PHYTOREMEDIATION OF HEAVY METALS CONTAMINATED SOIL USING *Brassica juncea (L.)* IN BANY EL- HARETH, SANA'A- YEMEN

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ABSTRACT

This study was carried out at Bany El-Hareth site north of Sana'a- Yemen to evaluated the ability of *Brassica juncea (L.),* which has already been recognized as a plant suitable for metal phytoremediation to extract Lead (Pb), Cadmium (Cd), Nickel (Ni), Zinc (Zn), and Copper (Cu) from waste water contaminated soil and investigated the effect of plant parts and contaminated soil on heavy metals accumulation in plant. Indian mustard plant (*B. juncea*) was grown in wastewater contaminated soil. This study showed that *Brassica juncea (L.)* was able to grow in heavy metals contaminated soils and also able to accumulate extraordinarily high concentrations of some metals such as Pb, Cd, Ni, Zn and Cu in its tissues. The results indicated that contaminated soil content of Pb, Cd, Ni, Zn and Cu decreased 45, 56, 73, 53and 79 % respectively after plant harvest. Plant parts and contaminated soil affected heavy metals accumulation in plant, the highest accumulation 54.0, 24.8 and 42.3 ppm of Pb, Cd and Ni respectively were in plant roots, whereas, the highest accumulation of Zn was 250.6 ppm in shoots and 35.6 ppm of Cu in fruits.

Keywords: Heavy metals, phytoremediation, hyperaccumulator, accumulation, Indian mustard.

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. 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 objectives of this research were to study the ability of *Brassica juncea* (L.) on remove Pb, Cd, Ni, Zn and Cu from waste water contaminated soil (Bany-El-Hareth soil) and study the effect of plant parts and soil on accumulation of previous heavy metals in *Brassica juncea* plant.

MATERIALS AND METHODS

The experiment was carried out at Bany El-hareth area which located at latitude (15°29 N) and longitude (44°13 E) north of Sana'a city - Yemen.

The study area has an elevation of around 2200 m above sea level, average annual precipitation about 400 mm/y, minimum temperature is 6.2 ⁰C in December and maximum temperature 27.7 ⁰C in July. The site has occupied approximately 4 Km^2, 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.

Seeds of Indian mustard (*Brassica juncea L.*) was planted on April, 28- 2012 in the wastewater contaminated soil from Bany El-Hareth site (soil flooded with wastewater since more than four years) where the soil with thickness of 50 cm, put on a layer of polyethylene for stop wash H.M. from soil by irrigate water, the planted area was 40m^2 and irrigation continued with clean water until the harvest on August, 12- 2012.In 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 completely (RCD) with two factors, the first was different soil (contaminated and uncontaminated soil) and the second was the part of plants (leaves, shoots and fruits). The experiment included 6 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 different parts of plants which planted in different soil. Using least significant difference (LSD at 5%) the method as mentioned by Gomez and Gomez (1984).

Sampling and Plant analysis

All the plant samples were randomly uprooted at maturity and separated into root, shoot and fruits parts for estimation of metals content. The samples were carefully washed with deionizer water and oven-dried at 70⁰C for 30 min, then ground into fine powder and sieved through a 1 mm nylon sieve. The concentrations of Pb, Cd, Ni, ZN and Cu in the plants were determined. 1 gram plant samples were digested by HNO3:HClO4 (3:1). The concentrations of Pb, Cd, Ni, ZN and Cu were determined by an Inductively Coupled Plasma Emission Spectroscopy (ICP-ES-710 Varian, Australia). Means of Pb, Cd, Ni, Zn and Cu were calculated from triplicate.

Sampling and soil analysis

Soil samples were taken before planting and after plant harvest from three levels (surface- depth 25cm- depth50cm.) of soil. Samples were air-dried at room temperature for 3 weeks, then ground into fine powder and sieved through a 2 mm nylon sieve. The concentrations of Pb, Cd, Ni, Zn and Cu in the soils were determined. 0.5 gram soil samples were digested by HNO3:HCI:HClO4 (1:2:2) to obtain a total extraction of the heavy metals. The total concentrations of Pb, Cd, Ni, Zn and Cu were determined by Inductively Coupled Plasma Emission Spectroscopy (ICP-ES-710 Varian, Australia). Means of Pb, Cd, Ni, Zn and Cu were calculated from triplicate.

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RESULTS AND DISCUSSION

The research is the first one in this site for using plant to cleanup contaminated soil.

1. Lead(Pb) accumulation:

1.1. Effect of soil on Pb accumulation (ppm) in organs of *Brassica juncea* plant.

The results in tables 1, indicated that, contaminated soil effect on Pb accumulation in *Brassica juncea* plant organs and there is a significant difference compare with control, they were 39.6 and 2.4ppm respectively. This result indicate that Bany El–hareth soil contains a high concentration of lead and *Brassica juncea* plant accumulated this element in its parts, in table 6, the concentration of Pb in soil after planted Indian mustard plant was 148.3 ppm compare with 271.3ppm before planting, about 45% of the amount of lead in the soil is absorbed by hyperaccumulator *Brassica juncea* during 104 days. Availability of organic compounds and mineral elements in contaminated soil with waste water enhancement growth and activity of *Brassica juncea* plant for remove Pb from contaminated soil. These results are in agreement with the results obtained by Lorestani *et al.*, (2011).

1.2. Effect of plant organs on Pb accumulation (ppm) in *Brassica juncea* plant.

Table1, showed that a significantly difference of Pb concentration in three plant parts, the highest value of Pb concentration was 28.7ppm in roots and the lowest value was10.0 ppm in shoots. Increasing of Pb concentration in roots may be due to deep of roots in soil and roots ability for Pb accumulation. These results were very similar to the study performed by Subhashini *et al.,* (2013).

1.3. Effect of interaction between soil and plant organs on Pb accumulation (ppm) in *Brassica juncea* plant.

The result showed that the highest value was in the interaction between contaminated soil and roots part, it was 54ppm and the lowest value was in the interaction between uncontaminated soil and shoots, it was 1.5 ppm.

Table 1. Pb concentration (ppm) in *Brassica juncea* organs planted in contaminated and uncontaminated soil.

Plant organ / Soil	Roots	Shoots	Fruits	Mean A
contaminated soil	54.0	18.4	46.3	39.6
Uncontaminated soil (control)	3.4	1.5	2.2	2.4
Mean B	28.7	10.0	24.3	
Lsd 5%	A = 0.75 B = 0.91 AB = 1.3			

2. Cadmium (Cd) accumulation:
2.1. Effect of soil on Cd accumulation (ppm) in parts of *Brassica juncea* plant.

Data in table 2, indicated that the increasing of Cd concentration in contaminated soil cause a significantly rise of Cd accumulation in plants parts compare with control, they were 10.9 and 1.4 ppm respectively, this result confirms the ability of plant for clean contaminated soil from this toxic element. In table 6, Cd concentration was significantly reduced from 62.3 to 27.4 in contaminated soil before planting and contaminated soil after plant harvest respectively, the low concentration of Cd in soil was 56%. *Brassica juncea* plant is effective in phytoextracting Cd from contaminated soils with relatively high Cd contamination. This result is agreement with Ebbs *et al.*, 1997 and Yanai *et al.*, 2004.

2.2. Effect of plant parts on Cd accumulation (ppm) in *Brassica juncea* plant.

From data in table 2, there is s significantly different of Cd concentration in plant parts, the highest value was in roots 13.1 ppm and the lowest value in shoots 2.1 ppm. Thus, the significantly decrease in the soil Cd content after *Brassica juncea* planting may result from the effective access of roots to Cd pools in soil and subsequent root accumulation. Whereas, Cd accumulation in shoots is driven mainly by mass flow due to the transpiration process.These results suggested that access to the Cd pool in the soil and subsequent Cd uptake by roots could be a more important factor for phytoremediation of soils with low Cd contamination.

2.3. Effect of interaction between soil and plant parts on Cd accumulation (ppm) in *Brassica juncea* plant.

As seen from Table 2, the interaction between roots and contaminated soil caused a high significantly effect on Cd accumulation in plant compare with interaction between fruits and control soil, the values were 24.8 and 1.3 respectively. Maybe, plant Cd accumulation is controlled by several factors, including root Cd uptake, shoot-to-root translocation, Cd tolerance and utilization of Cd in soil. Also, a decrease in soil pH may lead to the release of free ionic Cd^{2+} into the rhizosphere (Salam and Helmke 1998).

3. Nickel (Ni) accumulation:
3.1. Effect of soil on Ni accumulation (ppm) in parts of *Brassica juncea* plant.

The result in table 3, pointed that the accumulation of Ni in plant planted in contaminated soil has increased significantly compare with plant planted in control soil, the values were 32.5 and 5.1 respectively. In table 6, the concentration of Ni in contaminated soil decreased from 133.4 before planting to 35.9 after plant harvest, the percentage of decreasing was 73%. The increasing of phytoremediation of Nickel from contaminated soil may be due to high soil content of Nitrogen and organic compounds because of microbial activities change into NH3 (Sooknah, 2001 and Mahmood *et al.*, 2009d), this ammonia may increase the pH. The pH changes are related to Ni removal (Kashim and Singh, 2001). This result similar with the result obtained by Syed *et al.,*(2010).

3.2. Effect of plant organs on Ni accumulation (ppm) in *Brassica juncea* plant.

The values in table 3, showed that the concentration of Ni in roots has a significantly increasing compare with other parts, it was the highest value 24.7 whereas, the lowest value was 10.4 ppm in shoots. Higher Ni content in roots of a metal accumulator plant species is mainly dependent on at least two factors namely: sequestration and/or translocation. Ni could be transported as a nickel-citrate complex (Lee *et al.*, 1977) or as a nickel-peptide complex or as a nickel-histidine complex (Kramer *et al.,* 1996) to ensure high mobility of Ni within the plant.

3.3. Effect of interaction between soil and plant parts on Ni accumulation (ppm) in *Brassica juncea* plant.

Table 3, showed that the interaction between contaminated soil and plant parts caused a significant increase of Ni accumulation in plant, the highest value 42.3 ppm was in the interaction between contaminated soil and roots, whereas the lowest value 2.5 was in the interaction between uncontaminated soil and shoots.

The effectiveness of interaction for uptake Ni from contaminated soil probability due to the increase in soil pH that cause other mechanisms, such as ion exchange and roots exudation, may be responsible for the increased heavy metal uptake by plant. (Kashim and Singh, 2002).

4. Zinc (Zn) accumulation:

4.1. Effect of soil on Zn accumulation (ppm) in parts of *Brassica juncea* plant.

Data in table 4, cleared that the contaminated soil has a significantly effect on Zn accumulation in plant, the values were 223.5ppm in plant planted in contaminated soil compared with 22.7ppm in plant planted in control. According to Istvan and Benton (1997), toxic concentrations of heavy metals for various plant species are 300, 500, 300, 20 and 100 mg/kg for Pb, Fe, Mn, Cu and Zn, respectively, therefore the average contents of Zn in the sampled plants were higher than the toxic levels, but plant continues for accumulating Zn ,without suffering toxic effects.

Also, the result in table 6, indicated that Zn concentration in contaminated soil decrease from 1132.5 before planting to 533.5ppm after plant harvest, the percentage of decreasing was 53%, this result confirms the role of *Brassica juncea* plant on cleanup contaminated soil with heavy metals. This result in agreement with obtained result by Ebbs *et al.* (1997) who found *Brassica juncea* plant is effective in phytoextracting Cd and Zn from soils with relatively high Cd contamination.

4.2. Effect of plant organs on Zn accumulation (ppm) in *Brassica juncea* plant.

Also, data in table 4, pointed that plant parts has a significantly effected on Zn accumulation in plant, the highest value was in shoots and the lowest value was in roots, 139.5 and 111.2 ppm, respectively. In fact *Brassica juncea* plant is able to accumulate unusually high concentrations of Zn in their aboveground parts. This result is similar with result obtained by Lorestani *et al.*,(2011)

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Heavy metals are bound to soil components in varying degrees, depending on soil conditions such as pH, clay content, organic matter, redox potential. Plant available metals include species that are readily soluble or exchangeable, while metals that are more strongly adsorbed/bound to organic matter or co-precipitated with oxides are generally not available for plant uptake.

4.3. Effect of interaction between soil and plant organs on Zn accumulation (ppm) in *Brassica juncea* plant.

The result in table 4, showed that the interaction between soil and plant parts cause a significantly difference on Zn accumulation in plant, the highest accumulation of Zn was in the interaction between shoots and contaminated soil, where as the lowest accumulation of Zn was in the interaction between roots and control soil, 250.6 and 17.3ppm respectively.

5. Copper (Cu) accumulation:

5.1. Effect of soil on Cu accumulation (ppm) in parts of *Brassica juncea* plant.

Table 5, indicated that contaminated soil has a high significantly effect on Cu accumulation in plant compare with control soil, the values were 31.3 and 11.6 respectively. Table 6, pointed that a high significantly decreasing on Cu concentration in soil before planting and after plant harvest, the values were 114.2 and 24.3ppm respectively. The percentage of decreasing in Cu concentration in soil was 79%, this result confirm the role of *Brassica juncea* plant for phyroremediation of Cu from contaminated soil. The result is similar with the result obtained by Lorestani *et al.,*(2011).

5.2. Effect of plant parts on Cu accumulation (ppm) in *Brassica juncea* plant.

Table 5, cleared the effect of plant parts on Cu accumulation in plant, there a significantly difference between Cu accumulation in fruits and Cu accumulation in shoots, 24.4 and 17.0 ppm respectively, but there is no significantly difference between the values of Cu accumulation in fruits and roots, 24.4 and 23.0 respectively. Plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots, or through active transport crosses of the plasma membrane of root epidermal cells,(Kim *et al.,*2003).

5.3. Effect of interaction between soil and plant parts on Cu accumulation (ppm) in *Brassica juncea* plant.

Also Table, 5 pointed the interaction between soil and plan parts affected Cu accumulation in plant, the highest value was 35.6ppm in the interaction between contaminated soil and fruits, whereas the lowest value was 9.3 ppm in the interaction between control soil and shoots. May this due to pH of soil and the large biomasses of fruits in *Brassica juncea* plant. Also, *Brassica*ceae family contain different levels of GLS (glucosinolates) with diverse compositions, so their hydrolysis products also have different biocidal activities (Clarke, 2010).

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Table 2. Cd concentration (ppm) in *Brassica juncea* organs planted in contaminated and uncontaminated soil.

Plant organ / Soil	Roots	Shoots	Fruits	mean A
contaminated soil	24.8	2.5	5.3	10.9
Uncontaminated soil (control)	1.4	1.6	1.3	1.4
Mean B	13.1	2.1	3.3	
Lsd 5%	A =0.17 B =0.20 AB = 0.29			

Table 3. Ni concentration (ppm) in *Brassica juncea* organs planted in contaminated and uncontaminated soil.

Plant organ / Soil	Roots	Shoots	Fruits	mean A
contaminated soil	42.3	18.2	37	32.5
Uncontaminated soil (control)	7.0	2.5	5.8	5.1
Mean B	24.7	10.4	21.4	
Lsd 5%	A =0.76 B =0.93 AB =1.3			

Table 4. Zn concentration (ppm) in *Brassica juncea* organs planted in contaminated and uncontaminated soil.

Plant organ / Soil	Roots	Shoots	Fruits	Mean A
contaminated soil	205.1	250.6	214.9	223.5
Uncontaminated soil (control)	17.3	28.4	22.4	22.7
Mean B	111.2	139.5	118.7	
Lsd 5%	A= 2.15 B= 2.64 AB = 3.73			

Table 5. Cu concentration (ppm) in *Brassica juncea* organs planted in contaminated and uncontaminated soil.

Plant organ / Soil	Roots	Shoots	Fruits	Mean A
contaminated soil	33.6	24.7	35.6	31.3
Uncontaminated soil (control)	12.4	9.3	13.2	11.6
Mean B	23.0	17.0	24.4	
Lsd 5%	A =1.27 B = 1.56 AB =2.21			

Table 6. Heavy metals concentration (ppm) in contaminated soil before and after planted *Brassica juncea* plant.

Heavy metals concentration / Soil	Pb ppm	Cd ppm	Ni ppm	Zn ppm	cu ppm
Unpollution soil (control)	11.1	5.4	32.3	106.9	32.3
contaminated soil before planting	271.3	62.3	133.4	1132.5	114.2
Contaminated soil after plant harvest.	148.3	27.4	35.9	533.5	24.3
Low concentration%	45	56	73	53	79

CONCLUSION

Finally, it could be concluded that, this study showed *Brassica juncea (L.)* plant was able to grow in heavy metals contaminated soils and also able to accumulate extraordinarily high concentrations of some metals such as Pb, Cd, Ni, Zn and Cu in its tissues. Therefore contaminated soil content of Pb, Cd, Ni, Zn and Cu decreased 45, 56, 73, 53 and 79 % respectively after plant harvest. Plant organs and contaminated soil affected heavy metals accumulation in plant, the highest accumulation 54.0, 24.8 and 42.3 ppm of Pb, Cd and Ni respectively were in plant roots, whereas, the highest accumulation of Zn was 250.6 ppm in shoots and 35.6 ppm of Cu in fruits.

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إستخدام نبات الخردل (.L) *Brassica juncea* لإزالة المعادن الثقيلة من التربة الملوثة بمياه الصرف الصحي في منطقة بني الحارث – صنعاء – اليمن

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أجريت هذه التجربة في منطقة بني الحارث التي تقع شمال مدينة صنعاء - اليمن من أجل استخدام نبات الخردل (.L) *Brassica juncea* لإزالة المعادن الثقيلة مثل الرصاص (Pb) – الكادميوم (Cd)- النيكل (Ni) – الزنك (Zn) – النحاس (Cu) من التربة الملوثة بمياه الصرف الصحي في منطقة بني الحارث وقد تم استخدام هذا النبات بناءاً على أبحاث أثبتت القدرة العالية لهذا النبات على امتصاص تلك العناصر. كذلك لدراسة تأثير كل من الجذور والمجموع الخضري والثمار من ناحية و التربة من ناحية أخرى على تراكم المعادن الثقيلة في نبات الخردل. تمت زراعة نبات الخردل في التربة الملوثة بمياه الصرف الصحي وتمت المقارنة مع نفس المحصول الذي زرع في تربة نظيفة. أظهرت هذه الدراسة أن نبات الخردل له القدره على النمو في التربة الملوثة بمياه الصرف الصحي وتجميع كميات كبيره من المعادن الثقيلة مثل الرصاص والكادميوم والنيكل والزنك والنحاس في أنسجته . كما أشارت النتائج إلى أن محتوى التربة من الرصاص ، الكادميوم ، النيكل، الزنك ، النحاس انخفضت بنسبة ٤٥ ، ٥٦ ، ٧٣ ، ٥٣ , **79** ٪ على التوالي بعد زراعة الخردل في التربة الملوثة. أجزاء النبات المختلفة و التربة الملوثة أثرت وبشكل معنوي في تراكم المعادن الثقيلة في النبات، فقد كانت أعلى تراكيز للمعادن التالية الرصاص ، الكادميوم والنيكل هي ٥٤٫٠ ، ٢٤٫٨ ،٤٢٫٣ جزء بالمليون في جذور النبات ،في حين كان أعلى تراكيز لكل من الزنك ٢٥٠٫٦ جزء بالمليون في المجموع الخضري و ٣٥٫٦ جزء بالمليون من النحاس في الثمار.

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