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The effect of phytoremediation on the reduction of heavy metals in greenhouse scale

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Abstract

In recent years, the issue of heavy metal contaminated soils is one of the biggest environmental problems. The Environmental Protection Agency (EPA) identifies Nickel and Lead as two of the most important environmental pollutants. The phytoremediation of metal-contaminated soils is a low cost method for soil remediation.

This study was conducted to investigate the status of soil contamination in Gachsaran oil refinery using the pollution coefficients, the degree of contamination (DC), and the modified degree of contamination (MCD) indices. In order to evaluate the efficacy of alfalfa for phytoremediation of the Lead and Nickel from the oil-contaminated soil, transfer coefficients, bio concentration, and bioaccumulation factors were used. Four areas ranging from 0 to 500 meters, 500-1000 meters, 1000-1500 meters, and 1500-2000 meters were selected from the polluted site. Then, five soil samples were collected from each area. The inductively coupled plasma-optical emission spectroscopy (ICP-OES) was used to determine heavy metals. The analysis of the environmental indicators of the studied area showed a significant degree of contamination for Nickel and Lead. Due to the higher biostatic and bioaccumulation factors of Nickel (0.3 and 0.31, respectively) compared to those of Lead (0.09 and 0.11, respectively), alfalfa plant showed greater ability in refining Nickel contaminant both in aerial and root organs.

Conclusion: IN sum, the findings indicated higher ability of alfalfa plant in the phytoremediation of Nickel than Lead in oil-contaminated soil. Finally, the alfalfa is suggested for purifying other contaminants.

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Introduction

In recent years, there has been an increasing attention to heavy metals in soils due to their adverse effects on the metabolic and physiological activities of living organisms (Miretzky et al., 2004; Parnyan et al., 2011). Many of these elements are not necessary for biological life and have a very toxic effect. These metals have long term effects on the ecosystem due to their high toxicity and persistence in the environment. Nickel and Lead can be mentioned as heavy metals that play a major role in environmental contamination. Nickel is a heavy metal that is necessary at low concentrations for the life of plants, but at high concentrations it is harmful and dangerous. These metals can also accumulate in animal and plant tissues. Nickel causes headaches, dizziness, and nausea at low concentrations and, at high concentrations, can cause lung, respiratory tract and bone cancers (Parnyan et al., 2011). Lead is not necessary for biological life, and is very toxic for living organisms, even at low concentrations. It is the most slow-moving heavy metal in the soil. The concentrations of Lead in China, the average global shale, and the average global crust are 26, 20 and 20 mg/ kg, respectively. The toxic effects of Lead on plants at concentrations higher than 30 mg/kg can ultimately reduce the plant growth. This element is harmful for the kidneys, reproductive system, blood circulation and nerves and reduces the consciousness of living organisms. One of the human activities increasing the accumulation of heavy metals in the environment is releasing oil in the environment. As part of the environment containing heavy metals such as Nickel and Lead, oil can play a significant role in the spread of pollution. Oil contamination can affect human life through breathing the polluted air, using oil derivatives, feeding from hydrocarbon-polluted plants and animals, and drinking polluted water. In this regard, this study was carried out to investigate and monitor the soil pollution of Gachsaran oil refinery. Therefore, the soils of the studied area were explored for Nickel and Lead pollutions using indices of pollution coefficient and degree of pollution. Moreover, considering the native species of alfalfa plant in the studied area, the aim of this study was to determine the accumulation of these two elements in this plant, and its capacity to purify the soil polluted with these compounds. The following cases can be mentioned from the researches on Nickel and Lead contaminants in oil-rich regions. Also, the research by Kariminezhad et al. (2015) can be mentioned in this regard. According to Kilic et al. (2017), secondary metabolites, which play an important role in regulating the relationship between plants and the environment, are affected by soil pollution. Secondary metabolites often play a protective, ecological and survival role for plants, and are usually produced in protective response to various factors, such as environmental tensions, pests, and diseases. Islam et al. (2008) reported that plant tissues play an important role in moderating the toxicity of heavy metals like Lead. Depending on their age, the leaves of the plants are widely different in the ability to accumulate Lead. The maximum and minimum levels of Lead can be observed in the old leaves and young leaves, respectively. Hall et al. (2011) used different types of legume species to remediate hydrocarbon pollutants. The results of their research showed that the use of different types of legumes has been effective and economically suitable in eliminating such pollutants. Wang et al. (2008), with a 5-month study on the ability of plants to remediate soil polluted with hydrocarbons in the greenhouse scale, reported that about 30-40% of the concentration of oil hydrocarbons in the soil cultivated with plants was decreased. Kaimi et al. (2007) introduced Sorghum Vulgar and Zee mays plants for the treatment of soils contaminated with hydrocarbons. Khan et al. (2016) studied rare earth elements in different species of Cyperaceae, Gleicheniaceae and, Melastomataceae in various regions of Malaysia using plasma mass spectrometry (ICP-MS). They used the transfer coefficient, bioaccumulation factor, and bioconcentration factor, to evaluate the ability of plants for the absorption of the elements. The results showed that these local plant species had the ability to absorb the elements from the soil. It has been shown that Medicago sativa l. is able to tolerate heavy metals and grow in contaminated soils. Medicago sativa l. contains a large amount of seedling and root biomass and is reported to have high accumulations of heavy metals such as zinc, Lead, Nickel, chromium, cadmium, copper, and silver in its tissues (Peralta-Videa et al., 2002). Matko Stamenkovic et al. (2017) studied the concentration of Nickel in serpentine soil samples in different regions of central Bosnia and Herzegovina. In addition to Nickel, other elements like iron, magnesium, and zinc had high concentrations in the region, while cadmium, cobalt, and Lead were also detectable. Nickel concentration was high in all of the samples in the study area. The results of Matko phytoremediation showed that it had a high ability to accumulate and transfer heavy elements especially Nickel in the aboveground organs and root. Therefore, it can be used to clean the soil contaminated with heavy metals.

Material and Methods

Sampling and laboratory analysis

The study area was the city of Dogonbadan, the capital of Gachsaran County in Kohgiluyeh and Boyer Ahmad province, at the end of the oil-rich regions of Iran. In this applied research, in April 2020, 20 soil samples were collected from 0 to 30 cm depth of the soil in four study areas ranging from 0 to 500 m, 500 to 1000 m, 1000 to 1500 m and 1500 to 2000 m in the polluted area and towards out of the area. To determine the physical and chemical properties of the contaminated soil, each sample was placed in two polyethylene bags and transferred to the laboratory for preparation. The soil samples were dried under free air for 48 hours, and after passing through a sieve of 10-grade, were mixed to form a mixed soil.

Preparation of seed and plant cultivation in polluted soil

As one of the famous species of alfalfa genus, Medicago sativa has several cultivars. The present study was carried out using Nik Shahri cultivar obtained from Pakan Seed Co. The air of the soil was dried and after passing through the 10-grade sieve was distributed in 4 kg plastic pots. Four days a week, 150 gr of distilled water was used to irrigate the pots. The ambient temperature of the pots was 43 ± 5°C and 32 ± 5°C during days and nights, respectively. To reduce the leak of the contaminant from of the oil polluted soil during irrigation, the drainage holes of the pots were closed with paper glue. In the fourth and the eighth week, an amount of 2 gr of NPK fertilizer was added to the soil of each pot as soluble in irrigation water. A total of 10 pots were cultivated to check the remediation capacity of the alfalfa plant. The soil texture of the pots was determined by hydrometric testing.

Experimental cultivation site for Alfalfa

The cultivation of pots in this study was carried out in the Iemen Khak-e- Jonoub laboratory in Dehdasht from April to September 2017. Dehdasht is geographically located at 50 degrees and 33 minutes of the east, 30 degrees and 47 minutes north of the altitude, and is 800 m above the sea level. The climate of this city is generally hot and dry and the annual temperature is 21.4°C. The average annual rainfall is 390 mm and the average annual evaporation and transpiration from class A pan has been 268.28 mm. The maximum annual evaporation is about 3151 mm, corresponding to the year of 1970-71, and the least is 1656 mm, corresponding to the year of 1984-85.

Preparation of plants for experiment

After a five-month period, the plants were separated from the pot soil and their root organs were washed for 8 minutes with urban water. Then, they were transferred to the laboratory to determine the amount of the contaminants (Nickel and Lead) in the aboveground organs, root, and the soil.In this study, in order to determine the level of pollution in the Nickel and Lead contaminated soil (Table 1), the average shale concentration presented by Turkian and Wedephol (1961), was used.

Table 1. The concentration of Nickel and Lead in average shale (mg kg^{-1})

Element	Nickel	Lead
Average shale	50	20

To determine and interpret the sample soil contamination, the following factors were used:

Contamination factor: As shown in Table 2. Hakanson's degree of contamination (1980) is the ratio between the concentration of a pollutant in the environment and natural background concentration (Hakanson, 1980). Due to the limitations of the degree of contamination presented by Huckenson (1980), proposed the modified degree of contamination (the ratio of the degree of contamination to the number of studied pollutants). See Table 4.

Table 2. Hucnson's classification based on pollution factor

Contamination factor	Pollution situation
$cf < 1$	Low pollution factor
$1 \leq cf < 3$	Moderate pollution factor
$3 \leq cf < 6$	Significant contamination factor
$cf \geq 6$	High contamination factor

Table 3. Hucnson's classification based on degree of contamination

Degree of contamination	Sediment quality
$cd < 6$	Low degree of pollution
$6 \leq cd < 12$	Average degree of pollution
$12 \leq cd < 24$	Significant degree of pollution
$cd \geq 24$	Very high degree of contamination

Table 4. Grading the level of contamination based on mcd

Grade index range	Sediment contamination status
$\mathrm{mcd} < 1/5$	Very low degree of pollution
$1/5 \leq \mathrm{mcd} < 2$	Low degree of pollution
$2 \leq \mathrm{mcd} < 4$	Moderate degree of pollution
$4 \leq \mathrm{mcd} < 8$	High degree of pollution
$8 \leq \mathrm{mcd} < 16$	Very high degree of contamination
$16 \leq \mathrm{mcd} < 32$	Highly infected
$\mathrm{mcd} \geq 32$	Infected with high levels

Evaluation of remediation by alfalfa

To evaluate the ability of alfalfa plants to remediate the pollutants (Nickel and Lead) from the soil in the study area, the following indices are introduced. Transfer Coefficient: The ratio of pollutant concentration in the plant's aboveground organ to the pollutant concentration in the root (Doumett et al., 2008). Bio Accumulation Factor: The ratio of pollutant in the plant's aboveground organ to the concentration of the pollutant in the culture medium (Doumett et al., 2008). Bio Concentration Factor: The ratio of metal concentration in the plant's root to the concentration of metal in the soil. First, the normality assumption of the data was investigated using the Kolmogorov-Smirnov method. Statistical analysis was performed using SPSS 17 software. The t-test was used to compare the mean of soil data with critical level and also the difference between Lead and Nickel levels in above-ground organs and root.

Result

Chemical properties of the soil and concentration of Nickel and Lead in the four study areas are presented in Tables 5 and 6, respectively. The results of the hydrometric analysis of pot's soil for sand, silt, and clay were 60, 29 and 11%, respectively.

Table 5. the concentration of Nickel and Lead contaminant in soil contaminated (mg kg^{-1})

Test sample	Nickel	Lead
1	279.65	2900
2	247.78	2785
3	234.96	2640.45
4	211.66	2590

Results of the evaluation of environmental indicators

The results of the pollution coefficient indices related to the Nickel and Lead in the studied area are shown in Table 6. The results of the degree of contamination index and the modified degree of contamination index of Nickel and Lead are shown in Table 7. According to the obtained data, the pollution rate reduced from the first area to the fourth area. The results confirmed that the study area is highly contaminated.

Table 6. Results of the indicator of the coefficient of pollution related to Nickel and Lead elements

Test sample	Nickel	Lead
1	5.59	145.00
2	4.96	139.25
3	4.70	132.02
4	4.23	129.50

Table 7. Results of the index of degree of contamination index and corrected for Nickel and Lead elements

Elements	Nickel	Lead
Contamination coefficient index	19.48	545.77
Modified Degree of Contamination Index	4.87	136.44

Results of the evaluation of the Nickel and Lead concentrations in the study area

The results of total Nickel and Lead concentrations in the soil are shown in Figure 2. It was shown that there was a significant difference between the total Nickel and Lead concentrations in the study areas. As the figure shows, the concentration of Lead was reduced from the first area to the fourth one. In fact, the further the distance from the pollution center, the less Lead concentration was observed. In addition, as it can be seen in Figure 2, the same trend for Nickel was observed.

The results obtained from the comparison of the concentration of Nickel and Lead in the soil with the maximum permitted value

The results showed a significant difference between the average total concentration of Nickel and Lead in the soil and its maximum permitted value ($p<0.05$), See Table 8. In other words, the soil of the study area was highly polluted with these two metals.

Table 8. Comparison of Ni and Pb concentrations with maximum permitted values

Sources of changes	mean ±SD (mg kg^{-1})	Degrees of freedom	T test result
Average total Nickel concentration	243.51±28.35	3	13.65
Average total Lead concentration	2728.86±140.88	3	38.547

Results of concentration of Nickel and Lead in aboveground organs, root and the soil of the plant culture medium

At the end of the study (155 days after alfalfa cultivation), plants were slowly removed from the pot soil. The results of concentrations of the pollutants (Nickel and Lead) in aboveground organs, root and the soil of the ten pots are shown in Table 9 and Table 10, respectively.

Table 9. Concentration of Nickel pollutants in alfalfa roots and pots (mg kg^{-1})

Sample number	Nickel whole soil	Nickel Aerial	Nickel roots
1	126.0	39.98	40.0
2	134.3	37.40	39.8
3	132.0	37.80	39.5
4	135.5	37.10	39.2
5	129.0	36.20	37.4
6	147.3	47.35	46.9
7	146.0	44.30	48.2
8	145.0	47.30	47.6
9	145.0	45.20	46.8
10	142.0	46.60	48.4

Table 10. Concentration of Lead contamination in alfalfa roots and (mg kg^{-1})

Sample number	Lead whole soil	Lead Aerial	Lead roots
1	1990	179.5	195.3
2	1954.8	173.8	195.8
3	2028.5	177.4	198.7
4	2011.0	181.2	200.5
5	2006.1	170.1	202.0
6	2102.5	221.1	231.9
7	2122.4	189.6	249.5
8	2101.25	194.1	241.8
9	2087.0	204.4	276.0
10	2129.6	247.0	256.7

Results obtained from the evaluation of phytoremediation indices

The values of the transfer coefficient index, bioaccumulation factor and biological accumulation factor for Nickel and Lead are presented in table 11 and Table 12, respectively.

Table 11. Values of the ability to evaluate the refining of Nickel from polluted soil by alfalfa

Sample number	Transfer Coefficient	Bio Accumulation Factor	Bio Concentration Factor
1	0.99	0.32	0.32
2	0.94	0.28	0.30
3	0.96	0.29	0.30
4	0.95	0.27	0.29
5	0.97	0.28	0.29
6	1.00	0.32	0.32
7	0.92	0.30	0.33
8	0.99	0.33	0.33
9	0.97	0.31	0.32
10	0.96	0.33	0.34

The results of comparison of the average concentration of Nickel and Lead in soil of the plant culture medium with the study area

As Table 13 shows the total mean concentration of Nickel in the soil of ten pots was significantly different from that in the soil of four areas ($P \leq 0.05$). This means that the total concentration of Nickel in ten pots is lower than that in the soil of the four areas. Also, the results showed that the mean concentration of Lead in the soil of ten plots was significantly different from that in the soil of the four areas. This means that the total concentration of Lead in ten plots is lower than that in soil of the four areas.

Table 12. Values of the ability to evaluate the refining of the Lead element from soil contaminated by Alfalfa

Sample number	Transfer Coefficient	Bio Accumulation Factor	Bio Concentration Factor
1	0.92	0.09	0.10
2	0.89	0.09	0.10
3	0.89	0.09	0.10
4	0.90	0.09	0.10
5	0.84	0.09	0.10
6	0.95	0.11	0.11
7	0.76	0.09	0.12
8	0.80	0.09	0.12
9	0.74	0.10	0.13
10	0.96	0.12	0.12

Table13. Comparison of the average concentration of Nickel and Lead in soil of the cultivar with the studied area

Sources of changes	mean ±SD (mg kg^{-1})	Degrees of freedom	T test result
Total Nickel concentration	138.21±7.78	9	-7.318
Total Lead concentration	2053.32±62.17	9	-9.237

The results obtained from the comparison of the mean concentration of Nickel and Lead in aboveground and root organs of alfalfa

The results demonstrated that the mean concentration of Nickel in aboveground organs was significantly different from that in the root in ten pots. In other words, the concentration of Nickel in aboveground organs was lower than that in the root in ten pots. Also, according to Table 14, the results revealed that there was no significant difference between the mean concentration of Lead in aboveground organs and that in the root in 10 pots.

Table 14. Comparison of Average Nickel and Lead aboveground and roots of alfalfa

Sources of changes	mean ±SD (mg kg^{-1})	Degrees of freedom	T test result
Nickel Aerial	41.92±4.64	9	-3.621
Nickel roots	43.38±4.50		
Airline Lead	193.74±24.29	9	-1.938
Root of root	224.62±30.20		

The results of evaluation indices of the plant phytoremediation potential

Transfer factor values, bioaccumulation coefficient and bioaccumulation factor for the mean concentrations of Nickel and Lead for alfalfa are presented in *Table 15*.

Table 15. Average values of indices of evaluation of alfalfa plant's ability to refine Nickel and Lead elements

pollutant	Nickel	Lead
Transfer Coefficient	0.97	0.86
Bio Accumulation Factor	0.30	0.09
Bio Concentration Factor	0.31	0.11

Discussion

Contamination of soil with oil hydrocarbons is considered a serious threat to the environment and has been a concern for environmental activists due to its toxicity and carcinogenicity for living organisms. Heavy metals induced from hydrocarbon compounds can increase the concentration of the elements in the soil. Nickel and Lead are heavy pollutant metals that should have permitted levels in soil compared to other elements. In addition to its toxicity, Lead causes reduction in plant growth, disturbance in the distribution of nutrients, change in soil texture, disturbance in the water balance and enzymatic inhibition or activation. In addition, increasing Nickel concentration in the soil can reduce soil fertility and plant yield (Islam et al., 2007). In the present study, several sampling from polluted soil in the study area were taken to evaluate the environmental indicators and the potential of alfalfa for the bioremediation of Nickel and Lead. Based on the results of the contamination factor according to Hakanson's classification, it was revealed that both Nickel and Lead had a significant contamination factor. Also, the degree of contamination index showed that Nickel and Lead had a high degree of contamination. According to the modified index, Nickel showed an average degree of contamination, while Lead showed an ultra-high degree of contamination. As it's shown in *Table 8* and *Table 9*, the highest concentration of Nickel in the aboveground organs of alfalfa was 47.3 mg/kg, and the lowest was 36.2 mg/kg. In contrast, the highest and the lowest concentrations of Lead were 247 mg/kg and 170.1 mg/kg, respectively. In alfalfa roots, while the highest concentrations of Nickel and Lead were 48.4 mg mg/kg and 276 mg/kg, the lowest concentrations were 37.4 mg/kg and 195.3 mg/kg, respectively. The highest concentrations of Nickel and Lead in the soil of pots, after a five-week period, were 147.3 mg/kg and 2129. 6 mg/kg, respectively. Though, the lowest concentrations of these elements were 126 mg/kg and 1954.8 mg/kg, respectively. The results of the evaluation of transfer factors in alfalfa showed that the highest ratio of Nickel in the aboveground organ to root was 1, while the lowest ratio was 0.92. Besides, the highest ratio of Lead in the aboveground organ to root was 0.95, while the lowest ratio was 0.74. The values of the bioconcentration factor (BCF) and bioaccumulation factor (BASF) of alfalfa in all samples were less than one. The maximum and minimum values of the bioconcentration factor of the alfalfa plant were 0.33 and 0.27 for Nickel and 0.12 and 0.09 for Lead, respectively. The maximum and minimum bioaccumulation factors of the alfalfa were 0.34 and 0.29 for Nickel and 0.13 and 0.1 for Lead, respectively. Yoon et al. (2006) reported that plants with transfer coefficient index and bioaccumulation factor of more than one are suitable for the extractive process. Meanwhile, plants in which the value of the transfer coefficient is less than one and the value of the bioaccumulation factor is greater than one are suitable for the stabilizing process. The obtained transfer coefficients of Nickel and Lead in alfalfa (0.97 and 0.86, respectively) demonstrated that it is not suitable for extractive and stabilizing processes. Concerning the higher contamination factor of Nickel compared to Lead, alfalfa performs better in Nickel extraction than Lead.

Conclusions

According to the obtained ratio of Nickel and Lead contamination factor in alfalfa, which was less than 1, alfalfa acts as a repellent plant when used for the remediation of Lead and Nickel from the oil-contaminated soil of the study area and is not suitable for the extraction of contaminants (Nickel and Lead). This rate of Nickel absorption in the alfalfa plant shows the ability of alfalfa for remediating Nickel from oil-contaminated soils of Gachsaran. Aboveground organs and root showed an equal capacity in the absorption of Nickel and Lead from the contaminated soil. However, alfalfa performs better in absorbing Nickel in the root and transferring it to the aboveground organs. The limitations of alfalfa in the removal of Nickel and Lead include time taking reduction of the concentration of the pollutants, destruction of the contaminated alfalfa and producing a huge biomass, and in consequence feeding the livestock from the contaminated aboveground organs, that can lead to a threat for human being. But, due to its low cost and high efficiency, it can be a functional method for the remediation of soil contaminated with Nickel and Lead. However, further research with other plants, considering more heavy metal contaminants is suggested.

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