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Effect of biosludge and biofertilizer amendment on growth of *Jatropha curcas* in heavy metal contaminated soils

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Abstract The pot experiments were conducted to evaluate the effect of different concentrations of arsenic, chromium and zinc contaminated soils, amended with biosludge and biofertilizer on the growth of *Jatropha curcas* which is a biodiesel crop. The results further showed that biosludge alone and in combination with biofertilizer significantly improved the survival rates and enhanced the growth of the plant. With the amendments, the plant was able to grow and survive upto 500, 250 and 4,000mg kg^{-1} of As, Cr and Zn contaminated soils, respectively. The results also showed that zinc enhanced the growth of *J. curcas* more as compared to other metals contaminated soils. The heavy metal accumulation in plant increased with increasing concentrations of heavy metals in soil, where as a significant reduction in the metal uptake in plant was observed, when amended with biosludge and biofertilizer and biosludge alone. It seems that the organic matter present in the biosludge acted as metal chelator thereby reducing the toxicity of metals to the plant. Findings suggest that plantation of *J. curcas* may be promoted in metal contaminated soils, degraded soils or wasteland suitably after amending with organic waste.

Keywords Biofertilizer · Biosludge · Growth performance · Heavy metals · *Jatropha curcas*

Introduction

Heavy metal contamination has affected the biosphere drastically at many places worldwide (Cunningham et al. 1997; Meagher 2000; Raskin and Ensley 2000). Industrialized countries have regulated the emission of toxic substances in the environment. However, many sites around the world are still contaminated, posing a risk to humans and other organisms. In Indian soils, metal concentrations range from less than 1mg kg^{-1} to as high as 100,000mg kg^{-1}, due to the geological origin of the soil or as a result of human activity (NGRI 2004). Excess concentrations of some heavy metals in soils, such as As, Cr and Zn, have caused the disruption of natural terrestrial ecosystems (Meagher 2000; Gardea-Torresdey et al. 1996; Nanda et al. 1995). Currently, clean-up processes of heavy metal pollution are very expensive, cumbersome and environmentally destructive (Nanda et al. 1995; Moffat 1995). Recently, NEERI, India has started to develop cost-effective and eco-friendly technologies that use microorganisms and industrial wastes for cultivation of petro-crops in degraded lands due to metals (Juwarkar et al. 2006).

Among the various renewable energy choices, seed oil crops have a potential for meeting the increasing requirements of petroleum and its products (Banerjee

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et al. 1983). *Jatropha curcas,* commonly known as physic nut/Chandrajyoti/Chandraprabha/Nepalm, is today recognized as a petro-substitute. Its cultivation can also help in the reclamation of wastelands/degraded lands/mine contaminated lands. To meet the increasing demand of oil seed crops, cultivation of oil crops on large scale has to be initiated without scarifying the land under agriculture. Therefore other types of land, which we consider as wastelands, can be brought under cultivation following a suitable scientific approach.

The oil crises of 1970 and 1990 due to the rise in oil prices and the Gulf War, respectively, are instrumental to pave off way for exploitation of renewable sources of energy (Bhasbutra and Sutiponpeibun 1982). The planning commission of India (2003) has recommended launching of a national mission on bio-diesel with special focus on plantation of Jatropha on 3 Million hectare of wasteland, to meet the country's energy demand. At present, the demand for fossil fuels is increasing very rapidly and it is estimated that remaining reserves of fossil fuel will be exhausted by 2020 (Kumar and Kumar 1984). Among the various renewable energy choices, seed oil crops have a potential for meeting the increasing requirements of petroleum and its products (Banerjee et al. 1983). Industrial wastes play an important role in improving plant growth and amelioration of microclimatic conditions under stress conditions (Juwarkar et al. 1992). The interactions between microbes, plant root and amendments may have a great influence on both the increase of nutrients uptake and mitigation of the metal toxicity (Smith 1994).

In view of the above, the pot experiments were conducted in the present study to improve the growth of biodiesel species, *J. curcas* on different concentrations of heavy metal contaminated soils, such as As, Cr and Zn by using biosludge as an amendments and microbial cultures as biofertilizer. The study has high importance in the present context of Jatropha cultivation on wastelands/degraded lands/mined land to meet the raw material requirement for biodiesel.

Materials and methods

Collection of soil, biosludge and biofertilizer

The soil used for the pot experiments was black cotton (calcareous) soil, which was collected from the premises of National Environmental Engineering Research Institute (NEERI), Nagpur, India at a depth of 0–20cm. Biosludge was collected from Gujarat Refinery, Vadodara, India. The biosludge was fully matured (adequate decomposition) sludge, which is generated from the biologically treated effluent plant (ETP) of oil refinery. *Azotobacter chroococcum* was used as biofertilizer, which was isolated at NEERI from heavy metal contaminated soil.

Preparation of contaminated soil

Heavy metal contaminated soil was prepared with increasing concentrations of metals ranging from 0; 25; 50; 100; 250 and 500mg kg^{-1} for arsenic and chromium and 0; 100; 1,000; 2,000; 3,000; and 4,000mg kg^{-1} for zinc by using metal salts like $Na_2HAsO_4.7H_2O$, $K_2Cr_2O_7$ and $ZnSO_4.7H_2O$ respectively. The metal salts were dissolved in distilled water, sprayed on the soil sample and mixed thoroughly. Then, heavy metal treated soil samples were incubated in a pot and kept for 60days. Before the plantation, the actual metal concentrations in the incubated soils were determined using ICP-OES.

Pot experiment

Pot experiments were conducted with the incubated soil samples to evaluate metal tolerance and effect of different amendments on growth of *J. curcas.* The treatment details are given in Table 1. Each pot was filled with 14kg of soil thoroughly mixed with biosludge at the rate of 50tons/ha. For each treatment, four replicates were kept. Control pots were kept for all the four treatments without heavy metals. Fifteen days old healthy seedlings of *J. curcas* were transplanted to the experimental pots. Broth culture of *Azotobacter chroococcum* having titre value of 16 × 10^{8}CFU ml^{-1}

Table 1 Experimental design for pot experiments with As, Cr and Zn contaminated soils

Treatment details	
T1	Experimental soil + Heavy metal
T2	Experimental soil + Heavy metal + Biosludge[a]
T3	Experimental soil + Heavy metal + Biofertilizer
T4	Experimental soil + Heavy metal + Biosludge[a] + Biofertilizer

[a] at 50 t ha^{-1}, Replication=4, Controls were kept without metal

was used as biofertilizer for inoculation of seedlings at the time of plantation. Soil moisture was maintained at 40–50% of the maximum field water holding capacity by adding distilled water during the experimental period. Plants were grown under the net house with natural environmental conditions. The growth rate of plants was regularly recorded in each pot.

Sampling and analysis

Representative samples of initial soil and biosludge were collected, air dried and ground to 1mm before chemical analysis. The pH (solid: deionised water = 1:2w/v) and Electrical conductivity (EC; solid: deionised water = 1:2w/v) of the samples were measured using a glass electrode pH meter and EC meter, respectively. Percent organic carbon, total N, total P and total K were analyzed as per methods described in standard methods (Lindsay and Norvel 1978; Black et al. 1965). The control soil, contaminated soil and biosludge were analyzed for heavy metals concentration by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 4100 DV, Perkin Elmer, USA). The digestion of samples was accomplished in a microwave oven by using 6ml of HNO_3 following the USEPA 3051A method (1998).

Plant tissue analysis for metals

The plants were harvested after 180days for plant growth, biomass and heavy metal analysis. The stem and root lengths were recorded and stems were cut at the soil surface and all parts of plant were washed very carefully with deionised water four times and blotted dried. Plant tissues were oven dried at 70°C, and constant dry weights were recorded. The dried plant materials were ground to less than 1mm with a stainless steel mill for heavy metal analysis. Approximately 0.5g milled plant material was placed into a 100ml digesting tube, and then digested following the procedures described earlier and heavy metal concentrations in plants tissues were determined by ICP-OES.

Statistical analysis

The data of growth and heavy metal concentration of plants under different treatments were analyzed by using SPSS statistical package and MS excel. The tolerant index of the plants to As, Cr and Zn were calculated by the following equation (Wilkins 1978).

$$\text{Tolerant index} = \frac{\text{Mean height of the plant growing on metal contaminated soil X 100}}{\text{Mean height of the plant growing on garden soil alone (without metal, control)}}$$

Results and discussion

Physico-chemical properties of experimental soil and biosludge

Physico-chemical parameters of the experimental soil and biosludge are listed in Table 2. Many factors, such as poor physical texture, low water and nutrient holding capacity, deficiency of major nutrients (N, P, K), acidity and alkalinity, water supply, toxic materials, salinity, stability and surface temperature are known to affect plant establishment on heavy metal affected soils (Bradshaw 1987). The biosludge contained, high levels of total nitrogen (1,680 ± 122mg kg^{-1}), phosphorus (552 ± 96mg kg^{-1}), potassium (270 ± 22mg kg^{-1}), and organic carbon (17.2 ± 4), which were much higher than the experimental soil. This indicated that biosludge could be used as a nutrient source for the plant cultivation and also for the stabilization of the structural properties of the degraded soil.

Tolerant index and growth performance of *J. curcas* in As, Cr and Zn contaminated soils amended with biosludge and biofertilizer

Pot culture experiments were conducted over a short period of time and the tolerant index and growth performance of the *J. curcas* were observed in heavy metal contaminated soils. The tolerant index of *J. curcas* in different amendments subjected to various concentrations of As, Cr and Zn is listed in Table 3.

Table 2 Physico-chemical properties of experimental soil and biosludge (mean±sd, *n*=4)

Parameters	Experimental soil	Biosludge
Physical		
Bulk density (mg m^{-3})	1.05±0.05	3.045±0.62
Maximum water holding capacity (%)	59.45±2.8	67.86±3.2
Porosity (%)	74.28±5	99±6.8
Chemical		
pH	8.5±0.1	6.38±0.5
Electrical conductivity (dS m^{-1})	0.47±0.05	6.46±1.2
Total nitrogen (mg kg^{-1})	179±25	1,680±122
Available nitrogen (mg kg^{-1})	25±4	70±11.8
Total phosphorous (mg kg^{-1})	188±14	552±96
Available phosphorous (mg kg^{-1})	4.6±0.3	45.5±6.5
Total potassium (mg kg^{-1})	186±29	270±22
Available potassium (mg kg^{-1})	18±3.4	30±6.2
Organic carbon (%)	0.48±0.05	17.2±4
Trace metals (mg kg^{-1})		
Fe	3,512±722	1,668±471
Cu	93.6±5.9	372±15
Zn	30.6±3.2	34.6±2
Cr	1.2±0.02	ND
As	ND	ND
Cd	ND	ND
Pb	6.2±0.08	17±2.1

ND not detectable

The tolerant index was higher in T2 and T4 treatments than those of control plants (T1) in all the metal concentrations tested. The results are also in line with the view that the degree of tolerance is mainly governed by the specific metal concentration in contaminated areas (Bradshaw 1987). Results presented in Table 3, indicated that the application of biosludge combined with biofertilizer (T4) raised plant survival from 250 to 500mg kg^{-1} of As, 100 to 250mg kg^{-1} of Cr concentrations.

The growth performances and biomass yields of *J. curcas* in As, Cr and Zn contaminated soils are presented in Figs. 1, 2 and 3 respectively. Results depicted that soil concentrations of As and Cr above 250 and 100mg kg^{-1}, respectively, totally inhibited the growth of the plant in unamended soil. In case of Zn contaminated soil, very contrasting results was found, where Zn initially enhanced the growth of plants upto the concentration of 1,000mg kg^{-1} in soil, however, at higher concentrations, the growth of the plants was reduced. Heavy metal polluted soils display a large heterogeneity of metal distribution among soil constituents. Therefore, the toxic levels of heavy metals, deficiency of nutrients (N, P, K) and low organic matter in the heavy metal contaminated or degraded lands might be the major constraints for plant establishment (Balabane et al. 1999). Previous studies also demonstrated that phytotoxicity of heavy metals and extreme infertility of the contaminated soils were the major limiting factors for the plant growth (Lan et al. 1997; Shu et al. 1997). On the other hand, addition of amendments i.e. biosludge and biofertilizer increased the metal tolerance of the *J. curcas* at very high concentration of As, Cr and Zn, respectively (Table 3). It was also noticed that at higher concentrations of metal contamination, the combined application of biosludge and biofertilizer favored plant growth as reflected in plant height and biomass yields (Figs. 1, 2 and 3). A number of studies showed that organic amendment resulted in successful revegetation of mine spoils (Ye et al. 1999, 2000). Besides having high level of N, P and K, biosludge also improves the poor physical properties and microbial activities of the heavy metal contaminated soils. Finally, the results show that there was marked

Table 3 Tolerant index (%) of *Jatropha curcas* growing in different treatments of arsenic, chromium and zinc contaminated soil with and without amendments

Metal concentration (mg kg^{-1})	Treatments			
	T1	T2	T3	T4
Arsenic				
25	80	128	82	148
50	66	104	68	130
100	64	92	64	114
250	56	78	58	84
500	00	00	00	48
Chromium				
25	88	116	96	142
50	80	112	90	134
100	76	100	82	104
250	00	62	00	70
Zinc				
100	118	162	130	182
1,000	164	208	172	226
2,000	140	170	148	212
3,000	102	148	116	164
4,000	00	95	00	144

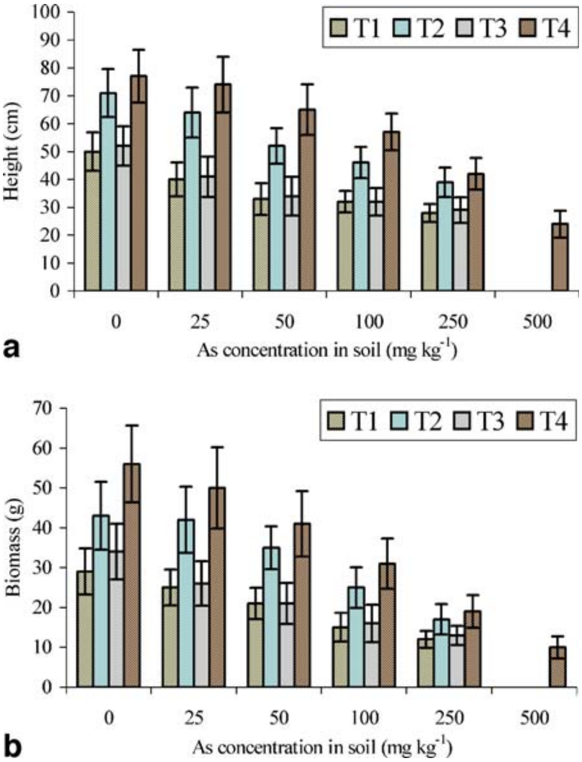

Fig. 1 **a** Height and **b** Biomass of *Jatropha curcas* in arsenic contaminated soils with and without amendments (Refer to Table 1 for the explanation of treatments and Error bars represent ± sd)

interactive effect of biosludge on biomass yield of *J. curcas*. The greatest yields occurred when biosludge was combined with biofertilizer application (T4), followed by intermediate yield with biosludge alone (T2) and the lowest yield was recorded when biofertilizer alone (T3) was applied. The results suggested that biosludge application was the primary factor influencing biomass yields, and when biosludge and biofertilizer were applied together, the growth was stimulated more than that of biosludge and biofertilizer supplied singly.

Metals uptake by plant

The concentrations of heavy metals in the root and stem of *J. curcas* are depicted in the Tables 4, 5 and 6. The results show that the treatments with low concentration of As and Cr (25mg kg^{-1}), did not show respective metal concentrations into the plant tissues, whereas with the increased metal concentrations in soils, there was a significant increase in the metal concentration in the plant in control treatments (T1). Results further indicated that the concentrations of As, Cr and Zn in both the root and stem tissues of *J. curcas* in metal contaminated soil amended with biosludge and biofertilizer (T4) were significantly decreased as compared to contaminated soil without amendments (T1). The treatment above 250mg kg^{-1}, resulted in high metal concentration (19.4 ± 2.3mg kg^{-1}) of As in control plants as compared to the soil amended with biosludge and biofertilizer (7.5 ± 1.4mg kg^{-1}). Similar, results were found in the case of Cr and Zn contaminated soils. In general, metal uptake by *J. curcas* was significantly affected by the concentration of metal in soil as well as biosludge and biofertilizer application. The higher metal concentration in soil significantly increased the metal uptake by the plant. The biofertilizer alone (T3) shows the highest metal uptake, compared to other amendments. The biosludge combined with biofertilizer (T4) and biosludge alone (T2) showed the most pronounced

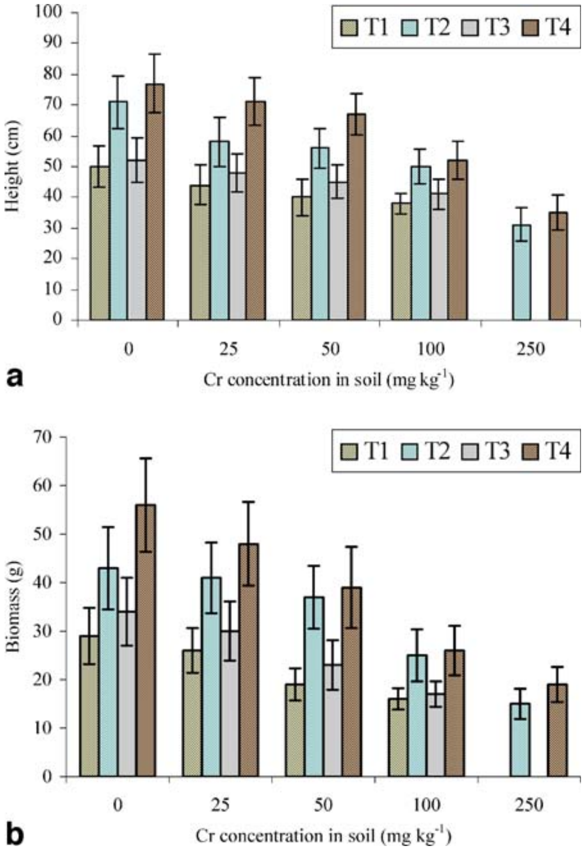

Fig. 2 **a** Height and **b** Biomass of *Jatropha curcas* in chromium contaminated soils with and without amendments (Refer to Table 1 for the explanation of treatments and Error bars represent ± sd)

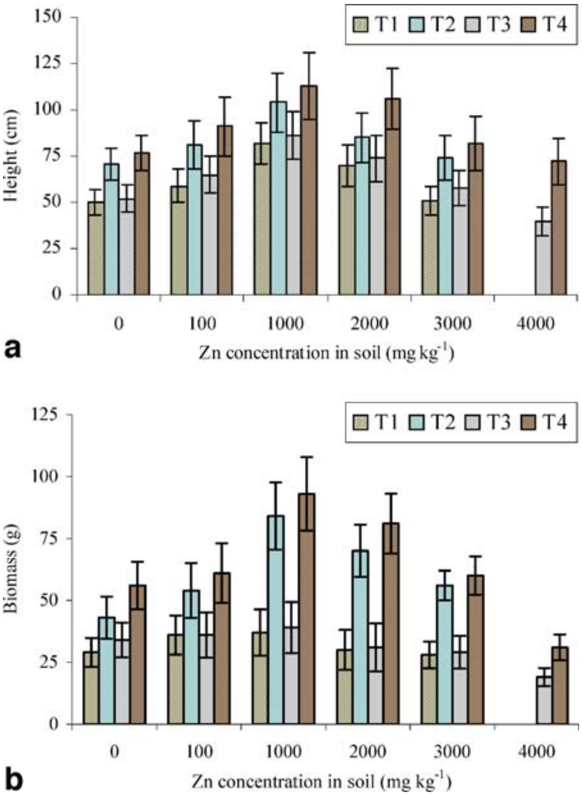

Fig. 3 **a** Height and **b** Biomass of *Jatropha curcas* in zinc contaminated soils with and without amendments (Refer to Table 1 for the explanation of treatments and Error bars represent ± sd)

effect on metal uptake. In these treatments, a significant decrease in metal uptake and high biomass yields were observed compared to all the other treatments tested. The application of biosludge in contaminated land not only provided nutrients for plant growth, but also stabilized the heavy metals in the soil and reduced metal toxicity to the plant. Hence, the high organic carbon content of biosludge led to the low uptake of metal by the plant. Organic amendments of industrial wastes have been reported to reduce the heavy metal toxicity to plants by complexing metals (Wong and Lau 1985). Role of organic amendments, such as fermented compost, which contains a high proportion of humid organic matter, has been reported for decreasing the bioavailability of heavy metals in soil, thus permitting the reestablishment of vegetation on contaminated sites (Tordoff et al. 2000; Walker et al. 2004). Accumulation and distribution of heavy metals in plant tissues are important aspects to evaluate the role of plant in remediation of metalliferous soils (Friedland 1989). In terms of stabilizing metal contaminated sites, a lower metal concentration in stem is preferred in order to prevent metal from entering the ecosystem (Taylor and Percival 2001). It is also preferred to grow non-food crops on metal contaminated soil and *J. curcas* being non-food crop is an important candidate to grown on such soils. Based on the above observations, it is inferred that the treatment of biosludge along with biofertilizer (T4) improved the sustainability of the *J. curcas* in As, Cr and Zn contaminated soils, as reflected by increased growth performance and decreased metal accumulation. Thus, the *J. curcas* can be successfully cultivated on metal contaminated soils using the biosludge application combined with the suitable biofertilizer.

Table 4 Arsenic concentration (mean±sd, *n*=4) in soil and plant tissues of *Jatropha curcas* grown in different treatments of arsenic contaminated soil with and without amendments after 180 days

Arsenic concentration (mg kg^{-1})	Treatment			
	T1	T2	T3	T4
Soil				
0	0.0±0	0.0±0	0.0±0	0.0±0
25	21.8±4.1	20.2±5.1	24.5±3.4	21.5±5.3
50	42.9±8.3	42.5±8.2	45.6±6.8	43.5±9.2
100	97.4±15.6	89.6±20.3	99.1±18.6	92.8±23.5
250	251.5±31.5	255.5±36.4	240.4±30.6	243.2±44.8
500	473.5±56.1	486.4±60.5	480.5±72.4	491.3±68.2
Stem				
0	ND	ND	ND	ND
25	ND	ND	ND	ND
50	ND	ND	ND	ND
100	1.3±0.6	0.06±0.01	1.1±0.6	0.06±0.01
250	3.12±0.8	1.47±0.3	3.05±0.8	1.25±0.4
500	NS	NS	NS	2.3±0.6
Root				
0	ND	ND	ND	ND
25	ND	ND	ND	ND
50	2.33±1.5	ND	2.06±0.8	ND
100	8.85±1.5	3.75±1.4	8.53±1.5	2.46±0.4
250	35.68±11.6	14.73±4.2	30.76±10.3	13.88±4.2
500	NS	NS	NS	26.55±8.8

ND not detectable, *NS* not survived

Table 5 Chromium concentration (mean±sd, n=4) in soil and plant tissues of *Jatropha curcas* grown in different treatments of chromium contaminated soil with and without amendments after 180 days

Chromium concentration (mg kg^{-1})	Treatment			
	T1	T2	T3	T4
Soil				
0	0.5±0.09	0.4±0.08	0.9±0.2	0.4±0.08
25	24.4±4.3	21.2±5.8	22.6±4.3	26.5±5.2
50	51.4±9.5	42.5±10.5	38.9±12.7	43.6±10.5
100	102.6±26.8	89.4±29.4	97.8±31.4	90.4±35.4
250	239.6±45.6	231.8±50.3	240.2±48.7	230.2±48.3
Stem				
0	ND	ND	ND	ND
25	ND	ND	ND	ND
50	1.26±2.5	ND	1.25±2.5	ND
100	15.55±4.7	3.11±1.6	14.94±5.5	1.75±0.4
250	NS	11.3±2.2	NS	9.64±2.1
Root				
0	ND	ND	ND	ND
25	ND	ND	ND	ND
50	23.14±2.3	8.26±2.3	20.45±3.5	8.12±1.5
100	134.55±12.5	20.54±5.6	133.45±26.5	15.30±4.3
250	NS	56.23±10.3	NS	36.31±5.8

ND not detectable, *NS* not survived

Conclusions

Pot culture experiments demonstrated that biosludge alone and in combination of biofertilizer applied to the heavy metal contaminated soils reduced the metal uptake in the plant tissues and enhanced the growth in terms of height and biomass of the *J. curcas* by reducing the phytotoxicity. The uptake of metals in plants was under acceptable limit i.e. upto 50mg kg^{-1} of As and Cr whereas for Zn, it was 2,000mg kg^{-1} of

Table 6 Zinc concentration (mean±sd, n=4) in soil and plant tissues of *Jatropha curcas* grown in different treatments of zinc contaminated soil with and without amendments after 180 days

Zinc concentration (mg kg^{-1})	Treatment			
	T1	T2	T3	T4
Soil				
0	26.2±14	27.4±15	26.4±10	21.5±16
1,000	1,130±246	1,024±213	1,065±249	1,102±186
2,000	2,036±356	2,107±375	2,069±300	2,160±345
3,000	2,645±517	2,596±436	2,638±348	2,703±312
4,000	3,521±645	3,746±611	3,566±625	3,711±510
Stem				
0	0.86±0.08	0.45±0.05	0.76±0.07	0.36±0.05
100	1.6±0.6	0.5±0.06	1.3±0.5	0.4±0.06
1,000	6.5±2.4	2.7±1.2	6.3±2.2	1.1±0.6
2,000	59.8±20.5	30.2±13.4	55.8±18.7	21.2±12.5
3,000	81.5±35.6	46.5±26.1	80.6±30.2	30.5±20.4
4,000	NS	51.6±27.8	NS	54.4±30.5
Root				
0	8.1±2.5	1.2±0.5	7.8±0.5	1.1±0.6
100	40.7±6.5	8.4±1.4	40.5±6.4	5.4±1.1
1,000	8.0±20.5	47.5±11.6	75.2±18.4	35.3±7.5
2,000	478.5±88.7	230.7±32.4	450.6±68.3	205.7±29.6
3,000	580.5±125.6	300.8±54.2	570.6±89.5	278.6±52.6
4,000	NS	311.2±58.4	NS	312.5±70.6

ND not detectable, *NS* not survived

metal contaminated soils. On the contrary, using biofertilizer alone could not effectively improve plant growth in metal contaminated soils. It was also evident from the results that plant grown in zinc contaminated soils amended with biosludge along with biofertilizer could survive at very high concentration as compared to control. The results are very encouraging for use of wasteland and heavy metal contaminated soils or degraded soils for cultivation of *J. curcas* after suitable amendment with organic waste when the constraints in the degraded soils or wastelands are identified.

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