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RESEARCH ARTICLE

The effect of irradiated and non irradiated sewage sludge application on Uptake of Heavy Metals by Jatropha curcas L plants

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Abstract

Soil contamination by heavy metals is a growing concern in many countries, especially in African continents. Phytoremediation of this polluted soil with non-edible plant like Jatropha *curcas L* offers an environmental friendly and cost-effective method for remediating the polluted soil. In this study, Research was conducted to elucidate the potential of Jatropha *curcas L*. to clean toxic heavy metals derived from sewage sludge. *J. curcas* seedlings were planted on six different planting media T0, (control); T1, (100 % gamma irradiated sewage sludge (GISS); T2, (rate 50% gamma irradiated sewage sludge (GISS) +50% compost); T3, (rate 100 % non - irradiated sewage sludge (NISS); T4, (rate50% non - irradiated sewage sludge (NISS) +50% compost); T5, (rate 100 %compost) was undertaken for a period of 180 days under field condition. This plant was able to remove heavy metals (Zn, Pb, Cd and Cu) effectively from the medium containing 100% sewage sludge and after harvesting, the concentrations of Zn, Pb, Cd and Cu in T3 (100% sewage sludge) were decreased by 216.8, 190, 40.8 and 3.5 mg/kg, respectively from the initial values. The highest levels of Zn (169 mg kg^{-1}), Cu (173.7 mg kg^{-1}), Pb (3.8 mg kg^{-1}) and Cd (3.2 mg kg^{-1}) accumulation were found in the roots. Concentrations of heavy metals in soil and plant were always higher in NISS treatment in compare to GISS. The results prove that the gamma irradiated sludge material was of better quality compared to the conventional NISS.

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Introduction

Planting Jatropha in Egypt started in 2004 **(SWERI, 2009).** It is still in the experimental stage, but it has been proved that the potential to plant this tree is high in the marginal areas and desert. The planting of this tree has succeeded in Upper of Egypt . Never the less the growth and blooming periods in this area is shorter than that in other countries; it produces flowers after 18 months while in other countries it needs three years. The planted area with Jatropha in Egypt is now about 500 ha located in three regions: Asyoot, Sohaj and Al-Swies. The industrial effluents have converted large agricultural areas into dry wasteland, which remains as such even after closing of industrial units in the area **(Ghavri and Singh, 2010).** The industrial effluents often contain large quantity of toxic heavy metals. These metals are non bio-degradable and persistent and can be differentially toxic to microbes **Giller *et al*., 2009),** plants **(Ghavri *et al*., 2010; Sharma *et al*., 2010),** animals **(Rainbow, 2007)** and human being **(Lim and Schoenung, 2010)** .Jatropha curcas L. (Family: Euphorbiacae) is a potential biodiesel plant, which **(Gunaseelan,2009)**, can survive harsh environments of semi-arid agro-climatic conditions, wastelands **(Mangkoedihardjo and Sunahmadia, 2008)** and grows fast with little maintenance. It can reach a height of 3-8 m Genus Jatropha with 172 species having significant economic importance is native to Central America and distributed in Africa and Asia **(Cano- Asseleih, 1989; Fairless, 2007).** Among the various Jatropha species, J. curcas, J. glandulifera, J. gossypifolia **(Achten, 2008),** identified as the most suitable oil bearing plant, and has been recommended for plantation on waste land. Jatropha curcas L., is a perennial Euphorbiaceae crop with potential such as medicinal and biodiesel crop recently and is recognized as potential oil seed **(Effendi *et al*., 2010; Rafii *et al*., 2012; Shabanimofrad *et al*., 2011).** This plant has proved it great important as a medicinal plant in treating tropical diseases of dermatological origin **(Igbinosa et al., 2009).** Also the attention on this crop has increased due to high rate of ozone layer depletion and global warming effect caused by increased usage of fossil fuel resulting in environmental pollution. Renewable biofuel feed stocks are perceived to be essential contributors to the energy supply portfolio as they contribute to the world energy supply security, reducing dependency on fossil fuel resources and provide opportunity for mitigating greenhouse gases **(Sudhakar and Nalini, 2011).** This newly introduced crop, which grows abundantly in wild and abandoned land, has its seed and oil yield unpredictable especially in tropical climate. Favourable environmental conditions that affect it production are yet to be known **(Ovando *et al*., 2011, Divakara *et al*., 2010).** In spite of the great potentials and attributes of Jatropha as a biodiesel crop, the full potentials of Jatropha have not been realized. One of the reasons for this, apart from the agronomic, social economic and institutional constraints is the facts that there is presently no planned rational conventional breeding and genetic programs. **Ginwal *et al*. (2005)** reported that, for the fact that Jatropha has adapted itself to wide range of environmental and ecological conditions suggests that, there exists considerable amount of genetic diversity yet to be detected for potential realization. **Rao *et al*. (2008)**

Plants in trace amount need heavy metal but their availability in the excess may cause plant toxicity **(Sharma and Katyal, 2006).** Phytotoxic concentration of the heavy metals referred in the literature does not always specify the levels **(Wua *et al*.2010),** upon reaching which, a tree become apparent lyvulnerable. The properties of soil/sludge transfer of heavy metal from the soil to the plants or ground water and phytoremediation potential of the various plants may also affect the toxicity of metals to the plants. The plants, which are less sensitive to the soil contamination, may be grown in such waste lands to remove the excessive toxic metals and to make the area green and cultivable. High heavy metal accumulations have been reported in roots **(Maurice and LayerKvist, 2000),** black alder (*Alrus glutinosa* L.) and pine (*Pinus sylvestris* L.) grown on sewage sludge **(Butkus and Baltrenaite, 2007)**, however, these plants may not survive in semi tropical conditions .

In this study J. curcas was selected due to its hardiness, its characteristics as non-edible plantwhich can growin tropical areas and its commercial viability for the production of biodiesel, therefore the objective of this study is to determine the potential of J. curcas in removing heavy metals from soil and to investigate effects of different planting media T0, (control); T1, (100 % gamma irradiated sewage sludge (GISS); T2, (rate 50% gamma irradiated sewage sludge (GISS) +50% compost); T3, (rate 100 % non - irradiated sewage sludge (NISS); T4, (rat50% non - irradiated sewage sludge (NISS) +50% compost); T5, (rate 100 %compost) on the ability of Jatropha in removing heavy metals.

2. Materials and methods

A field experiment was carried out at the soils & Water Research Department, Nuclear Research Center, Atomic Energy Authority, Inshas, Egypt, on Jatropha curcas L. as an indicator plant using the materials described below. Plants Jatropha curcas L**,** supplied by the Agriculture Research Centre (ARC), Giza, Egypt were used in the experiments carried out through the study. Jatropha plants were sown in January 2013 in seasons and spaced at 300cm apart. Chemical and physical analyses of tested soil samples were determined according to **Black (1965).** The physical and chemical properties of used sandy soil were 88.5% sand , Silt 2.7%, and 8.8% clay , pH (1: 2.5) 7.97, EC(dS /m) 0.27,O. C 0.017%, O.M 0.03%, T.N 0.007%, C/N Ratio 2.43, Ca CO_3 1.0%.The compost was collected from soils and Water Research Department, Nuclear Research Center were made by **(Moursy 2008).** The chemical properties of compost the used investigated were PH (1:5) 6.60, EC 8.50 ds/m, C/N ratio12.63, O.M 68.92%, N 1.25%, P 0.85%, K 0.658%.Fe 3020µg kg-1, Cu 215.58µg kg-1, Mn 203.42µg kg-1, Zn 163.92µg kg-1, swage sludge collected from El-Gabal El-Asfar farm and treated by Gamma cell at dose 10 KGy (Nuclear Research Center). The chemical properties of swage sludge the used investigated were PH (1:5) 6.80, EC 4.11 ds/m, C/N ratio 8.50, O.M 45.92%, N 3.2%, P 1.25%, K 0.23%, Fe 14650 mg kg^{-1}, Cu 593 mg kg^{-1}, Mn 413.42 mg kg^{-1}, Zn 1459 µg kg^{-1}.

2.2. Experimental setup

Experimental design was complete randomized block with three replicates. The experiment included six treatments, T0 treatment (Control), T1 rate 100 % gamma irradiated sewage sludge (GISS);T2 rate 50% gamma irradiated sewage sludge (GISS) + 50% compost, T3 rate 100% non-irradiated sewage sludge (NISS),T4 rate 50% non-irradiated sewage sludge (NISS) + 50% compost, T5 rate 100 % compost. Dose of organic fertilizer was applied to each tree spaces (3 x 3 m^2) at the rate of 10 m^3/ fed as organic material. No other fertilizer was used during plant growth. After 180 days, plants were harvested, leaves stem and roots then dried at 70ºC, weighed and digested.

The total metal removed from soil (mg kg^{-1}) was calculated by subtracting the amount of metal concentration in soil after planting from the amount of metal concentration in soil before planting.

2.3 Translocation Ratio (TR) and Transfer factor (TF):

This parameter is necessary for environmental transfer models which are useful in prediction of the pollutant concentrations in agricultural crops for estimating dose intake by man **(lofty and mostafa 2013).** TR is calculated by the relation: the ratio of concentration of metal in the shoot to the concentration of metal in the roots **(Cui *et al*., 2007).**

TR ={Concentration of metals}$_{shoot}$ /{Concentration of metals }$_{root}$ (1)

TF is given by the relation: the ratio of the concentration of metal in the shoots to the concentration of metal in the soil **(Chen *et al*., 2004).**

The transfer factor (TF) is a value used in evaluation studies on the impact of routine or accidental releases of pollutant into the environment.

TF ={Concentration of metals}$_{shoot}$ /{Concentration of metals }$_{soil}$ (2)

These factors were used to evaluate the Zn, Pb, Cd and Cu Phyto-extraction capacity of J. curcas plant.

2.4 Statistical analysis

Data were statistically analyzed to test the anova (two way and three way) and least significant different LSD using MSTAT software according to the standard statistical methods **(Power 1985).**

3. Results and Discussion

3.1 Heavy metal concentration in J. curcas growth media before planting and after harvesting:

The concentrations of heavy metals before planting and after harvesting are shown in Figs. 1-4. The J. curcas was found to be able to efficiently remove the heavy metals, such as Zn, Pb, Cd and Cu especially in T3 where the planting medium contained 100% sewage sludge.

Copper concentration decreased in the growth media, the highest reduction observed in T4 (30.2%) followed by T2 (30%) and T3 (26.8%), respectively. The lowest reduction (22%) was recorded in the T1 treatment (Fig.1). **Marcel (2006)** reported that Cu becomes more soluble in acidic soils. Therefore, Cu uptake by the plant would be more in acidic soil and as a result a higher reduction in the growth will be occurred. **Majid *et al*. (2011)** observed similar results in Cu contaminated soil planted with Acacia mangium. Copper is an essential micronutrient and constituent of many enzymes but in higher concentrations it creates toxicity to plants, humans and microorganisms **(Perk, 2006).**

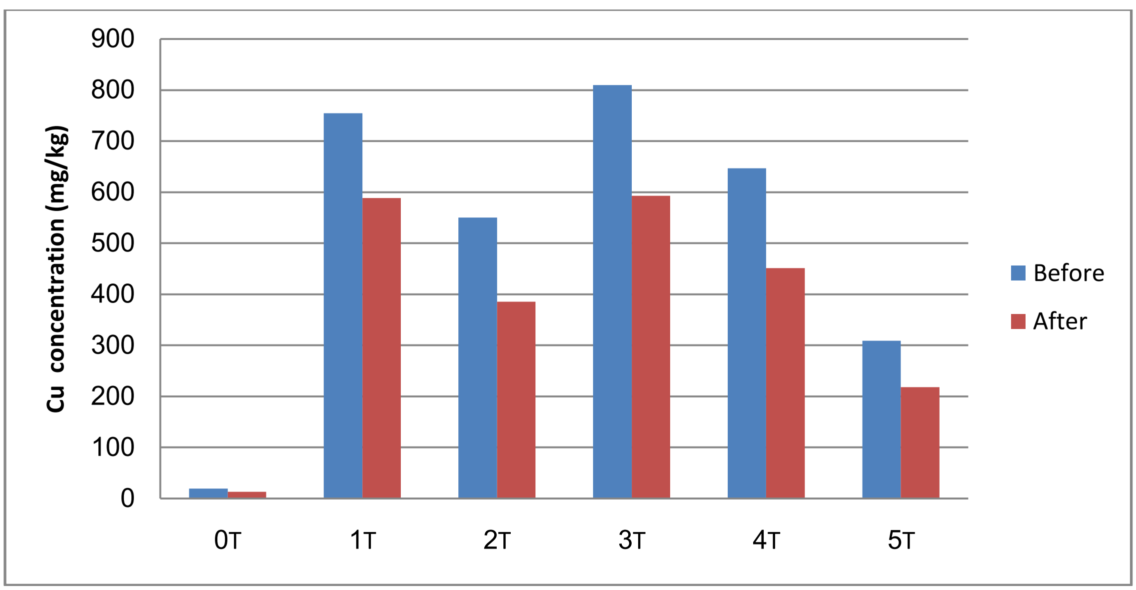

Fig. 1: Concentrations of Cu in growth medium before planting and after harvesting. Different letters indicate significant difference between means at each treatment before planting and after harvesting

according to a Student's t-test ($p<0.05$) ns, not significant difference ($p<0.05$). T0, (control); T1, (100 % gamma irradiated sewage sludge (GISS); T2, (rate 50% gamma irradiated sewage sludge (GISS) +50% compost); T3, (rate 100 % non - irradiated sewage sludge (NISS); T4, (rat50% non - irradiated sewage sludge (NISS) +50% compost); T5, (rate 100 %compost)

Zinc concentration decrease in all treatments, Treatment T3 showed highest reduction of Zinc concentration (11.5%) followed by T4 (10.1%) then T1 (8.1%). T2 showed the lowest reduction (7.8%) (Fig.2). Zinc concentration decreased in the growth media, which might be due to its higher uptake by the plant.

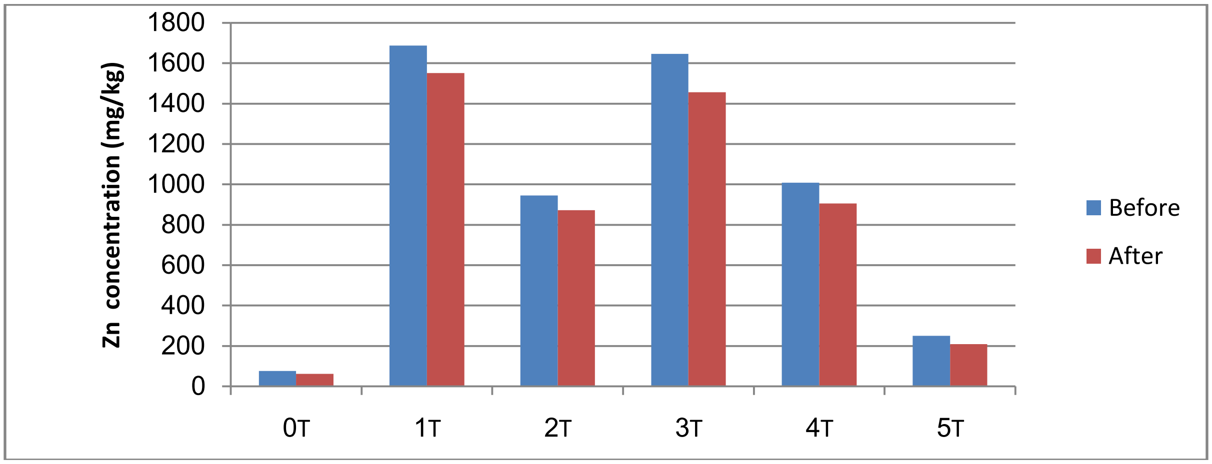

Fig. 2: Concentrations of Zn in growth medium before planting and after harvesting. Different letters indicate significant difference between means at each treatment before planting and after harvesting according to a Student's t-test ($p<0.05$) ns, not significant difference ($p<0.05$). T0, (control); T1, (100 % gamma irradiated sewage sludge (GISS); T2, (rate 50% gamma irradiated sewage sludge (GISS) +50% compost); T3, (rate 100 % non - irradiated sewage sludge (NISS); T4, (rat50% non - irradiated sewage sludge (NISS) +50% compost); T5, (rate 100 %compost)

Lead (Pb) is one of the most frequently inorganic pollutants in the soils **(Alkorta *et al*., 2004).** The normal levels of Pb in soils are the range of 0.85 to 65.8 % **(Zarcinas, 2003).** It is potentially toxic even at low concentrations and above 400 mg Pb kg^{-1}, the soil is considered hazardous to human health **(US-EPA, 2001).** Lead is not an essential element and potentially is toxic to plant, animal and human. J. Curcas was found to remove lead (Pb) efficiently. Lead (Pb) concentration was significantly decreased in the growth media as the highest reduction recorded in T2 (40.8 mg/kg) followed by T4 (37.5 mg/kg) followed by T1 (37 mg/kg) and the lowest in the treatments T0 (2.1 mg/kg) and T5 (3.6 mg/kg) (Fig.3). Cadmium (Cd) concentration was also decreased in the growth media after harvest

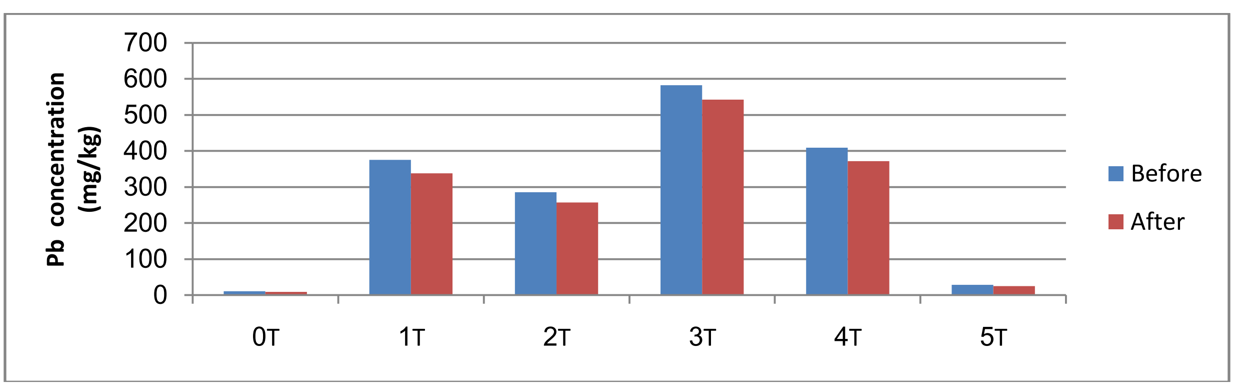

Fig. 3: Concentrations of Pb in growth medium before planting and after harvesting. Different letters indicate significant difference between means at each treatment before planting and after harvesting according to a Student's t-test ($p<0.05$) ns, not significant difference ($p<0.05$). T0, (control); T1, (100 % gamma irradiated sewage sludge (GISS); T2, (rate 50% gamma irradiated sewage sludge (GISS) +50% compost); T3, (rate 100 % non - irradiated sewage sludge (NISS); T4, (rat50% non - irradiated sewage sludge (NISS) +50% compost); T5, (rate 100 %compost)

(Fig.4). It takes the same trend which was observed with the other elements under investigation

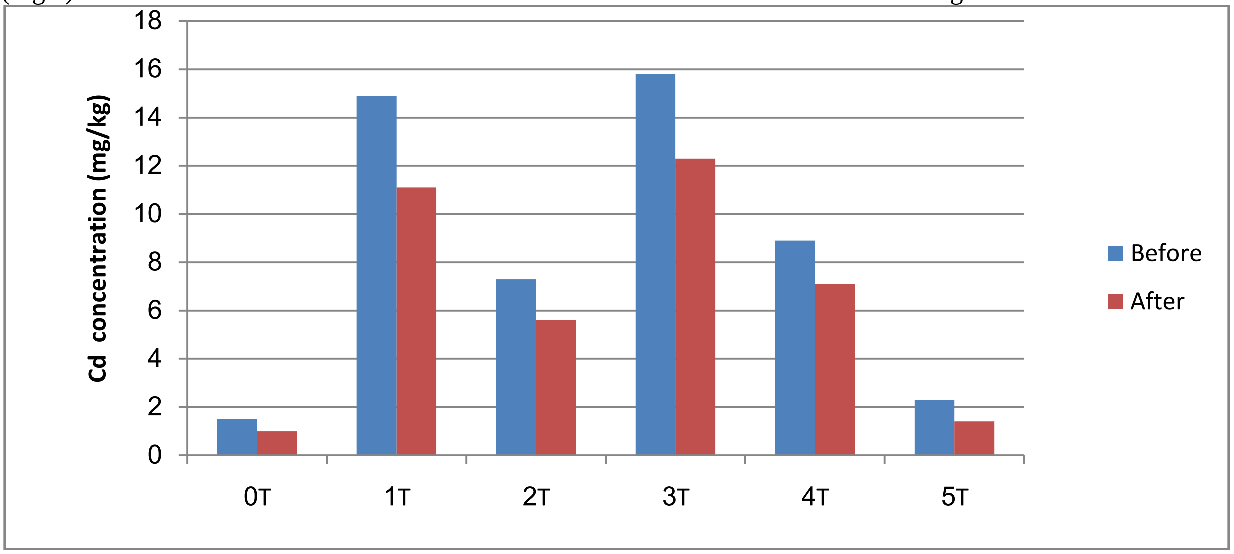

Fig. 4: Concentrations of Cd in growth medium before planting and after harvesting. Different letters indicate significant difference between means at each treatment before planting and after harvesting according to a Student's t-test ($p<0.05$) ns, not significant difference ($p<0.05$). T0, (control); T1, (100 % gamma irradiated sewage sludge (GISS); T2, (rate 50% gamma irradiated sewage sludge (GISS) +50% compost); T3, (rate 100 % non - irradiated sewage sludge (NISS); T4, (rat50% non - irradiated sewage sludge (NISS) +50% compost); T5, (rate 100 %compost)

3.2 Heavy metal concentration in plant parts

The heavy metal concentration in the plant parts was significantly variable among treatments ($p\leq0.05$). The roots showed highest Cu accumulation (173.7 mg/kg) followed by the steam and leaves (43. mg/kg) (table 1). The lowest accumulation (1.7 mg/kg) was in the leaves. **Qihang *et al*. (2011)** also reported highest Cu absorption by the roots for J.curcas grown on metal contaminated acid soils. Generally, The heavy metal concentration in the plant parts of treatment T1 and T3 was significantly variable among treatments ($p\leq0.05$)

In root, T3 showed the highest Cu concentration (173.7 mg/kg) followed by T4 (158.5 mg/kg), T1 (135.7 mg/kg), T2 (122.87 mg/kg), and T5 (68.7 mg/kg). The lowest accumulation of Cu (4.5 mg/kg) was detected in the control. Among the treatments, similar trend was observed with steam and leaf. Where, T3 showed the highest concentration of Cu (43.1 mg/kg) followed by T2 (42.33 mg/kg), T4 (36.8 mg/kg), T1 (30 mg/kg) and T5 (22.33 mg/kg) and the lowest (1.7 mg/kg) was recorded in the control. It was observed that higher concentrations of Cu in soil caused a high Cu translocation in the shoot and roots. The uptake and translocation of Cu is highly dependent on pH and phytoavailability of Cu increases with the decrease in pH **(Sheldon and Menzies, 2005).** The metal transfer mechanism and accumulation pattern in different ecosystems vary from plant to plant and metal to metal **(Jamil *et al*., 2009).** The present findings showed that total root Cu concentration more than shoot Cu concentration. These results confirmed former studies showing that plants can restrict Cu translocation towards their shoots **(McBride, 2001; Chaignon and Hinsinger, 2003; Chaignon *et al*., 2003; Kopittke and Menzies, 2006)** even for field-grown cereals **(Michaud [et al]., 2007).**

The highest Zn concentration was found in the roots (169 mg/kg) followed by stems and leaf (20.3 mg/kg), whereas the minimum Zn recorded in the steam and leaf (1.2 mg/kg) (Table 1). Among the treatments, T3 showed the highest Zn concentration in the roots (169 mg/kg) followed by T1 (116.2 mg/kg), T4 (84 mg/kg), T2 (56.2 mg/kg), T5 (35.3 mg/kg) and T0 (12.5 mg/kg). In stems and leaf, the maximum accumulation (21 mg/kg) was detected in T3 followed by T1 (20.3 mg/kg), whereas the minimum (1.2 mg/kg) was in the control.

Generally, the normal concentration of Pb in the plant ranged from 0.1 to 5.0 ppm **(Reeves and Baker, 2000).** Similar trends were observed with Pb and Cd , where The roots exhibited highest absorption compare with the aboveground part, the highest Pb and Cd accumulation (3.7 and 3.8 mg/kg respectively) were observed in T3 and the lowest (1.8 and 0.4 mg/kg respectively) was recorded in the control (table 1). It was observed that Pb and Cd absorption increased with increase of sewage sludge percentage in the growth media. Accumulation and distribution of heavy metals in plant tissues are important to evaluate the role of plant in remediation of heavy metals in soils **(Friedland, 1989).** To stabilize metal contaminated sites, a lower metal concentration in stem is preferred, in order to prevent the metals which enter into the ecosystems **(Taylor and Percival, 2010). Qihang *et al*. (2011)** reported highest Pb accumulation by the Jatropha curcas root, which agrees with the findings of our results. The success of J. curcas in taking up Pb and Cd from soil is paralleled with the capacities of Euphorbia cheinrandenia , which comes from the same family **(Chehregani and Malayeri, 2007).** In addition, Increasing soil zinc is known to reduce cadmium availability to plants because Zn inhibits cadmium uptake and cadmium translocation from roots to shoots of plants **(Chaney, 1983).** Zn and Cu are both essential for man, plants and animals but in high concentration can be toxic. in addition, zinc plays an important role in many biochemical functions in the plants **(Fox and Guerimot, 1998).**

Table 1: Heavy metal concentrations in leaves and root of J. curcas at three months after planting

Treatment	Concentration of Cu (mg/kg)		Concentration of Zn (mg/kg)		Concentration of Pb (mg/kg)		Concentration of Cd (mg/kg)	
	Root	Steam and Leaf	Root	Steam and Leaf	Root	Steam and Leaf	Root	Steam and Leaf
T0	4.5E	1.7D	12.5F	1.2B	1.8C	0.3C	0.4D	0.1B
T1	135.7C	30BC	116.2B	20.3A	33.6A	3.4AB	2.9A	0.9A
T2	122.87C	42.33A	56.3D	17.8A	26B	3B	1.3BC	0.4AB
T3	173.7A	43.1A	169A	21A	37A	3.8A	3.2A	0.3B
T4	158.5B	36.8AB	84C	18.2A	34A	3.5AB	1.6B	0.2B
T5	68.7D	22.33C	35.3E	5.8B	3C	0.6C	0.8CD	0.1B
LSD	14.17	11.17	16.02	11.91	4.96	0.72	0.74	0.52

Table 2. Translocation ratio (TR) and transfer factor(TF) of heavy metals in Jatropha curcas as influenced by different treatments.

Treatment	TR (translocation ratio)				TF (transfer factor)			
	Cu	Zn	Pb	Cd	Cu	Zn	Pb	Cd
T0	0.38A	0.10D	0.17AB	0.25A	0.09A	0.02A	0.03A	0.07A
T1	0.22C	0.17BC	0.10C	0.31A	0.04A	0.01A	0.01A	0.06A
T2	0.34AB	0.32A	0.12B	0.31A	0.08A	0.02A	0.01A	0.05A
T3	0.25BC	0.12CD	0.10C	0.09B	0.05B	0.01A	0.01A	0.02A
T4	0.23C	0.22B	0.10 C	0.13B	0.06B	0.02A	0.01A	0.02A
T5	0.33AB	0.16BCD	0.20C	0.13B	0.07B	0.02A	0.02A	0.04A
LSD	0.09	0.006	0.006	0.115	0.009	0.02	0.03	0.02

3.3 Translocation ratio (TR) and transfer factor(TF) of heavy metals in Jatropha curcas as influenced by different treatments.

The translocation ratio of metals under investigation ranged between 09 to 38. the lowest translocation ratio was observed in the T3 (0.12, 0.10 and 0.09 for Zn, Pb and Cd, respectively), which may imply the restriction in soil-root and root-shoot transfer at higher metal concentrations in the soil. Similar results were found by **Yoon *et al*. (2006)** **(Table 2). Majid *et al*. (2012)** also reported lowest TR at higher metal concentrations on cultivation of Justicia gendarussa in textile sludge contaminated soil. In average the transfere factor of cu under different treatments equal 0.06

We can conclude that J. curcas can uptake heavy metals such as Zn, Pb, Cd and Cu efficiently, especially in T3, in which the planting medium contained 100% sewage sludge (Table 1). Before planting, the higher accumulation of Cd, Cu, Zn and Pb in the sewage sludge containing soils were out of- the permissible limits stated by **World Health Organization (1998).** The level of Pb in T3 also showed the highest decrease compared to the other treatments and control medium, with 40.8 mg/kg less in the growth medium after harvesting (542.2 mg kg^{-1}) compared to the initial Pb level (583 mg kg^{-1}). The success of J. curcas in taking up Pb and Cd from soil is paralleled with the capacities of Euphorbia cheinrandenia, which comes from the same family **(Chehregani and Malayeri, 2007).** The highest decrease in Cu levels in the J. curcas growth medium was observed in T3, in which the level of Cu after harvesting was 593.1 mg kg^{-1}, while the initial level was 809.9 mg kg^{-1}, indicating a 26.8% loss.

The TR of Cu and Zn are similar (0.29 and 0.18, respectively). while the TR of Pb and Cd are similar, although lower than those of Cu and Zn. These results may be justified by the fact that the first two are oligolements, that are necessary for the plant growth **(He *et al*., 2005),** whereas the other two are rather harmful from the physiological point of view. So, and as reported by **Denaix (2007),** the plant keeps the highest quantity of them in the root system, either precipitated in the cells or chelated with an organic compound, thus lowering the trace element translocation to the outer parts.

Concerning treatments and the control sample, at a preliminary stage, one should note that the TR of all the elements are lower than one for Pb, Cd, Zn and Cu); which means that the physiological need of the plant for these elements is rather limited. However, similarity in the TR

is observed for Cu and Zn, on the one hand, and for Pb and Cd, on the other, with TR (Cu ,Zn) being superior to TR (Pb,Cd), showing that the first elements are necessary for the growth of the plant.

In general, the more contaminated plants are the more accumulated heavy metals we notice in root cells, and specifically in the apoplasm. The uptake of these elements is regulated by active (metabolic) and passive (non-metabolic) mechanisms at the soil-root interface **(Denaix, 2007).** Trace elements are translocated from roots to shoots via a number of physiological processes, including metal unloading into root xylem cells, long-distance carrying from the xylem to the shoots and metal reabsorption, by leaf mesophyll cells, from the xylem stream. Once the trace metals have been unloaded into the xylem vessels, the metals are carried to the shoots by the transpiration stream **(Blaylock and Huang, 2000).** So, the contrasts observed, with respect to the biogeochemical characteristics of the two plant speciesnder consideration, are the illustration of difference

in physiological behavior, either during the acquisition of element at the soil/root interface, or at the time of the root/upper part transfer **(Marschner, 1995).**

4- Conclusion

Phytoremediation of contaminated soil with heavy metals using non-edible plant like Jatropha *curcas L* offers an environmental friendly and cost-effective method for remediating the polluted soil. The J. curcas was found to be able to efficiently remove the heavy metals, such as Zn, Pb, Cd and Cu especially in T3 where the planting medium contained 100% sewage sludge. In addition our results prove that the gamma irradiated sludge material was of better quality compared to the conventional NISS. Where the uptake of heavy metals by plant parts were always higher in non - irradiated sewage sludge (NISS) compared to irradiated sewage sludge.

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