Phytoremediation potential of *Jatropha curcas* for removal of heavy metals from refinery sludge

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Abstract- Heavy metal pollution in soil is one of the most important environmental problems throughout the world, which causes significant toxic effect on humans, animals, microorganisms and plants. Phytromediation is an emerging technology in which non-edible plants are used to remove, stabilize and eradicate organic and inorganic contaminant from soil, sediment and water. This research aims to examine the growth response, metal tolerance and phytoremediation ability of *Jatropha curcas* for heavy metals present in oily sludge of petroleum refinery. The seedlings of *J. curcas* were planted in the growth media (soil) at six treatment levels with sludge. The seedlings showed the growth performance comparable to control in first three treatments in terms of shoot height, number of leaves and root length. Among different treatments, the highest plant biomass was recorded in S1 and S2 treatment, depicting its tolerance in multi-metal contaminated soils. In S2 and S3 treatments, the maximum percent removal of cadmium was observed, followed by chromium, copper and nickel. Being a biodiesel and non-edible plant, *J. curcas* can be an ideal option to be grown for phytoremediation in multi-metal contaminated sites and to mitigate the soil pollution for sustainability of land resources.

Keywords – Biomass, Biodiesel, Heavy metals, *Jatropha curcas*, Metal Tolerance, Phytoremediation, Refinery sludge,

1 INTRODUCTION

Rapid industrial development and urbanization during the past two decades have increased the quantity and diversity of toxic and hazardous wastes on land. In the developing countries, heavy metal pollution becomes serious and this dramatic acceleration of heavy metal contamination in soil is mainly as a result of industrial revolution e.g. mining, mineral, smelting and tannery industries [1-2]. Most common heavy metal contaminants are lead, cadmium, chromium, copper, mercury and zinc [3]. Lead and cadmium are non-essential elements, but zinc at lower concentration is an essential micronutrient for plants. Higher doses of these metals may cause metabolic disorders and growth inhibition for most of the plant species, often leading to death [4-5]. Moreover, heavy metals are not subjected to degradation and therefore, remain almost indefinitely in the environment [6].

Remediation of heavy metals polluted soil could be carried out using physico-chemicals processes such as ion-exchange, precipitation, reverse osmosis, evaporation and chemical reduction; however, these measures are costly and leave toxics in soil [7]. Phytoremediation seems to be an alternative to all these methods by which plant is applied to absorb, transform and detoxify heavy metals and this method is simple, efficient, cost effective and environmental friendly [8]. Investigation on the ability of plants in removing heavy metals from soil has been investigated, using *Lolium perenne* [10], *Brassica juncea* [11], *Helianthus annus* and *Brassica napus* [12], *Streptanthus polygaloides, Sebertia acuminata, Armeria maritima, Aeollanthus biformifolius* and *Eichornia crassipes* [13].

For effective phytoremediation process, the plant species should be non-edible and can be grown abundantly in large scale on wastelands. Considering these factors, *Jatropha curcas* L. (Ratanjyot) has been selected for this study. J. curcas is a bush/small tree belonging to the family of Euphorbiaceae [14] and this might be effective in removing Pb and Cd as demonstrated for *Euphorbia cheirandenia* [15]. Jatropha is native of Central America and has become naturalized in many tropical and subtropical areas including India, Africa and North America. *J. curcas* is a multipurpose species with many attributes and considerable potential that grows practically all over India under a variety of agro-climatic conditions. In addition, Jatropha has been found to be able to remove hexavalent chromium [16]. Therefore, planting of *Jatropha curcas* L. could have many advantages, i.e. remediation of metal polluted soil, provision of green space and a source of alternative biofuel.

The objective of this research was to assess the effectiveness of *J. curcas* for removal of chromium, copper, nickel and lead from refinery sludge contaminated soil by determining (1) its growth parameters (2) accumulation of heavy metals in different plant components and (3) change in conc. of heavy metals in growth medium (soil) after harvesting of plants.

2 MATERIALS AND METHODS

2.1 Soil and Oily Sludge

The pot experiment was carried out in the green house of Institute of Environmental Studies at Kurukshetra University, Kurukshetra, Haryana, India. The soil used was neither saline nor sodic with pH-7.32, moisture content–21%, water holding capacity–43%, organic carbon-0.85%, available nitrogen-6.35mg/kg, organic matter–1.36% and available phosphorous–0.35 mg/kg. The refinery sludge used in this study was taken from Indian Oil Petroleum Refinery, Haryana, India. The composition of sludge was pH-5.85, moisture content–52%, organic carbon-2.95%, available nitrogen-15.5mg/kg, organic matter–5.08%.

2.2 Pot experiment

The phytoremediation potential of *Jatropha curcas* was assessed by pot experiments. Jatropha seeds were collected from local agriculture agency and plants were grown in nursery. Healthy plants with a height of 10-15 cm were selected as test plants for this study. Two plants were placed in each pot containing 5 kg test medium (soil). Six different levels of metal contamination were obtained by mixing refinery sludge and soil (w/w) as 10% sludge (S1), 20% sludge (S2), 30% sludge (S3), 40% sludge (S4), 50% sludge (S5) and 60% sludge (S6). Soil with no sludge was treated as control. There were three replicates of each treatments and the duration of the pot experiment was 90 days. After the end of pot experiment the shoot length, root length, number of leaves and plant biomass were observed and heavy metal analysis of different components of plants and of soil before and after harvest was performed.

2.3 Preparation of soil and plant samples for heavy metal analysis

After 90 days of experimental set up, soil and harvested plant samples were analysed for heavy metals by Atomic Absorption Spectrophotometry, which is an ideal, sensitive and accurate method for quantification of heavy metals. One gram of the soil from each pot was digested in acidic mixture of HNO_3: $HClO_4$ and the concentration of heavy metals was analysed. After harvesting, root, shoot and leaves of Jatropha plant were separated, converted in to ash and digested as in case of soil samples.

3 RESULTS

The set up of pot experiment is shown in Fig.1 and the effect of sludge with multi-metal contamination on the growth parameters of Jatropha plants is shown in Table 1.

Fig. 1. Growth of *Jatropha curcas* at different treatment levels of refinery sludge

3.1 Growth performance of J. curcas cultivated in a soil containing refinery sludge

The growth parameters of Jatropha plants were affected more or less by the toxicity of refinery sludge and the multi-metal contamination in it. But at lower level of contamination up to S3 treatment, the observed effect was negligible. The plants in the control soil had highest average shoot height (62±2.0 cm), followed by S2 and S1, whereas S6 had the shortest shoot height (31±1.0 cm). The plants in control and S1 treatment also had highest number of leaves, followed by S2 treatment and the plants in S5 and S6 showed up to 40 - 60% decrease in no. of leaves.

Highest root length was observed in S1 treatment (21±1.2 cm), followed by S2 treatment and control. Treatment S5 and S6 had lowest root length. Plant biomass estimation showed significant difference between different treatment levels. Almost same plant biomass was observed in control and first treatment (S1), but at higher level of contamination, biomass decreased subsequently up to 65% in S4 and up to 35% in S6 treatment.

3.2 Heavy metal concentrations in growth medium (soil) before planting and after harvesting of *J. curcas*

The concentrations of heavy metals in initial soil samples (before planting) and after harvesting of *J. curcas* (final) are shown in figure 2.

TABLE 1: GROWTH PARAMETERS OF JATROPHA CURCAS AFTER 3 MONTHS GROWTH IN DIFFERENT TREATMENT LEVELS

Treatment Level	Shoot Length (cm)	Root Length (cm)	No. of Leaves (cm)	Plant Biomass (g)
Control	62±2.0	19±1.2	10±0.6	15.93±0.5
10% S (S1)	60±1.0	21±1.2	10±0.6	15.23±0.8
20% S (S2)	60±1.0	20±1.0	9±0.6	13.43±0.6
30% S (S3)	52±1.5	15±1.5	8±0.6	11.57±1.2
40% S (S4)	47±3.5	14±0.6	7±0	10.03±0.6
50% S (S5)	38±2.0	10±1.0	6±0.6	7.70±0.8
60% S (S6)	31±1.0	9±1.0	4±1.0	6.13±0.6

Jatropha curcas was found to be capable of efficient removal of all observed heavy metals, such as Cd, Cr, Cu and Ni from soil but the percent removal was highest in case of cadmium. The Cd content in treatment S1 was 0.08 mg/kg initially and after 3 months growth of Jatropha, Cd levels. Almost same trend was observed in case of chromium and nickel removal.

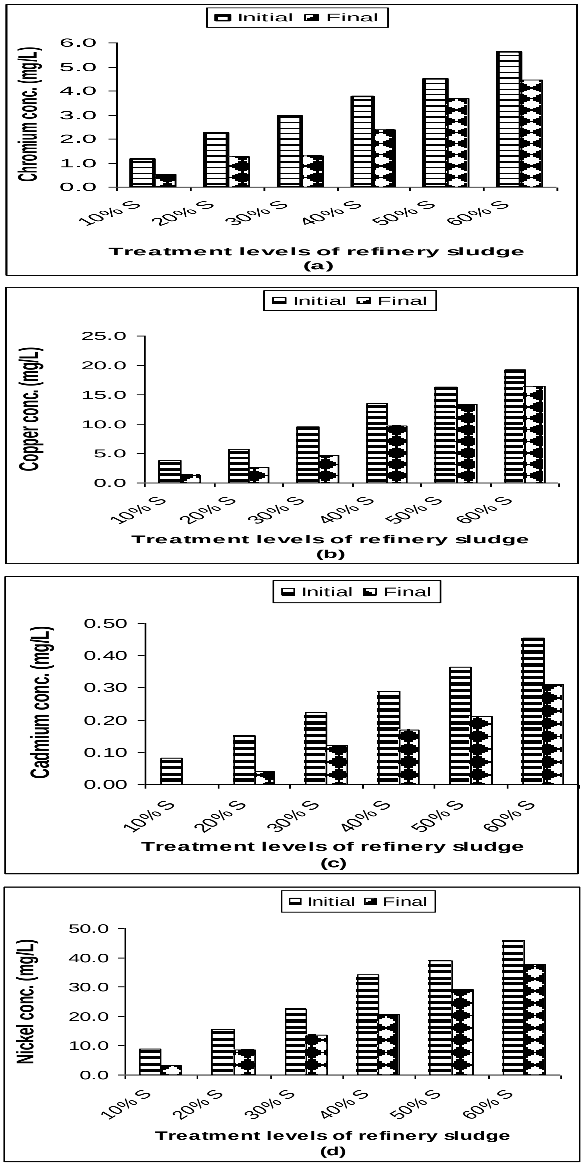

Fig. 2. Change in heavy metals concentrations (a) chromium (b) copper (c) cadmium (d) nickel in the soil (initial) and after cultivation of Jatropha curcas (final)

In general, at lower contamination level, up to treatment S3 (30% sludge), the observed percent removal was higher, but at treatment S5 and S6, the percentage of heavy metals removal were subsequently reduced.

3.3 Heavy metal accumulation in different plant parts of Jatropha curcas:

The concentrations of Cd, Cr, Cu and Ni in plant parts (leaves, stem and roots) at three months after planting are shown in Fig. 3. The accumulation of Cd was observed in the only roots of the plants up to S4 level, but at S5 and S6 level, the accumulation was also reported in shoots. The highest Cd accumulation was observed in the roots of J. curcas at S2 treatment level (0.80 mg kg-1), followed by S3 (0.70 mg kg-1) and S1 (0.50 mg kg-1) respectively. Furthermore, the roots of J. curcas were found to absorb high levels of Cu and Ni as compared to the stem and leaves. The amount of copper accumulation was found to be very small in leaves of Jatropha plants.

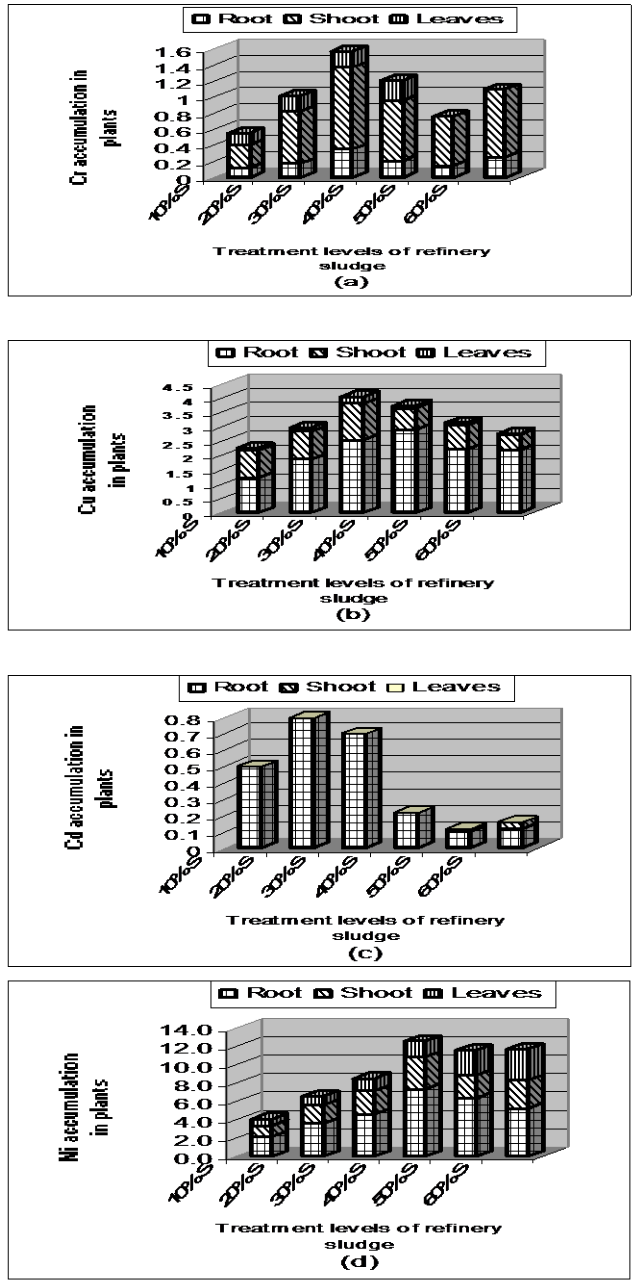

Fig. 3. Heavy metals concentrations (a) chromium (b) copper (c) cadmium (d) nickel in different plant parts of *Jatropha curcas* after harvesting

4 DISCUSSION

According to results of growth parameters of *J. curcas*, it was observed that the growth of this plant was not significantly affected at lower level of metal contamination, but higher level of contamination decreased the growth parameters up to some extent. The highest average shoot length (62±2.0cm) was found in control, followed by S2 (60.5±1.0 cm) and S1 (60±1.0 cm). These results indicate that the treatments S2 (20% sludge + 80% soil) and S1 (10% sludge + 90% soil) were found to be suitable and ideal for achieving optimum *J. curcas* growth. The shoot growth of *J. curcas* was found to be slow in the first 2 weeks, which likely occurred due to the plants attempting to acclimatize themselves to their new growing medium. The number of leaves were observed to be similar in the control medium and S1, but declined up to S6, which may have been caused by the high heavy metal level in the soil, creating an acidic state in the growing medium and resulting in lesser growth of leaves. The higher root length in first and second treatment level as compared to control, has depicted the adaptation capability of plants for accumulation of metals from contaminated soil. However, there was a significant reduction of plant dry matter at highest level of metal contamination because of high level toxicity of metals. This has also been reported by [17-18] for phytoremediation of cadmium and lead. Other workers have also found that low nutrient and water holding capacities, toxic substances, salinity, stability, nutrient deficiencies (especially N, P and K), poor physical properties and excess acidity and alkalinity are some of the contributing factors that inhibit or disrupt plant growth in soil containing high amounts of heavy metals [19].

J. curcas can uptake heavy metals such as Cd, Cr, Cu and Ni efficiently, especially in first three treatment levels, in which the planting medium contained 10%-30% sludge. After three months of Jatropha growth Cd was not detected in the soil at S1 level, imply complete removal. At S2 treatment also, percent removal of Cd was 76% compared to the initial level. The level of Cr in S3 also showed the highest decrease compared to the other treatments and control medium, with 56.08% removal of Cr from the growth medium after harvesting compared to the initial level. The success of *J. curcas* in taking up Cd from soil is paralleled with the capacities of Euphorbia cheinrandenia, which comes from the same family (Chehregani and Malayeri, 2007) [15]. Other scientists have also reported that the J. curcas plant has the capability to grow well in soil with high heavy metals concentrations of heavy metals, [20-21]. The highest decrease in Cu levels in the *J. curcas* growth medium was observed at S1 level with 67.5% decline from initial concentration.

The higher Cu accumulation was recorded in roots of J. curcas as compared to shoot and leaves at S3, S4 and S5 treatment levels. In case of Cr, the shoots of *J. curcas* were found to absorb higher levels, compared to the root and leaves. However in case of Cd, accumulation was only found in the roots of the plants, with the higher level observed at S2 and S3 level. The restriction of heavy metals in roots of *J. curcas* also affects the uptake of Cd. The Cd level was found to be low in the soil after harvest, with the highest uptake of Cd observed in the roots and the stems and leaves to a lesser degree at higher levels of treatment; this finding was supported by the fact that plants with extensive root systems have the advantage of being able to absorb greater amounts of heavy metals due to better soil penetration [22]. Also, it was found that *J. curcas* is not a good Ni accumulator because it absorbed small amounts of it, in comparison to the other elements.

Regarding the potential use of *Jatropha curcas* for the phytoremediation of heavy metals from refinery sludge contaminated soils; we have discerned that *J. curcas* is very efficient in accumulating heavy metals, causing almost no damage to the plant biomass. All parts of *Jatropha curcas* were able to absorb and store Cr, especially the stems, which showed the highest levels of Cr uptake in S3 level. The accumulation of heavy metals in *J. curcas* tends to occur mainly in the roots and to a lesser degree in the leaves and stems. Plant roots stimulate the degradation of toxic organic materials [23]. Furthermore, roots act as a medium for soil microorganisms to speed up the biodegradation rates of organic pollutant [24].

5 CONCLUSION

Phytoremediation may offer a viable solution to the problems of contamination and is drawing significant attention of scientists. In-situ remediation (plants / microbes) has drawn great values, because it is alternative to the soil removal and replacement. The hyper - accumulator plants could be grown in heavy metal contaminated soils to clean up such sites. The roots of *J. curcas* were found to be suitable for taking up heavy metals from refinery sludge. Cadmium was found to be effectively absorbed by the roots of *J. curcas*. However, the plant could be used for phytoremediation of copper and chromium polluted soil provided the initial concentrations of toxics were not very high. Therefore, *Jatropha curcas* can be an ideal option for phytoremediation in multi-metal contaminated sites. However, it is suggested that in such plants where accumulation is higher in roots, the whole plant should be uprooted or removed after the phytoaccumulation process.

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