Phytoremediation of heavy metals for contaminated soil with wastewater in Bani Al- Harith- Sana'a

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ABSTRACT.

The contamination of soils due to the presence of toxic metals can result in serious negative consequences, such as damage of ecosystems and of agricultural productivity deterioration of food chain, contamination of water resources, economic damage and, finally, serious human and animal health problems. This study was carried out at farm of agriculture faculty at Sana'a University to evaluate the ability of four plants namely, *Brassica juncea*, *Brassica oleracea* , *Medicago sativa* and *Helianthus annuus*, The four plants were grown in wastewater contaminated soil. The design of experiment was Randomized Complete Block Design (RCBD) with two factors, the first was different soils (contaminated and uncontaminated soil) and the second was the four plants. The experiment included eight treatments each treatment included three replicates. Irrigation continued with clean water during the period April- June, 2014. The thickness of wastewater-contaminated soil was 50 cm, placed on a layer of polyethylene of 2mm to prevent seeping heavy metal from soil by irrigate water. Soil samples were collected from contaminated and uncontaminated soil before and after the experiments from three levels (surface, at a depth of 25cm,and at a depth of 50cm) of soil. Samples of each soil were mixed, air-dried at room temperature for two weeks, then ground into fine powder and sieved through a 2 mm nylon sieve. Triplicate soil samples were collected for analyses. The concentrations of Pb, Zn, Ni, Mn, Cu and Fe in the soils were determined using Inductively Coupled Plasma Emission Spectroscopy. The analysis of variance (ANOVA) was applied to compare the significant difference of the mean concentrations of heavy metals in the samples. The study showed that *Brassica juncea* was the best plant for cleanup contaminated soil with some heavy metals as, Zn, Mn, Cu and Fe. the values were 44.2, 290.2, 29.0, and 9781.2 PPb, respectively. At the same time, *Helianthus annuus* was the best plant for cleanup contaminated soil with some other heavy metals as, Pb and Ni. The values were 3.3 and 12.8 PPb, respectively. The results indicated that contaminated soil content of Pb, Zn, Ni, Mn, Cu and Fe decreased from 17, 95, 36, 472, 52 and12644 before applying plants to 3.5, 41.9, 18.1, 355.4, 31.8 and10828.9, respectively after applying the plants. The study concluded that *Brassica juncea*, *Brassica oleracea* , *Medicago sativa* and *Helianthus annuus* plants were able to grow in wastewater - contaminated soils, also able to decrease concentrations of Pb, Zn, Ni, Mn, Cu and Fe metals in wastewater - contaminated soils. However, the concentrations was not decreased up to the WHO recommended concentrations for only one time experimental setup. It is therefore, recommended that, further research should be conducted by repeating the experimental set up till reaching the safe concentrations.

Keywords: Heavy metals, Phytoremediation, Hyperaccumulator plants, Accumulation, contaminated soils.

Introduction:

The contamination of soils due to the presence of toxic metals can result in serious negative consequences, such as damage of ecosystems and of agricultural productivity deterioration of food chain, contamination of water resources, economic damage and, finally, serious human and animal health problems (Raicevic *et al*.,2005).

With the development of urbanization and industrialization, soils have become increasingly polluted by heavy metals which threaten ecosystems, surface, and ground waters, food safety, and human health (Moon *et al*., 2000; Chen *et al*., 2005; Davydova, 2005; Krishna and Govil, 2005; Kachenko and Singh, 2006).

Phytoremediation is a relatively new approach to removing contaminants from the environment. It may be defined as the use of plants to remove, destroy or sequester hazardous substances from environment. It has become a topical research field in the last decades as it is safe and potentially cheap compared to traditional remediation techniques (Salt *et al*., 1998; Mitch, 2002; Glick, 2003; Pulford and Watson, 2003).

The basic idea that plants can be used for environmental remediation is very old, and cannot be traced to any particular source. However, a series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of this idea into a promising, cost-effective, and environmentally friendly technology (Baker *et al.*, 1991).

Although phytoremediation has received considerable attention recently and there are an increasing number of reports suggesting that it should become the technology of choice for the cleanup of various types of environment contamination, this technology is still in its infancy (Glick, 2003).

This absorption by plants facilitates use of plants for removal of excessive heavy metals from the soil. About 450 plants were identified absorb excessive concentrations of heavy metals and accumulate these absorbed heavy metals in their parts such as roots, stems and leaves (Prasad and Freitas,2003)

There have been many reports of hyperaccumulating plant (Berti and Cunningham, 1993; Brown *et al.*, 1995; Shen and Liu, 1998; Ozturk *et al.*, 2003).

A hyperaccumulator has been defined as a plant that can accumulate 1000 mg/kg of Cu, Co, Cr, Ni and Pb, or 10000 mg/kg of Fe, Mn and Zn in their shoot dry matter (Baker and Brooks, 1989). The Indian mustard, *Brassica juncea* was found to significant amount of lead (Bishop,1995).

Phytoremediation is proposed as a cost-effective alternative for the treatment of contaminated soils. Topsoil would be preserved and the amount of hazardous materials reduced significantly.(Ensley,2000).

The main factors controlling the ability of phytoextraction are plant species, metal availability to plant roots, metal uptake by roots, metal translocation from roots to shoots and plant tolerance to toxic metals. There are many types of plants currently used in phytoextraction, such as *Thlaspi caerulescens*, *Alyssum murale*, *Alyssum lesbiacum,* and *Alyssum tenium,* which can accumulate high levels of Zn and Cd in shoots. However, the remediation potential may be limited due to the slow growth and low biomass of these plants.(Baker,1994).

Recently phytoremediation researchers have discovered that Indian mustard (*Brassica juncea* (L.) can accumulate high levels of metals, including Zn and Se. The metal accumulating ability of this plant, coupled with the potential to rapidly produce large quantities of shoot biomass, makes this plant ideal for phytoextraction.(Montes-Bayon, *et al.*, 2002)

In Yemen, research on the metal-accumulating efficiency of crop plants and weeds is still too limited, only the study that planted *Brassica juncea* in wastewater-contaminated soil in Bani Al- Hareth the concentrations of Pb, Cd, Ni, Zn and Cu were 271, 62, 133, 1132 and 114 ppm respectively before planted, concentrations of heavy metals decreased after planted to 148, 27, 35, 533 and 24ppm respectively (Tahish A. H.,2013).

In this study, *Brassica juncea* (L.) was selected because it has desirable characteristics such as high shoot biomass, metals tolerance, short life cycle and handling ease.

The objective of this research was to study the ability of four plants namely *Brassica juncea*, *Brassica oleracea* , *Medicago sativa* and *Helianthus annuus* on removing Pb, Ni, Zn, Mn, Cu and Fe from waste water contaminated soil (from Bani-Al-Hareth site).

Materials and Methods

This study was carried out at farm of agriculture faculty in Sana'a University. The wastewater-contaminated soil was transported from Bani Al-Hareth area which is far from Sana'a about 25Km north of Sana'a city that located at latitude (15°29 N) and longitude (44°13 E) to Farm of agriculture college on April, 5, 2014.

Bani Al-Hareth area has occupied approximately 4 Km2, it was estuary of untreated wastewater of Sana'a city, and therefore the discharging of industrial wastewater contaminated by toxic heavy metals is a serious concern. Contamination by heavy metals was mainly concentrated in the top 20 cm of the soil (soil flooded with wastewater since more than four years).

Plants seeds of *Brassica juncea*, *Brassica oleracea* , *Medicago sativa* and *Helianthus annuus* were planted in wastewater-contaminated soil on April 9, 2014. The thickness of wastewater-contaminated soil was 50 cm, placed on a layer of polyethylene of 2mm (Fig. 1) to prevent seeping heavy metal from soil by irrigate water.

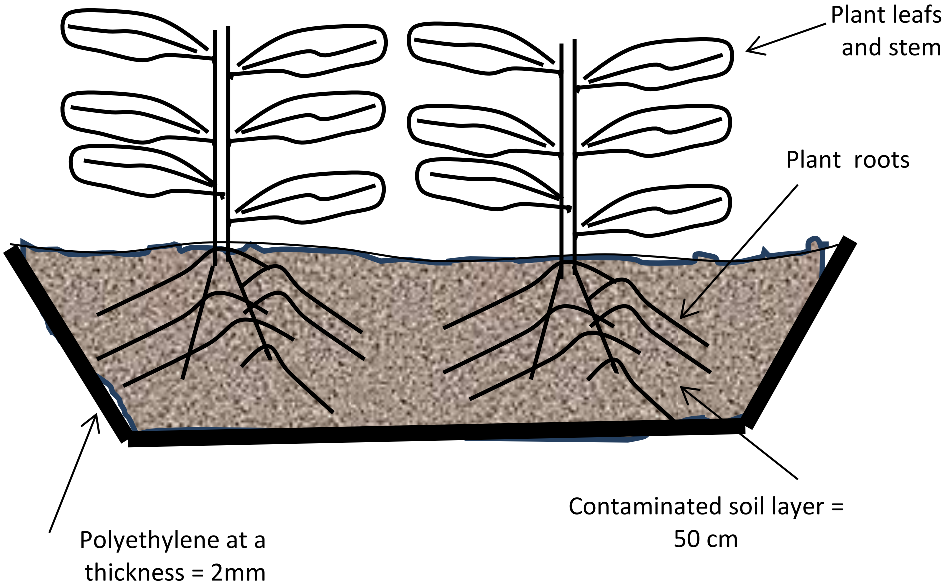

Fig. 1. Cross section of the plantation

Irrigation continued with clean water until the harvest on June,12, 2014. At the same time the experiment replicated in uncontaminated soil from faculty of agriculture Sana'a University site served as control.

Statistical analysis:

The design of experiment was Randomized Complete Block Design (RCBD) (Fig. 2) with two factors, the first was different soil (contaminated and uncontaminated soil) and the second was plants (*Brassica juncea*, *Brassica oleracea* , *Medicago sativa* and *Helianthus annuus*). The experiment included 8 treatments each treatment included 3 replicates. The analysis of variance (ANOVA) was used to compare the significant difference in the mean concentration of heavy metals in the samples that taken from soils after planted different plants and compare the significant difference in the mean concentration of heavy metals in the samples that taken from contaminated and uncontaminated soil. Using least significant difference (LSD at 5%) the method as mentioned by Gomez and Gomez (1984).

P1 R3 S2	P3 R2 S1	P2 R1 S2
P4 R3 S3	P1 R2 S1	P4 R1 S2
P2 R3 S2	P3 R2 S1	P3 R1 S2
P3 R3 S2	P2 R1 S1	P1 R1 S2
P4 R2 S1	P1 R2 S2	P2 R1 S1
P2 R3 S1	P3 R2 S2	P4 R1 S1
P1 R3 S1	P4 R2 S2	P3 R1 S1
P3 R3 S1	P2 R2 S2	P1 R1 S1

P1: *Helianthus annuus*; P2: *Brassica oleraceae*; P3: *Brassica Juncea*; P4: *Medicago sativa*; R: Replicate; S1: un-contaminated soil; S2: contaminated soil

Fig. 2. Experimental set up

Sampling and soil analysis

Soil samples were collected from contaminated and uncontaminated soil before planting and after plants harvested from three levels (surface, at a depth of 25cm,and at a depth of 50cm.) of soil. Samples were air-dried at room temperature for 2 weeks, then ground into fine powder and sieved through a 2 mm nylon sieve. The concentrations of Pb, Zn, Ni, Mn, Cu and Fe in the soils were determined. 0.5 gram soil samples were digested by HNO3:HCI:HCIO4 (1:2:2) to obtain a total extraction of the heavy metals. The total concentrations of Pb, Zn, Ni, Mn, Cu and Fe were determined by Inductively Coupled Plasma Emission Spectroscopy (ICP-ES-710 Varian, Australia) using standard method (ICARDA, 2003). Means of Pb, Zn, Ni, Mn, Cu and Fe were calculated from triplicate.

Results and Discussion

Table 1 shows the different concentrations before the experiments.

Table 1. Pb, Zn, Ni, Mn, Fe and Cu concentrations (in PPb) in contaminated and uncontaminated soils Before planted.

Soil \ metal	Pb	Zn	Ni	Mn	Cu	Fe
Uncontaminated soil (S1)	8	72	17	332	39	10728
contaminated soil (S2)	17	95	36	472	52	12644

1. **Effect of *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago* plants on cleanup contaminated soil with Pb.**

Table 2 shows the different concentrations of Pb in soils after the experiments.

Table 2. Pb concentration (in PPb) in contaminated and uncontaminated soils after planted *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago* plants.

Plant \ Soil	Uncontaminated soil (S1)	contaminated soil (S2)	Mean B
Helianthus annuus	4.5	2.0	3.3
Brassica oleracea	4.3	6.7	5.5
Brassica juncea	6.0	2.3	4.2
Medicago sativa	7.0	3.0	5.0
Mean A	5.5	3.5	
Lsd 5%	A =1.7 B=2.5 AB= 3.5		

The results in table 2, indicated that, *Helianthus annuus, Brassica oleracea*, *Brassica juncea* and *Medicago sativa* plants effect on cleanup contaminated soil with Pb significantly. The concentration of Pb in contaminated soil before planted was 17 PPb (table.1) compare with 3.5 PPb (table.2) after planted. *Helianthus annuus* was the best plant for cleanup contaminated soil, Pb concentration after planted was 3.3 PPb compare with *Brassica oleracea* that Pb concentrations after planted was 5.5 PPb.

Availability of organic compounds and mineral elements in contaminated soil enhancement growth and activity of plants for removing Pb from contaminated soil. This cause decrease Pb concentration in contaminated soil compared with uncontaminated soil, that were, 3.5 and 5.5, respectively. These results are in agreement with the results obtained by Lorestani *et al*. (2011).

2. **Effect of *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago* plants on cleanup contaminated soil with Zn.**

Table 3 shows the different concentrations of Zn in soils after the experiments.

Table 3. Zn concentration (in PPb) in *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago sativa* planted in contaminated and uncontaminated soil.

Soil / Plant	Uncontaminated soil (S1)	contaminated soil (S2)	Mean B
Helianthus annuus	67.7	32.5	50.1
Brassica oleracea	49.0	69.7	59.3
Brassica juncea	61.3	27.0	44.2
Medicago sativa	66.0	38.5	52.3
Mean A	61.0	41.9	
Lsd 5%	A=10.9 B= 15.5 AB=21.9		

The results in table 3, showed that, *Helianthus annuus, Brassica oleracea*, *Brassica juncea* and *Medicago sativa* plants effect on cleanup contaminated soil with Zn significantly. The concentration of Zn in contaminated soil before planted was 95 PPb (table.1) compare with 41.9 PPb (table.3) after planted This result in agreement with obtained result by Ebbs *et al.* (1997).

Brassica juncea was the best plant for cleanup contaminated soil, Zn concentration after planted was 44.2 PPb compare with *Brassica oleracea* that Zn concentrations after planted was 59.3 PPb, but plants effects on cleanup contaminated soil were not significantly.

Availability of organic compounds and mineral elements in contaminated soil enhancement growth and activity of plants for removing Zn from contaminated soil. This cause decrease Zn concentration in contaminated soil compare with uncontaminated soil, that were, 41.9 and 61.0, respectively.

3. **Effect of *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago* plants on cleanup contaminated soil with Ni.**

Table 4 shows the different concentrations of Ni in soils after the experiments.

Table 4. Ni concentration (in PPb) in *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago sativa* planted in contaminated and uncontaminated soil.

Soil / Plant	Uncontaminated soil (S1)	contaminated soil (S2)	Mean B
Helianthus annuus	10.0	15.5	12.8
Brassica oleracea	9.0	18.7	13.8
Brassica juncea	10.7	15.7	13.2
Medicago sativa	21.7	22.7	22.2
Mean A	12.8	18.1	
Lsd 5%	A= 6.1 B= 8.6 AB= 12.5		

The results in table 4, pointed that, *Helianthus annuus, Brassica oleracea*, Brassica juncea and Medicago sativa plants effect on cleanup contaminated soil with Ni significantly. The concentration of Ni in contaminated soil before planted was 36 PPb (table.1) compare with 18.1PPb (table.4) after planted This result in agreement with obtained result by Ebbs *et al.* (1997).

Helianthus annuus was the best plant for cleanup contaminated soil, Ni concentration after planted was 12.8 PPb compare with ***Medicago sativa*** that Ni concentrations after planted was 22.2 PPb, plant effect on cleanup contaminated soil were significantly.

4. **Effect of *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago* plants on cleanup contaminated soil with Mn.**

Table 5 shows the different concentrations of Mn in soils after the experiments.

Table 5. Mn concentration (in PPb) in *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago sativa* planted in contaminated and uncontaminated soil.

Soil / Plant	Uncontaminated soil (S1)	contaminated soil (S2)	Mean B
Helianthus annuus	304.5	360.0	332.3
Brassica oleracea	314.0	432.7	373.3
Brassica juncea	320.3	260.0	290.2
Medicago sativa	462.0	369.0	415.5
Mean A	350.2	355.4	
Lsd 5%	A= 77.9 B= 110.2 AB= 155.9		

The data in table 5, showed that, *Helianthus annuus, Brassica oleracea*, *Brassica juncea* and *Medicago sativa* plants effect on cleanup contaminated soil with Mn significantly. The concentration of Mn in contaminated soil before planted was 472.0 PPb (table.1) compare with 355.4 PPb (table.5) after planted This result in agreement with obtained result by Ebbs *et al.* (1997).

Brassica juncea was the best plant for cleanup contaminated soil, Mn concentration after planted was 260.0 PPb compare with *Brassica oleracea* that Mn concentrations after planted was 432.7 PPb, plant effect on cleanup contaminated soil was significantly.

5. **Effect of *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago* plants on cleanup contaminated soil with Cu.**

Table 6 shows the different concentrations of Cu in soils after the experiments.

Table 6. Cu concentration (in PPb) in *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago sativa* planted in contaminated and uncontaminated soil.

Soil / Plant	Uncontaminated soil (S1)	contaminated soil (S2)	Mean B

Helianthus annuus	33.0	31.7	32.3
Brassica oleracea	35.1	40.4	37.7
Brassica juncea	32.3	25.7	29.0
Medicago sativa	32.5	29.5	31.0
Mean A	33.2	31.8	
Lsd 5%	A=6.1 B=6.1 AB= 13.2		

The results in table 6, pointed that, *Helianthus annuus, Brassica oleracea*, *Brassica juncea* and *Medicago sativa* plants effect on cleanup contaminated soil with Cu significantly. The concentration of Cu in contaminated soil before planted was 52 PPb (table.1) compare with 31.8 PPb (table.5) after planted. . The result is similar with the result obtained by Lorestani *et al.*,(2011).

Brassica juncea was the best plant for cleanup contaminated soil, Cu concentration after planted was 29.0. PPb compare with *Brassica oleracea* that Cu concentrations after planted was 37.7 PPb, plant effect on cleanup contaminated soil was significantly.

Availability of organic compounds and mineral elements in contaminated soil enhancement growth and activity of plants for remove Cu from contaminated soil this cause decrease Cu concentration in contaminated soil compare with uncontaminated soil, that were, 31.8 and 33.2 respectively. These results are in agreement with the results obtained by Lorestani *et al.*, (2011).

6. **Effect of *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago* plants on cleanup contaminated soil with Fe.**

Table 7 shows the different concentrations of Fe in soils after the experiments.

Table 7. Fe concentration (in PPb) in *Helianthus annuus, Brassica oleracea, Brassica juncea* and *Medicago sativa* planted in contaminated and uncontaminated soil.

Soil / Plant	Uncontaminated soil (S1)	contaminated soil (S2)	Mean B
Helianthus annuus	9099.0	10785.5	9942.3
Brassica oleracea	10282.5	11240.7	10761.6
Brassica juncea	9696.0	9866.3	9781.2
Medicago sativa	10558.5	11423.0	10990.8
Mean A	9909.0	10828.9	
Lsd 5%	A= 1747 B= 2741 AB=3494		

The results in table 6, pointed that, *Helianthus annuus, Brassica oleracea*, *Brassica juncea* and *Medicago sativa* plants effect on cleanup contaminated soil with Fe significantly. The concentration of Fe in contaminated soil before planted was 12644.0 PPb (table.1) compare with 10828.9 PPb (table.6) after planted.

Brassica juncea was the best plant for cleanup contaminated soil, Fe concentration after planted was 9781.2. PPb compare with *Medicago sativa* that Fe concentrations after planted was 10990.8 PPb, plant effect on cleanup contaminated soil was significantly.

Conclusion:

Finally, it could be concluded that, this study showed *Brassica juncea*, *Brassica oleracea* , *Medicago sativa* and *Helianthus annuus* plants were able to grow in wastewater - contaminated soils, also able to decrease concentrations of Pb, Zn, Ni, Mn, Cu and Fe metals in wastewater - contaminated soils.

Recommendations:

Since the heavy metals concentrations in the soil during the experiment were not decreased up to the safe concentrations by only one time experimental setup, it is therefore, recommended that, further research should be conducted by repeating the experimental set up till the concentrations is reaching the safe concentrations.

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