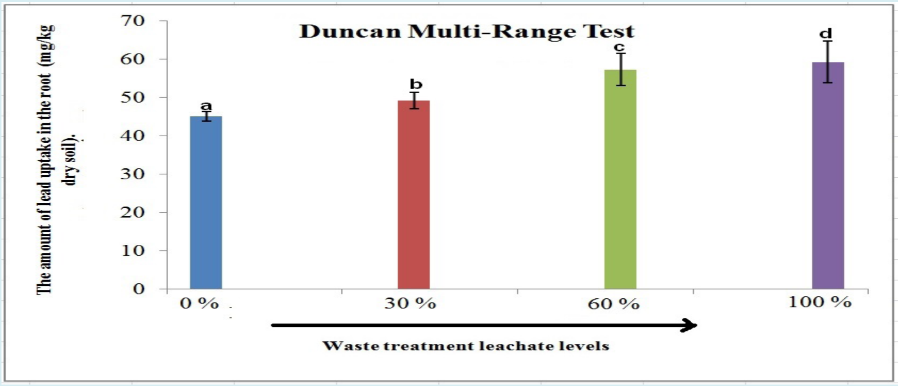

Figure 1: Duncan Multi-Range Test for different waste leachate treatments and its relationship to lead uptake in root of Vetiver plant.

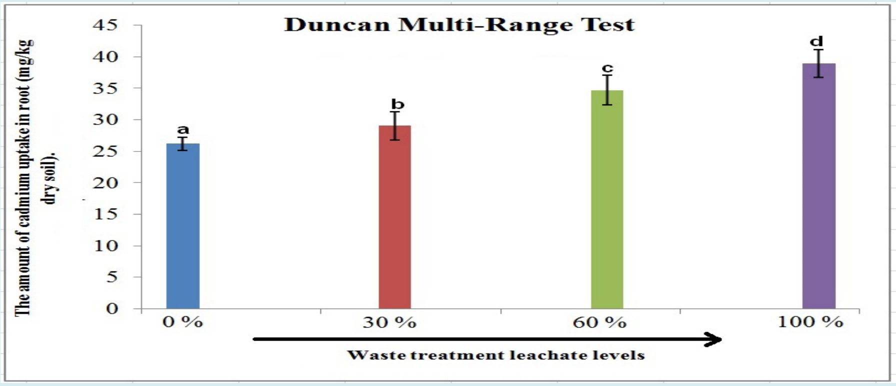

Figure 2: Duncan Multi-Range Test for different waste leachate treatments and its relationship to cadmium uptake in root of vetiver plant.

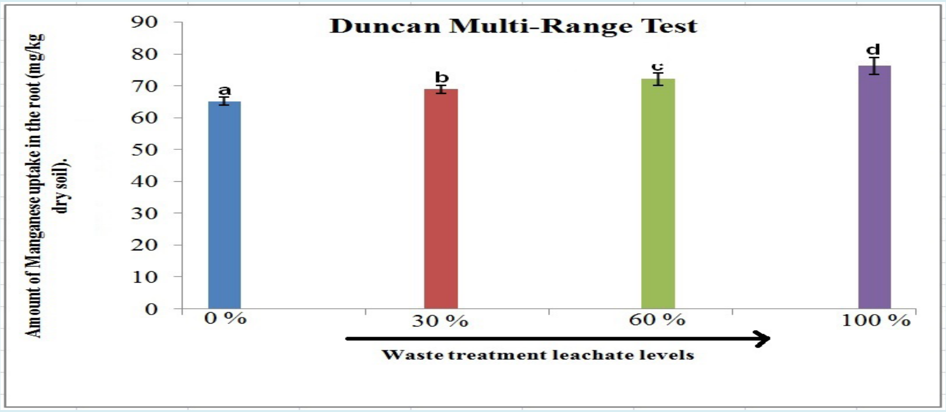

Figure 3: Duncan Multi-Range Test for different waste leachate treatments and its relationship to manganese uptake in root of Vetiver plant.

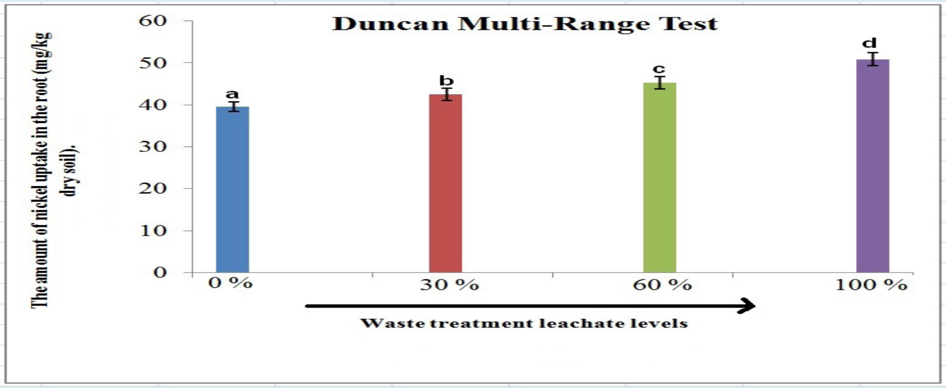

Figure 4: Duncan Multi-Range Test for different waste leachate treatments and its relationship to nickel uptake in root of vetiver plant.

*In Figures 1-4, different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in nickel absorption.

*Each number in the graph is average of three repetitions

Considering the result shown in Figures 1-4 Evaluating the Duncan's test regarding the effect of variation of different concentrations of leachate in soil (leachate treatment levels), it was revealed that with the increase of different concentrations of leachate, the accumulation and uptake rate of the heavy metals lead, cadmium, manganese and nickel in the root there is a significant difference in the level of 95%, which shows that with the increase of leachate treatment levels (increase in leachate concentration), the uptake of these heavy metals in the roots increases.

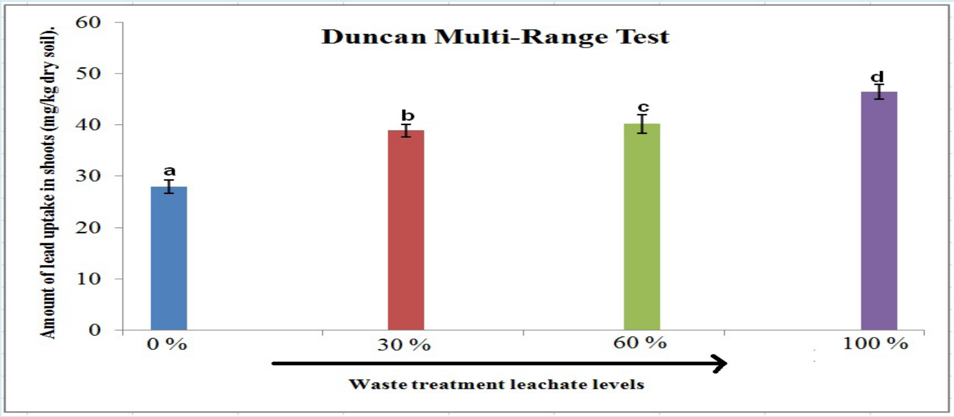

Figure 5: Duncan Multi-Range Test for different waste leachate treatments and its relationship to lead uptake in shoot of Vetiver plant.

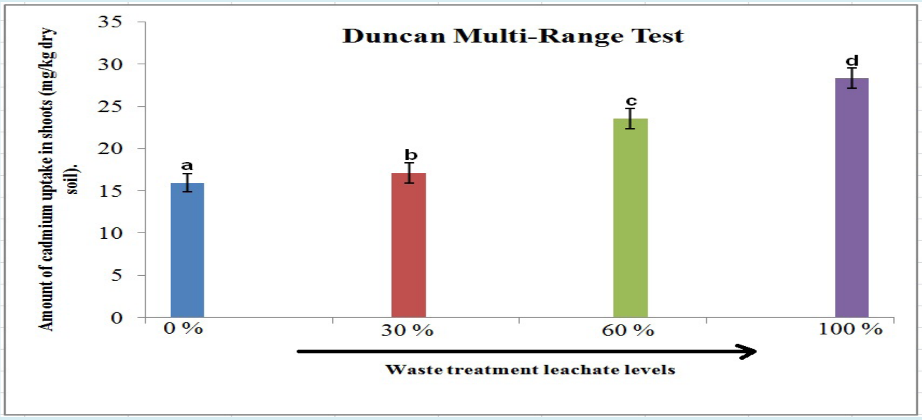

Figure 6: Duncan Multi-Range Test for different waste leachate treatments and its relationship to cadmium uptake in shoot of vetiver plant.

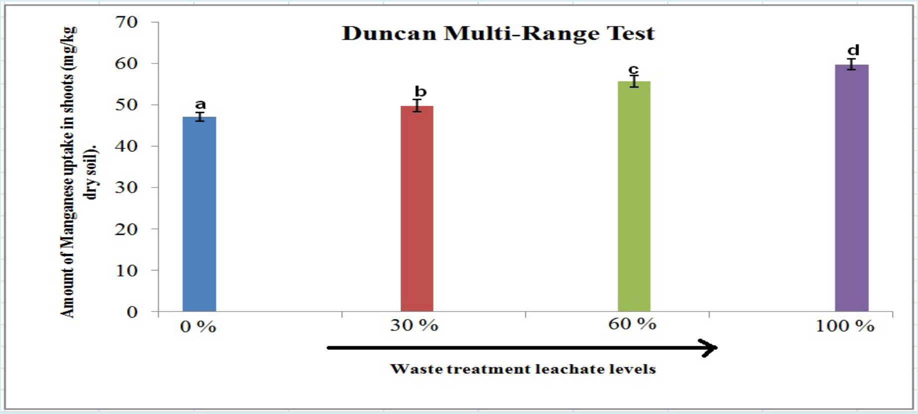

Figure 7: Duncan Multi-Range Test for different waste leachate treatments and its relationship to manganese uptake in shoot of Vetiver plant.

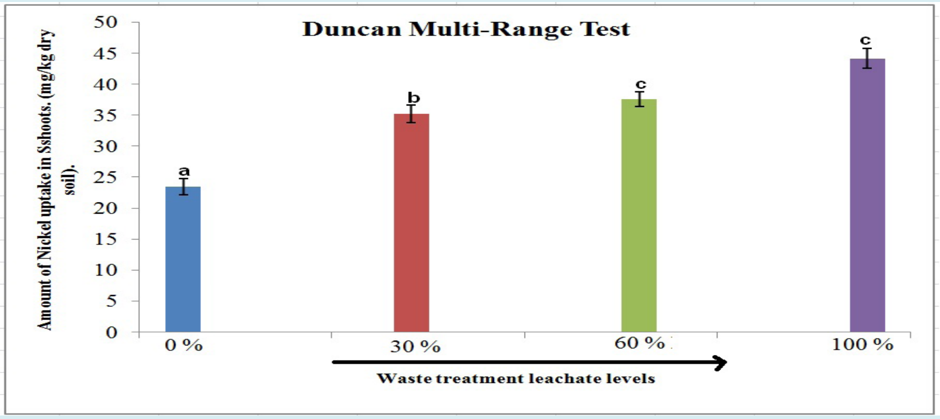

Figure 8: Duncan Multi-Range Test for different waste leachate treatments and its relationship to nickel uptake in root of vetiver plant.

* In Figures 1-4, different letters indicate a significant difference in the Duncan Multi-Range Test at a probability level of 5% between different levels of treatment in nickel absorption.
* Each number in the graph is average of three repetitions.

Based on the Figures 5-8 Evaluating the Duncan's test regarding the effect of variation of different concentrations of leachate in soil (leachate treatment levels), it was revealed that with the increase of different concentrations of leachate, the accumulation and uptake rate of the heavy metals lead, cadmium, manganese and nickel in shoots, there is a significant difference in the level of 95%, which shows that with the increase of leachate treatment levels (increase in leachate concentration), the uptake of these heavy metals in the aerial organs increases.

Ability for Metal Translocation and Accumulation

In this study the ability for metal translocation and accumulation were evaluated by the biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF), as follows:

BCF = HMs concentration in root - shoot / HMs concentration in soil

BAC = HM concentration in shoot / HM concentration in soil

TF = HM concentration in shoot / HM concentration in root [36,37,63,64].

Treatments	Total uptake of heavy metals (lead, cadmium, manganese and nickel) in roots and shoots (mg/kg dry soil)	BCF_{root}	BAC	TF
		bio-concentration factors in roots	**Bioaccumulation factor**	**translocation factor**
Blank	290.82^{c}	1.3	0.58	0.65
Leachate 30%	220.85^{a}	1.14	0.65	0.74
Leachate 60%	266.65^{b}	1.16	0.66	0.75
Leachate 100%	404.27^{d}	1.22	0.77	0.8

Table 4: Total average of adsorption and the effect of various HMs treatments on biological concentration factor (BCF), biological accumulation coefficient (BAC), and translocation factor (TF).

Based on the results of Table 4 to determine the potential of Vetiver phytoremediation, through translocation and bio-concentration factors (TF and BCF), it was found that with increasing levels of leachate treatment, translocation and bio-concentration factor, there is a significant difference at the level of 99%. And the highest translocation factor and biological concentration is related to leachate treatment with 100% concentration. The results showed that the translocation factor is less than one (TF<1) and the bio-concentration factor is more than one (BCF>1).

Morphological Changes of Vetiver Plant against Leachate Treatment Levels

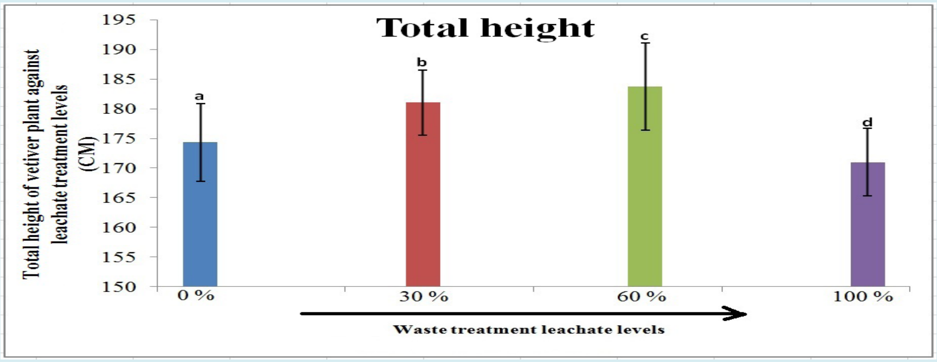

Figure 9: Changes of vetiver (total height) against leachate treatment levels.

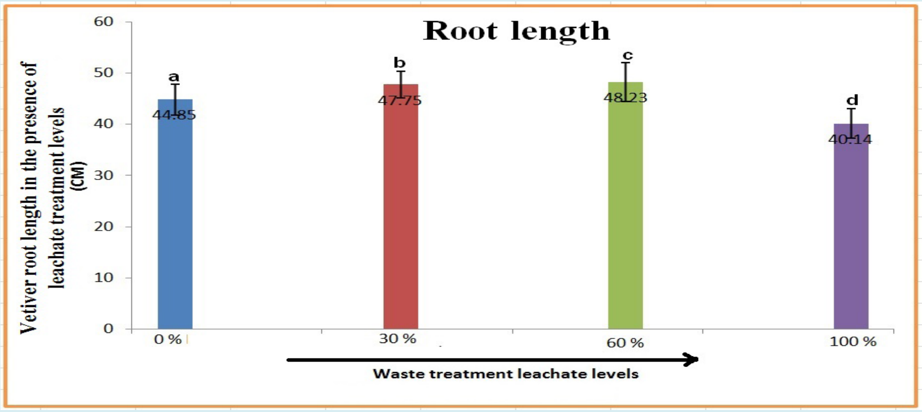

Figure 10: Morphological changes of vetiver roots against leachate treatment levels.

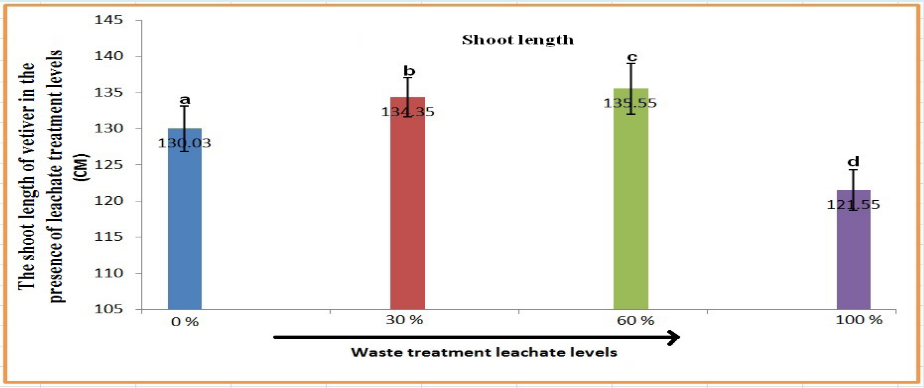

Figure 11: Morphological changes of vetiver shoots against leachate treatment levels.

- **Effect of heavy metals on vegetative traits (length root, shoot and total height) of vetiver seedlings**

Based on the results in Figures 9 to 11, it was shown that different levels of leachate treatment have a significant effect on the vegetative characteristics (length root, shoot and total height) of the studied seedlings and with increasing leachate concentration from 0 to 60%, the length of roots and shoots increases and when Vetiver plant is treated with 100% leachate or pure leachate, root and shoot length as well as total height are significantly reduced compared to 0, 30 and 60% treatments. The maximum increase in root and shoot length as well as total height is 60% for leachate treatment. And the minimum length of roots and shoots as well as total height is attributed to 100% leachate treatment.

Discussion

Physiological Function of Plant Roots and Shoots in the Uptake of Heavy Metals

Lead, cadmium, manganese and nickel are toxic heavy metals in the body when present beyond required levels it can bind with important enzymes and inactivate them [65]. Lead, cadmium, manganese and nickel in untreated leachate was found to be beyond WHO limit [66]. However in this study, it was found that Vetiver grass significantly reduced manganese, lead, nickel and cadmium after leachate treatments waste bringing it to concentration within acceptable limit.

Root length is an important indicator to measure of plant stress against any pollutant [67]. Root length decreased with the increase in the concentration of leachate treatments in the soil. Absorption of heavy metals in plants included transfer of metals outside the cells of the root, storing in tissues of xylem, and then subsequent detoxification, translocation and sequestration of metals occurred at both cellular and whole-plant level [68]. Results obtained from the present study revealed that roots of the plants absorb more concentration of heavy metals than the shoots of the plant (Figures 1 to 8). The highest adsorption of heavy metals under different leachate treatments for Vetiver is for manganese > lead > nickel> cadmium, respectively. And maximum plant uptake was observed in 100% treatment. In plants, the transfer of ions through cell membranes is first mediated by proteins called transporters. These ion carriers are specific ion transmitters and have specific function and are transported to the shoots of the plant [69]. But reason for the increase of lead, cadmium, manganese, and nickel) in the root of the Vetiver plant is its accumulation in the vacuoles; the accumulation of elements in cell vacuoles prevents their transmission to the aerial parts [70]. Therefore, the value of these heavy metals in the roots is more than the shoot [70]. However, absorption of HMs in the vacuole and especially in the cell wall has less toxic effects on the plant [70].

In Vetiver plant, lead cadmium, manganese, and nickel, remain attached to the cell wall or are stored in the root vacuole. It appears that lead and other elements (cadmium, manganese, and nickel) reduce water transfer to the shoots and following that, a portion (less than root absorption) is transferred to the shoot, and most of their accumulation has occurred in the root.

In fact, the stabilization and accumulation of HMs in the roots and less transmission to the shoot compared to the roots, which may be due to the sequestering of metal pollutants in root vacuole and the cells, is a strategy that some plants adopt to counteract the toxicity of heavy metals. In this way, organs that are involved in the metabolism of plants are protected from the damage of HMs [71] but in this research, According to the data in Figures 1 to 8 and Table 4, although the Vetiver plant was more successful in absorbing HMs leachate in the root compared to the shoot and this is consistent with the research mentioned above, it has also been successful in the absorption and transfer of HMs to the shoot and this has not caused a disruption in the growth of Vetiver plants Figures 9 to 11, and the statistics of approximately 100% survival in Vetiver plants signifies this claim, therefore, according to its characteristics, Vetiver plant can be effective in clearing soils contaminated with HMs leachate.

Evaluation of Vetiver Phytoremediation Potential in Refining Heavy Metal Contaminated Soils Based on BCF and TF

TF and BCFs are important indicators to determine the phytoremediation potential. TF is the ratio of metal in shoots to the concentration of that metal in the roots of the plant BCF is used to measure the ratio of heavy metals in the shoots or root of the harvested plant to the (HMs) concentration in the soil [72]. BCF has four criteria which value less than 0.01 mean has no accumulation, value between 0.01–0.1 means low accumulation of heavy metals, value between 0.1-1.0 as medium accumulation and value more than 1.0 is considered as high accumulation of heavy metals [36,37,63,64]. Maximum BCF values was observed in 100% treatment The difference in the BCF values for the understudy heavy metals through these plants was attributed to the mobility and the forms of these metals in which they exist. Overall, Vetiver had a relatively high concentration of heavy metals in its tissues as compared. The maximum TF value for the treatment was 100%. Then it was related to leachate treatments 60, 30 and control, respectively. That is, the transfer factor of the studied treatments was significantly higher than the control treatment. Which shows the effect of leachate and the ability of vetiver to deal with the toxic effects of heavy metals in leachate and to fight for survival against these effects In Vetiver, BF> 1 and TF <1 were reported (Table 4).

The low ability of plants translocation, from roots to shoots might be due to the reason that these plants possess multifaceted vacuoles in root tissues [73]. Interaction of different metals not only affects the rate of translocation but also alters the uptake efficiency and distribution of. This is confirmed by the Duncan multi-domain test and variance analysis. In the current study, vteiver store maximum concentration of heavy metals in roots while a smaller portion of these metals translocated in higher parts such as shoots. Significant metal immobility occurs due to the binding of metals to root cells, which can be considered a type of plant tolerance mechanism [74,75].

These indicators (BCF, TF, BAC), the plant's ability purification of contaminated soils from pollutants is assessed. Due to the fact that the Phytoremediation potential is determined according to the translocation factor (TF), biological concentration factor (BCF), biological accumulation coefficient (BAC) [36,37,63,64,76]. If the TF value is less than one and the BCF value is more than one the plants are suitable for the Phytoestablization process, and if the TF is greater than one, the plant is suitable for Phytoextraction. Also, the plants with TF and BAC index values greater than one are suitable for the Phytoextraction process. Also, the plants with TF and BCF values greater than one are suitable for the Phytoextraction and Phytoestablization process in the Phytoremediation process. And they can be a *hyper-accumulating* [76]. In this research, indices such as biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF), together with transfer efficiency indices were considered. In this study, it was found that with the increase of leachate concentration in the soil, BCF, and finally the TF have showed a significant increase. That is, when the plant is exposed to more heavy metals (when the concentration of leachate increases and we move towards treatments with a higher concentration of leachate) the vetiver plant reacts against it reacts with more accumulation of metals in the roots and their relative transfer to the shoot and resists this behavior against heavy metals. The results of this study showed that the BCF level was more than 1 (Table 4).

This factor indicates the ratio of HMs in plant organs to the soil bed [77]. In fact factor BCF has important value for estimating plant potential for plant extraction or plant stabilization [36,37,64]. If the value of BCF in the root is higher than that of the shoot, it indicates that the HMs is more accumulation in the roots [76]. According to the results of this study, the root accumulation factor is higher than the shoots, indicating that the Vetiver plant has stored HMs mostly in the roots, (Table 4). Based on the findings in Table 4, in the vetiver plant, due to having BCF > 1 and TF < 1, It can be said that it is suitable for refining leachate HMs pollution as an Phytoestablization.

The Amount of Heavy Metal Uptake in Roots and Shoots in Contaminated Soils under the Influence of Leachate

According to Tables (3-4), which shows the average absorption at different levels of treatments, it was found that the highest amount of adsorption, among the treatments, was related to Mn metal, which was 123.88 mg/kg in root and shoots, of this amount, 70.70 mg/kg in roots and 53.18 mg/kg in shoot were accumulated, then, the highest absorption, respectively, was related to Pb > Ni > Cd Of course, this amount of absorption of metals is due to their amount in leachate and soil. Of course, this amount of metal absorption is due to the amount and nature of them in leachate and soil and in all treatments, root uptake was significantly higher than shoot. This is due to the fact that, first, the root of the first organ is in contact with toxic elements, and typically the accumulation of heavy metals in plant roots is higher than in shoots. This is because, the root of the first organ is in contact with toxic elements, and usually the accumulation of heavy metals in the roots of the plant is more than the shoots and in this study, by increasing the concentration of leachate, (leachate treatments) the absorption of metals increases. However, due to the amount of metals remaining in the soil, the percentage of metal uptake has decreased, and the first level of treatment had the highest percentage of absorption.

The mechanism of metal uptake from the roots is that plants are highly absorbent of metals and around their rhizosphere, they release protons that, by acidifying the soil, increase the mobility of metal ions and make it available to the roots [78]. 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 [79]. Some of the metals adsorbed to the apoplast bind 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 [80]. 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 roles in cells, 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. Numerous studies have been performed on the pathways of proline biosynthesis and catabolism. Vetiver seems to use the mechanism of metal excluders to reduce the toxicity caused by heavy metals, because it prevents the increasing accumulation of metal ions in the shoots and accumulates them more in the roots. To do this, the vetiver releases more proline to defend against the stresses of the heavy metals lead, cadmium, manganese and nickel.

Investigation of Organs Length of Vetiver Plant in Different Concentrations of Leachate

In general, changes in root morphology due to increased leachate concentration due to the presence of heavy metals and changes in root structure reduce nutrient uptake and reduce growth [81]. Reduction of sub-branches of plant root due to increase in nickel concentration, change in root color and decrease in root diameter are among the effects of nickel metal on Petroselinum crispum, which has been confirmed in other plants [82]. Given the long time that vetiver plants were under leachate treatments and as seen in (Figures 9-11) with increasing the leachate concentration to 60%, the root length of vetiver plants increased after 5 months of leachate irrigation. The highest root length was observed in vetiver plants irrigated with 60% leachate, which was equal to 48.23 cm. Root lengths of plants irrigated with 100% leachate were the shortest and was equal to 40.14 cm. The slope of root length changes in leachate treatment 60% was higher than other treatments and we face an increasing slope and then decreases. Differences in root length of control vetiver plants with plants irrigated with different percentages of leachate; it was statistically significant. Plants irrigated with leachate were also significantly different from each other. According to (Figures 9-11) it was found that the amount of heavy metals studied in leachate treatments, initially (up to 60% leachate treatment) increased the root length of vetiver plants. Then, by increasing the concentration of leachate, in 100% leachate treatment, it reduced the root length of plants irrigated with leachate.

Paraltau, et al. suggested that root damage caused by heavy metals was the main cause of growth reduction. Reduction of root length due to accumulation of heavy metals, increase in the process of disruption of plant biosynthesis, destruction of root tip meristems, or Cell division disorders and mitosis is abnormal [83]. Toxic concentrations of nickel, cadmium and manganese, by changing the membrane structure of root cells and reducing water absorption levels, have a negative effect on physiological processes such as transpiration, respiration, photosynthesis and ultimately reduce plant growth [81,84]. Heavy metals have a negative effect on root structure and function and reduce the absorption of water and salts, reducing water absorption and creating secondary drought stress in plants [85]. According to the results of this study, a study performed on *Petroselinum crispum* showed that high concentrations of nickel have a significant effect on root length. So that with increasing nickels concentration, root length decreased [86]. In this study, the effect of leachate treatments on the height of vetiver organs was statistically significant at the level of 1%. Heavy metal stress caused a significant reduction in growth traits. Reduce 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 [87]. In this study, it seems that heavy metals have reduced the height of the plant by affecting the photosynthesis of the plant. On the other hand, Vetiver has minimized this lack of growth and shortening of the organs by releasing large amounts of proline. Reduced growth of roots and shoots under lead stress can be due to high accumulation of lead in the roots, ligninization of the wall under the influence of heavy metal, direct impact of heavy metal on the cell nucleus, and interaction of heavy metals with sulfhydryl groups of cell membranes and inactivation. Other negative effects of lead on plant growth include its reduction in root and aerial part biomass and reduced function. Toxic effects of lead on plant photosynthesis can be applied in various ways, including reducing chlorophyll biosynthesis by reducing the concentration of essential elements magnesium and iron in leaves, complexing with photosynthetic proteins and increasing chlorophylase activity for chlorophyll degradation. Lead reduces the uptake of essential nutrients such as calcium, magnesium and iron by disrupting the normal activity of root cell membrane carriers, and as a result, lead-treated plants show signs of deficiency of these essential elements. Lead toxicity is due to the fact that it mimics many aspects of calcium metabolic behavior and inhibits the activity of many enzymes. Studies have shown that cadmium affects cell division and growth, overall plant growth, cell division in the meristem area, and regulates plant growth and development. Cadmium also causes chlorosis and necrosis, decreased total chlorophyll, a and b, carotenoids in plants, and impaired carbohydrate metabolism. The most important cause of the destructive effect of cadmium is that it cause produces reactive oxygen radicals such as superoxide (-2O) hydroxide (-OH) and hydrogen peroxide free radicals. 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 [88]. High concentrations of nickel and manganese are considered as stressors factor for plants that can affect the physiological and biochemical properties of plants as a growth limiting factor. Nickel competes with the cations of calcium, magnesium, iron and zinc, so high the amount of nickel in the root environment of contaminated soils may lead to iron and zinc deficiency in the plant. One of the reasons for the toxicity of nickel and manganese in plants is its effect on iron homeostasis in roots and shoots with different mechanisms. High levels of nickel are involved in iron homeostasis. Vetiver seems to use the mechanism of metal excluders to reduce the toxicity caused by heavy metals, because it prevents the increasing accumulation of metal ions to the shoots and accumulates them more in the roots. Vetiver plant to reduce the toxicity of metals (lead, cadmium, manganese and nickel) studied in waste leachate, among non-enzymatic antioxidants, it benefits from the presence of proline. Proline is an antioxidant that scavenges free radicals, and by binding to heavy metals and forming a heavy metal-proline complex, it prevents the toxicity of this element [89-105].

Conclusion

Based on the results mentioned above, which was obtained under greenhouse conditions, certainly can be concluded that Vetiver has been very specialized in reducing heavy metals and other pollutants and has the ability to reduce the harms of heavy metals caused by waste leachate. Vetiver was found efficient accumulator for the heavy metals for the treatment of landfill leachate. In general, according to the results of the present study, it can be stated that, considering the optimum growth, acceptable viability, acceptable transfer of HMs from soil to roots and from roots to shoot, Interaction of metals in their uptake by plants, along with accumulation improvement of lead, cadmium, manganese and nickel in Vetiver plant organs grown in contaminated soil, vetiver have the ability to filtrate soils contaminated with leachate contains heavy metals lead, cadmium, manganese and nickel [106-109].

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