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Jatropha curcas: A potential crop for phytoremediation of coal fly ash

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ABSTRACT

A greenhouse pot experiment was conducted to test the heavy metal phytoremediation capacity of *Jatropha curcas* from fly ash. Both natural accumulation by *J. curcas* and chemically enhanced phytoextraction was investigated. Plants were grown on FA and FA amended with fertile garden soil, in presence and absence of chemical chelating agent EDTA at 0.1 g kg^{-1} and 0.3 g kg^{-1} of soil. EDTA enhanced the uptake of all five elements (Fe, Al, Cr, Cu and Mn) tested. Fe and Mn were retained more in roots while Cu, Al and Cr were translocated more to the shoot. Metal accumulation index indicates that the effect of EDTA at 0.3 g kg^{-1} was more pronounced than EDTA at 0.1 g kg^{-1} in terms of metal accumulation. Biomass was enhanced up to 37% when FA was amended with GS. Heavy metal uptake was enhanced by 117% in root, 62% in stem, 86% in leaves when EDTA was applied at 0.3 g kg^{-1} to FA amended with GS. Study suggest that *J. curcas* has potential of establishing itself on FA when provided with basic plant nutrients and can also accumulate heavy metals many folds from FA without attenuating plant growth.

1. Introduction

Combustion of coal in thermal power plants, create environmental pollution by producing huge amount of fly ash (FA). In India FA generation has increased from 89 million tones in 1999–2000 to about 112 million tones in 2005–2006.The quantity of ash in ash ponds has increased from about 450 million tones in 1999–2000 to about 900 million tones in 2005–2006. The land area under active ash ponds has grown to about 16,000 hectares [1]. In India the utilization of fly ash is hardly 13%. It is mainly used in building material industry, in civil engineering, in road construction and for restoration of open cast mines [2]. Rest of the fly ash remain unutilized and is dumped in the landfills and ponds constructed for this purpose. The major elements found in FA are Si, Al, and Fe with a considerable concentration of trace elements like As, Ba, B, Ca, Cd, Cr, Cu, Hg, Mg, Mn, Mo, Ni, Na, Pb, Se, Sr, Sb, Ti, V, and Zn [3,4]. These toxic heavy metals, upon disposal, cause contamination of the ground water, surface water, and agricultural land, posing hazards to the entire population living in the area [5].

Vegetating the land fill seems to be the most feasible in situ remediation technique that will lead to: stabilization against wind and water erosion; decontamination of toxic heavy metals by plants; and development of aesthetically pleasing landscape that will provide shelter and habitat for wildlife [6]. Phytoremediation of heavy metal contaminated soil is a developing technology and has attracted much attention because it is an environment friendly and relatively cheap technique. One of the phenomena of phytoremediation, the 'phytoextraction', involves the use of plants to remove metals from the soil and concentrate them in the harvestable parts. Another phenomenon, the 'phytostabilization', involves the use of plants to arrest the contaminant in the rhizosphere and thus reduce its bioavailability in the environment [7,8].

In several cases it has been observed that inspite of presence of high concentration of metals in soil, they have low mobility into the plant system [9]. For such cases chelate induced phytoextraction was developed, with the objective of desorbing heavy metals from soil matrix into soil solution to facilitate the transport of metals into xylem, and increase translocation of metals from the roots to shoots of some fast growing, high biomass producing plants [10,11]. Several chelating agents like ethylene diamine tetra acetic acid (EDTA), *trans*-1,2-diaminocyclohexane-N′,N′,N′-tetraacetic acid (CDTA), diethylene triamine pentaacetic acid (DTPA), citric acid, maleic acid, etc. form more soluble complexes with the metal ions, and facilitate their uptake [12]. Out of these EDTA has been the most widely used chelating agent in studies of phytoremediation because of its high efficiency in extracting many metals [13].

Researchers all over the world are searching new plant species suitable to be used in phytoremediation. While selecting a species for phytoremediation several factors have to be taken into account. The species should be fast growing, high biomass producing, with profuse root system, tolerant to adverse environment condition, and non edible and economically beneficial [7,9]. Taking all these

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Table 1
Treatments given to *J. curcas*.

Code	Treatments
C	Garden soil (GS) 100% as control
F	Fly ash (FA) 100%
FL	FA 100% + EDTA @ 0.1 g kg^{-1}
FH	FA 100% + EDTA @ 0.3 g kg^{-1}
M	FA 50% + GS 50%
ML	FA 50% + GS 50% EDTA @ 0.1 g kg^{-1}
MH	FA 50% + GS 50% EDTA @ 0.3 g kg^{-1}

factors into consideration we have chosen *Jatropha curcas* and tested its suitability for phytoremediation of fly ash. *J. curcas* is a large shrub or small tree belonging to the family Euphorbiaceae. It is regarded as a potential biofuel crop for future due to its low moisture demands, pure hardiness and stress handling ability [14,15]. It grows fast with little maintenance and can reach a height of 3–8 m [14,16]. It has been identified in India as the most suitable oil bearing plant and has been recommended for plantation on wasteland as it requires minimal inputs for its establishment [15,16].

Fly ash landfills are vast wastelands in India. Our aim is to exploit this plant for phytoremediation. Firstly, for phytostabilization of fly ash, to reduce its blowing in nearby crop fields; and secondly, for phytoextraction of heavy metals through the use of chelator. This would restrict the leaching in the water bodies. The present study would identify (i) multi element accumulation pattern by *J. curcas* in different plant parts; (ii) effect of EDTA chelating agent on translocation of heavy metal from FA to different plant parts (enhanced phytoextraction); and (iii) the effect of substrate composition (pure FA and FA amended with fertile soil) on growth and phytoremediation efficiency of *J. curcas*.

2. Materials and methods

2.1. Experimental set up

Three types of substrate were chosen for the experiment, (i) fertile garden soil serving as control (C); (ii) 100% fly ash (F); and (iii) mixture of garden soil and fly ash (M) in the ratio of 1:1. FA was brought from the fresh dykes of Renu-Power Plant, HINDALCO Ind. Ltd., Renusagar, District Sonebhadra, U.P., India. The experiment was set up inside the glass house, in plastic pots of 0.25 kg capacity. Ten replicates were taken per treatment. The seeds of *J. curcas* (IC-468910) were obtained from the Seed Bank of National Botanical Research Institute, Lucknow, India. The seeds were collected from the wild from Lucknow district of U.P. The seed germplasm and passport information related to the mother plant was deposited at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi for medium and long-term conservation and allotment of accession number. The accession is also being maintained at NBRI by clonal propagation. These seeds were soaked in water for 2 h and then sown in vermiculite, inside germination chamber. After 15 days of germination, seedling was transferred in pot containing C (10 pots), F (30 pots) and M (30 pots) substrate (one seedling in each pot). The plants were allowed to grow in glass house and watered at regular intervals to keep the soil saturated. After 30 days of transplantation, 10 pots each of F and M were treated with 0.1 g kg^{-1} EDTA and another 10 pots each of F and M were treated with 0.3 g kg^{-1} EDTA. The treatments are listed in Table 1. After 30 days of EDTA application the plants were harvested to study the changes in growth, heavy metal content and other biochemical changes. All the substrates were also analyzed to observe the changes in heavy metal content.

2.2. Physico-chemical analysis of substrate

Physico-chemical analyses of GS, FA and FA–GS mixture were carried out taking five replicates. The pH was measured in soil–water suspension using Thermo-orion pH meter (Model 920); electrical conductivity (EC) was measured using cyberscan 500 EC Meter .The bulk density (BD), particle density (PD) and porosity were measured using the method of Blake [17]. Water holding capacity (WHC) was determined by the method of Klute [18]. Fe, Cu, Mn, Cr, and Al were determined by atomic absorption spectrophotometer (PerkinElmer Analyst 300) after di-acidic digestion using concentrated HNO_3 and $HClO_4$ in the ratio of 5:1.

2.3. Biochemical study

Chlorophyll content was estimated after extraction in 80% chilled acetone following the method of Machalachlan and Zalik [19] and carotenoid content was determined by the method of Duxbury and Yentsch [20]. Protein contents in leaves were estimated as per the procedure of Lowry et al. [21] using bovine serum albumin as a standard. The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content by the thiobarbituric acid (TBA) reaction [22].

2.4. Biomass and metal content

Harvested plants were repeatedly washed with double distilled water. The plants were separated in to roots, shoots and leaves. The biomass was estimated by drying the leaves, stem and roots to a constant weight at 80 °C in an oven. The plant materials were then ground into a fine powder for digestion. The samples were digested with a mixture of nitric acid and perchloric acid in the ratio of 5:1. Heavy metal (Fe, Al, Cu, Mn and Cr) analysis was performed using a PerkinElmer AAnalyst 300 atomic absorption spectrophotometer (AAS).

The analytical grade reagents were used. Certified aqueous standards of the elements (Sigma, traceable to NIST) were used to prepare standard curve for AAS. The standard reference material of metals (E-Merck, Germany) was used for calibration and quality assurance for each analytical batch. Analytical data quality of metals was ensured through repeated analysis ($n = 5$) of EPA quality control samples for metals in water and the results were found to be within ±2.79% of certified values. Recoveries of metals from the plant tissues were found to be more than 98.5% as determined by digesting three samples each from control plant with a known amount of metals.

2.5. Metal accumulation index

Metal accumulation index (MAI) of plant is a tool to obtain total metal accumulation by plants. It is calculated by the formula developed by Liu et al. [23].

$$\mathrm{MAI} = \left[\frac{1}{N}\right] \sum_{j=1}^{N} I_j$$

The mean value of each metal (Fe, Al, Cr, Cu and Mn) was divided by its standard deviation to get the sub index (I_j) of all the five metals studied. The sum of sub index of all the metals divided by total number of metals analyzed (N) gives the MAI. MAI identify the total elemental concentration in plant. In our study we have studied 5 elements, therefore the value of $N = 5$.

Table 2
Physico-chemical analysis of garden soil, fly ash and soil mixture.

Parameters	Garden Soil (C)	Fly ash (F)	Fly ash + garden soil (M)
pH	7.7 ± 0.1	8.2 ± 0.0	7.83 ± 0.05
EC (μs)	134.56 ± 6.85	326.2 ± 2.8	147.5 ± 5.26
BD (g cc^{-1})	1.38 ± 0.05	0.98 ± 0.02	1.24 ± 0.02
PD (g cc^{-1})	1.62 ± 0.05	1.9 ± 0.16	1.80 ± 0.06
Porosity (%)	14.81	48.42	31.11
WHC (%)	35.58 ± 0.37	65.53 ± 2.06	47.82 ± 0.48
N (%)	0.193 ± 0.005	ND	0.087 ± 0.005
Available P (μg g^{-1})	40.33 ± 0.39	1.72 ± 0.03	28.37 ± 0.90
Available K (μg g^{-1})	36.37 ± 0.37	16.31 ± 0.39	27.14 ± 0.38
Fe (μg g^{-1})	6353 ± 469	12407 ± 976	9333 ± 306
Al (μg g^{-1})	2900 ± 264	7366 ± 115	5533 ± 251
Cu (μg g^{-1})	27.3 ± 6.9	80.5 ± 6.1	54.9 ± 6.1
Mn (μg g^{-1})	85.33 ± 19.7	295.5 ± 12.8	200.2 ± 4.95
Cr (μg g^{-1})	11.7 ± 1.27	86.00 ± 1.74	56.9 ± 2.12

'ND' indicates no detection.

2.6. Translocation of heavy metals

The translocation of heavy metals from roots to above ground parts was determined by translocation factor which is defined as the ratio of metal concentration in shoots to that in roots [24].

2.7. Statistical analysis

The data was statistically analyzed using statistical software SAS (9.1.3, Cary, USA). Statistical difference in the concentration and translocation of Fe, Al, Cu, Mn, and Cr in different plant plants under different treatments were determined by one way ANOVA ($P \leq 0.05$) followed by Duncan's Multiple Range Test at $P \leq 0.05$ level. The same was also applied to see the statistical difference in terms of growth, biomass, photosynthetic pigments, protein and lipid peroxidation.

3. Results and discussion

3.1. Physico-chemical properties of substrate

The physico-chemical characteristics of FA, GS and FA–GS mixture are mentioned in Table 2. The analysis showed that the pH of FA is alkaline. The pH value of FA depends on the S content of parent coal. Coal with high S produces acidic ashes and with low S content produces alkaline ashes [25]. The EC estimates the amount of total dissolved salts in soil solution. EC of FA was generally higher than that of normal soil because of the presence of more soluble salts in FA [26].

The mixing of FA to the GS had increased the porosity and WHC and decreased the BD when compared with control. The BD determines the soil compaction, higher the value of BD more compact will be the soil and lesser will be the pore space and aeration in the soil [27]. The WHC depends on soil texture, finer the texture more is the WHC [27]. The WHC of 100% FA was highest, since FA possesses a finer texture with particle size ranging from 0.01 μm to 100 μm [28].

The major factors that limit the growth of plants on FA include: lack of essential nutrients usually N and P; toxicity caused by high pH and high concentration of heavy metals [5]. Since FA is deficient in essential plant macro nutrient N, P and K, as also evident from our analysis results (Table 2), mixing FA with fertile GS enhanced the nitrogen from 0% to 0.08%, phosphorus from 1.72 μg g^{-1} to 28.37 μg g^{-1} and potassium from 16.31 μg g^{-1} to 27.14 μg g^{-1}. The heavy metal analysis of the substrates showed that the concentration of Fe, Cu, Mn, Cr, and Al was greater in FA when compared with GS (Table 2).

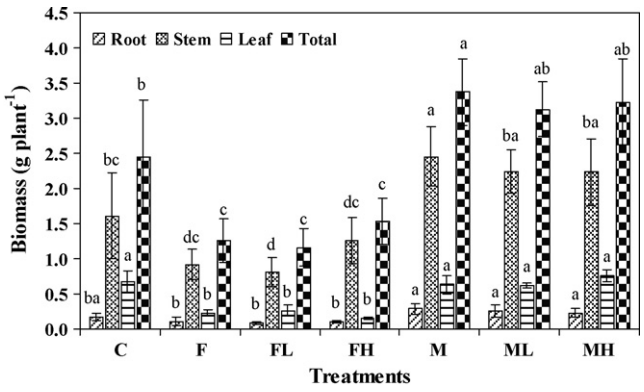

Fig. 1. Root, stem, leaf and Total biomass (g/plant) under different treatments.

3.2. Growth performance of J. curcas

Our study showed that although *J. curcas* survived in 100% FA but the biomass, height and photosynthetic area was much lower in 100% FA when compared with control (Fig. 1, data for height and leaf area not shown). The total biomass decreased by 48%, 52% and 37% under treatments F, FL and FH, respectively. However, there was no significant difference in biomass ($P \leq 0.05$) under these treatments, indicating that there is no clear effect of EDTA on plant growth. The growth enhanced significantly when FA was mixed with soil. The total biomass increased by 37%, 27% and 31% under treatment in M, ML and MH, respectively. The biomass under the treatment M (without EDTA dose) was slightly higher than the statistically similar ML and MH ($P \leq 0.05$).

The root biomass slightly decreased in 100% FA, whereas it had increased in FA amended with GS, despite high level of toxic metals in substrate, when compared with control. The increment in root biomass is in the order of 62%, 44% and 31% in M, ML and MH, respectively, indicating resistance of plants against high metal concentrations in rhizosphere. The root density and spatial distribution is an important factor in assessing phytoextraction efficiency of a plant. Plants with extensive root system are capable of trapping more heavy metals due to better exploration in soil [29]. The performance of plants improved in FA amended with GS, due to improved physicochemical properties of the substrate and increased availability of nutrients to the plants resulting in increased biomass.

3.3. Phytoextraction of heavy metals

Plants can absorb toxic ions along with the beneficial ones because of the chemical similarity between the two. Some plants have adopted exclusion mechanisms, where there is a reduced uptake by the roots or a restricted transport of the metals from root to shoot [30]. The EDTA solubilizes the elements and transport it through the xylem [13,31]. In our study the elevated concentration of all the elements had been observed when EDTA was applied. The accumulation of Fe, Al, Mn, Cr and Cu in root, stem and leaf in different treatments is shown in Fig. 2A–E. Different treatment showed significant difference ($P \leq 0.05$) in concentration of elements in different plant parts, indicating impact of EDTA doses and substrate composition on accumulation of heavy metals (Fig. 2A–E). The impact of EDTA on metal accumulation and transfer to different plant parts is clearly observed if we compare the plants where EDTA was applied with the ones to which no EDTA was applied:

3.3.1. Impact of EDTA on FA substrate

In case of FA the application of EDTA enhanced the uptake and accumulation of Fe, Al, Mn and Cr in roots. Cu was not retained but has been translocated to stem and leaves (Fig. 3A). This is clear from

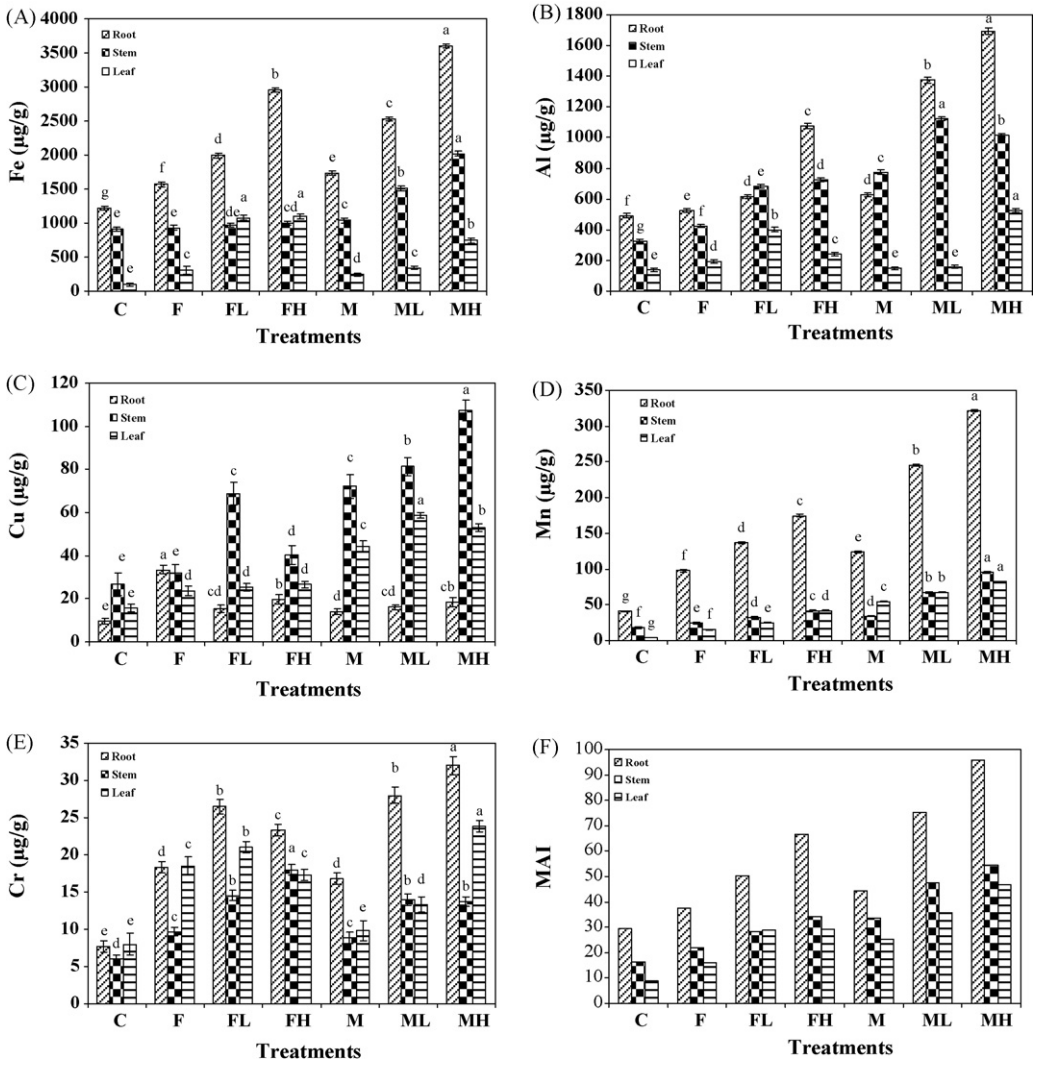

Fig. 2. Metal concentrations ($\mu g\,g^{-1}$ dry weight) in root, stem and leaves of *J. curcas*: (A) iron; (B) aluminum; (C) copper; (D) manganese; (E) chromium; and (F) metal accumulation index of root, stem and leaf.

the translocation factor value which is 1.66 for F (no EDTA), 6.23 for FL and 3.45 for FH (Table 3). Fe, Al, Mn and Cr accumulation in roots increased on addition of EDTA. The stems did not retain much Fe, it was only 4.27% (FL) and 7.06% (FH) higher than non EDTA treated plants. Al, Cu, Mn and Cr retention in stem enhanced in FL and FH, when compared to plants where no EDTA was added (Fig. 3B). EDTA enhanced the Fe accumulation in leaves by 244% and 250% in FL and FH, respectively. However, Fe in leaves is still less when compared with roots (Fig. 2A). EDTA had very little impact on accumulation of Cu and Cr in leaves. They are enhanced only by 8.62% and 12.88% in FL and by 13.51% and (−) 6.31% in FH, respectively (Fig. 3C).

3.3.2. Impact of EDTA on FA + GS substrate

The pattern of accumulation in root, stem and leaves of plant in this substrate on application of EDTA has been observed to be different from that of pure FA. This may have occurred because of dilution in concentration of heavy metals in the rhizosphere, due to addition of soil and enhanced biomass of root, stem and leaves, due to enrichment of nutrients in the rhizosphere (Table 2). In case of roots all elements showed a dose dependent accumulation with enhanced accumulation at higher EDTA dose (Fig. 3A). In stem Fe, Cu and Mn accumulation was more enhanced at higher EDTA dose. But Al and Cr accumulation was more at lower dose (ML), 45% and

Table 3
Translocation factor for different elements in different treatments.

Treatments	Fe	Cu	Mn	Al	Cr
C	0.83 ± 0.06^{b}	4.47 ± 0.09^{c}	0.53 ± 0.04^{b}	0.94 ± 0.01^{d}	1.83 ± 0.26^{a}
F	0.79 ± 0.04^{cb}	1.66 ± 0.05^{d}	0.40 ± 0.01^{d}	1.18 ± 0.06^{c}	1.54 ± 0.10^{b}
FL	1.03 ± 0.01^{a}	6.23 ± 1.02^{b}	0.42 ± 0.01^{d}	1.76 ± 0.03^{a}	1.34 ± 0.03^{cb}
FH	0.71 ± 0.02^{d}	3.45 ± 0.44^{c}	0.48 ± 0.01^{c}	0.90 ± 0.03^{d}	1.52 ± 0.04^{b}
M	0.74 ± 0.02^{cd}	8.41 ± 1.39^{a}	0.71 ± 0.01^{a}	1.48 ± 0.06^{b}	1.11 ± 0.08^{d}
ML	0.73 ± 0.03^{cd}	8.77 ± 0.53^{a}	0.55 ± 0.02^{b}	0.93 ± 0.02^{d}	0.98 ± 0.07^{d}
MH	0.77 ± 0.01^{cd}	8.81 ± 0.52^{a}	0.55 ± 0.01^{b}	0.91 ± 0.02^{d}	1.17 ± 0.04^{cd}

Data are average ± SD. Average with the same letter as superscript are statistically similar (within individual column) at $P \leq 0.05$ level according to Duncan's Multiple Range Test.

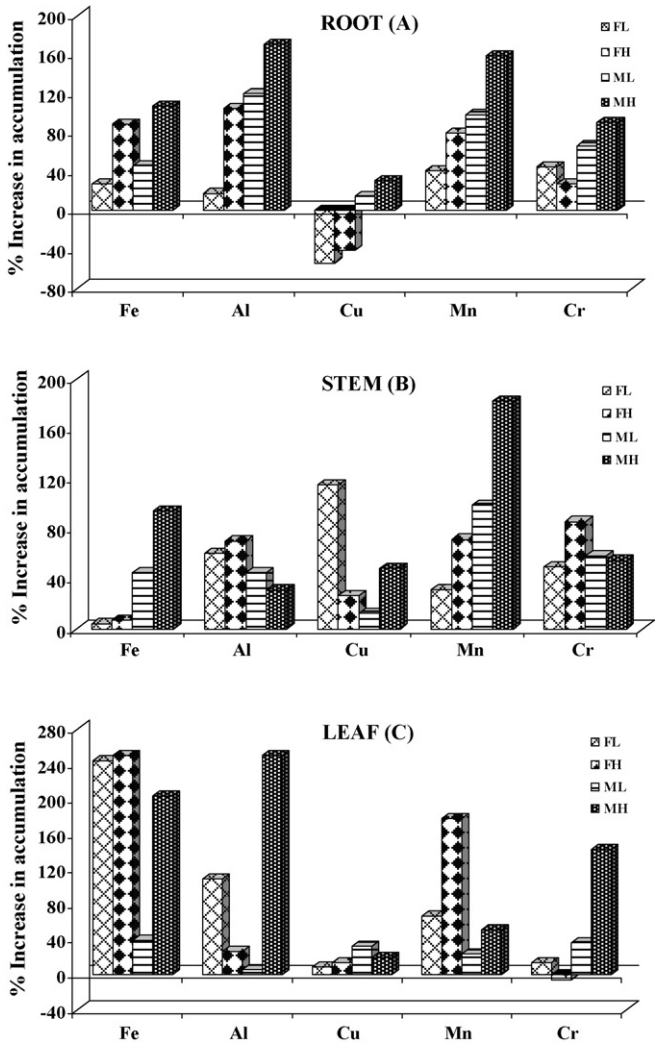

Fig. 3. Percent increase or decrease in accumulation of heavy metals in (A) root; (B) stem; and (C) leaves, on application of EDTA at 0.1 g kg^{-1} (FL and ML) and 0.3 g kg^{-1} (FH and MH).

59% against 31% and 55% at higher dose (MH), respectively (Fig. 3B). This is because higher dose transported Al and Cr more to the leaves. It is clear from their corresponding concentrations in leaves where Al and Cr showed an increase by 250% and 142%, respectively at higher dose in comparison to 6% and 36%, respectively, at lower dose (Fig. 3C). Fe, Cu and Mn showed a dose dependent increase in accumulation in stem and leaves.

Table 4
Heavy metal removal efficiency of *J. curcas* after 60 days of exposure (% of the total substrate metal).

Treatments	Roots	Stem	Leaves	Total
F	0.05	0.27	0.06	0.37
FL	0.04	0.42	0.08	0.55
FH	0.07	0.52	0.05	0.64
M	0.17	1.85	0.33	2.35
ML	0.26	2.18	0.43	2.87
MH	0.29	2.75	0.60	3.64

3.3.3. Heavy metal translocation, accumulation and removal efficiency

For phytoextraction it is essential, that the metals must be transported from root to shoot. Movement of metal-containing sap from root to shoot is termed translocation. Following translocation to leaves, metals can be reabsorbed from the sap into leaf cells [32]. The translocation factor (TF) value greater than 1, indicates the translocation of element from root to above ground part. In our study it was observed that Fe and Mn were retained more in root while Cu, Al and Cr were translocated more to the shoot, indicating phytoextraction of these three elements from substrate (Table 3).

The metal accumulation index showed that the accumulation of element was more in roots followed by stem and then in leaves, in each treatment (Fig. 2F). Maximum amount of elements accumulated in plants grown on MH substrate. The ANOVA revealed that, regardless of more accumulation of element in roots in this substrate, no damaging effect has been observed since the root biomass was not reduced. However, it was slightly higher when compared to control.

Analysis of substrate after 60 days of exposure showed that 0.37%, 0.55%, 0.64%, 2.35%, 2.87% and 3.64% heavy metals were removed from F, FL, FH, M, ML and MH substrate, respectively. The metal removal efficiency was found to be highest for MH substrate (Table 4), where EDTA was added at the rate of 0.3 g kg^{-1}. The percent removal in this treatment was found to be 26% higher when compared to its corresponding lower dose (EDTA 0.1 g kg^{-1}). The data indicates that heavy metal removal efficiency depends on the biomass produced and dose of EDTA applied. The M substrate produced higher biomass as compared to F substrate (Fig. 1). Although the biomass in ML and MH treatment was found to be statistically similar, but higher heavy metal removal efficiency at MH substrate indicates clear effect of higher EDTA dose. The heavy metal removal efficiency of different plant parts is in the order of stem < leaf < root and this is in accordance to their respective biomass being highest for stem and lowest for root. Our results indicates that on 60 days of exposure, *J. curcas* plants with a total biomass of 3.37 g can remove 2.35% of heavy metals from substrate and this efficiency can be enhanced to 3.64% in presence of EDTA.

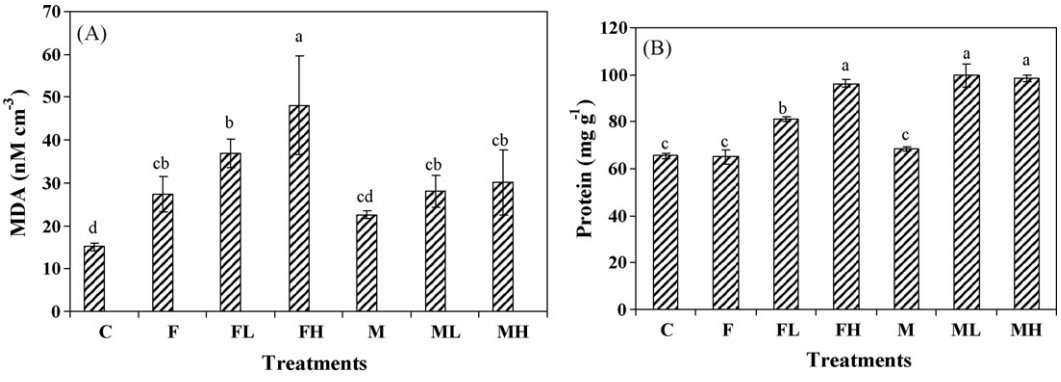

Fig. 4. (A) MDA (nM cm^{-3}) content and (B) protein content (mg g^{-1} fresh weight) in leaves of *J. curcas*.

Table 5
Chlorophyll a (mg g^{-1}), b (mg g^{-1}), a + b (mg g^{-1}), a/b ratio and carotenoids (mg g^{-1}) in leaves of *J. curcas* in different treatments.

Treatments	Chl. a	Chl. b	Chl. a + b	Chl. a/b	Carotenoids
C	2.03 ± 0.08^{a}	0.87 ± 0.05^{a}	2.89 ± 0.10^{a}	2.34 ± 0.16^{ab}	0.62 ± 0.10^{ba}
F	0.86 ± 0.20^{d}	0.36 ± 0.06^{d}	1.22 ± 0.16^{d}	2.45 ± 0.95^{ab}	0.40 ± 0.13^{b}
FL	1.31 ± 0.06^{cb}	0.53 ± 0.02^{dc}	1.84 ± 0.07^{cb}	2.45 ± 0.09^{ab}	0.63 ± 0.05^{ba}
FH	1.09 ± 0.20^{cd}	0.53 ± 0.10^{dc}	1.61 ± 0.30^{cd}	2.07 ± 0.03^{b}	0.58 ± 0.03^{ba}
M	1.40 ± 0.24^{cb}	0.49 ± 0.16^{dc}	1.88 ± 0.40^{cb}	2.88 ± 0.45^{a}	0.52 ± 0.25^{ba}
ML	1.61 ± 0.15^{b}	0.66 ± 0.09^{bc}	2.27 ± 0.23^{b}	2.46 ± 0.16^{ab}	0.65 ± 0.09^{a}
MH	1.49 ± 0.27^{b}	0.73 ± 0.16^{ba}	2.22 ± 0.23^{b}	2.04 ± 0.11^{b}	0.69 ± 0.05^{a}

Data are average ± SD. Average with the same letter as superscript are statistically similar (within individual column) at $P \leq 0.05$ level according to Duncan's Multiple Range Test.

3.4. Biochemical changes

3.4.1. Lipid peroxidation

In various higher plants lipid peroxidation has been reported to be induced due to heavy metal stress [33]. The heavy metal induces the production of free radicals mainly the reactive oxygen species that may distort the membrane architecture causing an oxidative damage [34]. Aldehydes such as thiobarbituric acid reacting substances (TBARS) have been widely accepted as a general marker of free radical production. The most commonly measured TBARS is malondialdehyde. In our study MDA content increased with the increase in concentration of heavy metals in both type of substrate (Fig. 4A). Maximum MDA production was recorded in case of pure FA treated with higher dose of EDTA (FH) indicating maximum toxicity and membrane damage in this treatment. However, if we see MAI, we find that maximum accumulation of metals occurred in treatment marked MH, but lower MDA production in this treatment indicates diluted toxicity of elements as a result of better plant growth and enhanced biomass.

3.4.2. Protein

In plants one of the common features of stress response is the induction (increased synthesis) of a group of proteins called heat shock proteins or referred to more generally as stress proteins [35]. These proteins are synthesized in order to confer resistance to further damage. The DNA of heavy metal stressed cells produces specific mRNA transcripts which regulates the synthesis of stress proteins [35]. Heavy metal induced synthesis of stress protein has been reported in several higher plants [33,36]. ANOVA shows that the protein content of plants growing on non EDTA treated substrate is statistically similar ($P \leq 0.05$) to that of control (Fig. 4B). However the protein content of EDTA treated plants is higher than that of control. This may be due to the production of stress protein, as a resistance against enhanced heavy metal accumulation in EDTA treated plants.

3.4.3. Chlorophyll and carotenoids

A reduction in chlorophyll pigment (a + b) had been observed in all the treatments when compared with control. However, Chl. (a + b) content had been found to be more in EDTA treated plant than in non EDTA treated plants (Table 5). This may be due to increased uptake of essential elements responsible for chlorophyll synthesis on addition of EDTA. The chlorophyll a/b ratio had increased in F, FL, M and ML treatment and decreased in FH and MH treatment. An increase in chlorophyll a/b ratio indicates reduction of Chl. b to Chl. a in order to maintain Chl. a pool [37]. As metal accumulation in leaf cells progress, e.g., in case of FH and MH the damage created by the metals may have exceeded the ability of the leaves to maintain chlorophyll levels resulting in a decrease in Chl. a/b ratio [37].

The carotenoid content of the treatment FL, FH and M was statistically similar to control ($P \leq 0.05$). Whereas, the carotenoid content in treatment ML and MH showed an increase and it dropped in the treatment marked F (100% FA). Increase in the carotenoid content is considered as a plant's defense mechanism towards metal stress, as happened in case of ML and MH (Table 5). They act as a non enzymetic antioxidants and play an important role in protection of chlorophyll under stress condition [38].

4. Conclusions

Our study shows that *J. curcas* is capable of extracting heavy metals from FA and the extraction is enhanced many folds in presence of chemical chelants like EDTA. In 100% FA the growth is greatly stunted due to lack of essential nutrients in FA. *J. curcas* can be established on FA when provided with essential plant nutrients such as N, P and K. The nutrients enhanced the growth many folds and hence the biomass, which is essential for effective phytoextraction to occur. Biochemical analysis directly indicates resistance offered by *J. curcas* plants against metal stress. *J. curcas* is a high biomass producing crop with ability to accumulate heavy metals as evident from our study and hence may be recommended for plantation on barren FA landfills, when provided with essential plant nutrients. Since EDTA is a synthetic chelating agent with low biodegradability, further experiments are under progress to assess the affect of biodegradable organic acids on metal phytoextraction from fly ash.

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