

Growth, biomass production and remediation of copper contamination by *Jatropha curcas* plant in industrial wasteland soil

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Abstract

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The survival, biomass production and copper (Cu) remediation efficiency of *Jatropha curcas* L. was evaluated in Cu rich industrial wasteland soil (IWLS), collected from a local town, Sandila (Hardoi), Uttar Pradesh, India. The IWLS had high bulk density, water holding capacity (WHC), pH, electrical conductivity (EC), organic carbon and NPK. The Cu and Mn contents in IWLS were about 3 and 2 fold higher than that in the normal field soil (control). Stem cuttings of the *J. curcas* clones (BTP-A, BTP-N and BTP-K) were planted in IWLS as well as the same amended with cowdung or sand. The percent survival, net elongations and biomass accumulation of *J. curcas* were decreased slightly in IWLS, as compared to the control soil. The translocation of Cu from soil to the plants was higher in IWLS grown plants, which was more pronounced in IWLS amended with cowdung. *J. curcas* clones BTP-N, showed better survival and Cu removal efficiency from IWLS.

Key words

Bioaccumulation, Copper toxicity, Industrial wasteland, *Jatropha curcas*

Introduction

Sandila town in district Hardoi (Uttar Pradesh), India, represents the ill planned proliferation of small industries resulting in the formation of wasteland in their surroundings due to ad hoc disposal of the effluents. 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 (Singh *et al.*, 2003, 2007; Leon *et al.*, 2006; Kumar *et al.*, 2009; Ghavri *et al.*, 2010; Sharma *et al.*, 2010), animals (Rainbow, 2007) and human being (Butkus and Baltreinaite, 2007; Eren 2008; Lim and Schoenung, 2010).

Though a limit of 2 ml l⁻¹ of Cu in drinking water has been proposed the World Health Organization (WHO) as the provisional guideline value, entry of large doses of Cu in ecosystem can be toxic to microbes, plants and human being (Eren, 2008). Intake of excessive Cu in human being leads to severe mucosal irritation and corrosion, widespread capillary

damage, hepatic and renal damage and central nervous system irritation followed by depression. Severe gastrointestinal irritation and possible necrotic changes in liver and kidney could occur (Nogue *et al.*, 2000; Eren, 2008). Copper is needed by plants in trace amount but their availability in the excess may cause plant toxicity (Ghasemi *et al.*, 2005; Sharma and Mukhopadhyay, 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 apparently vulnerable. 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 Cu accumulations have been reported in *Betula pendula* roots (Maurice and Layerkvist, 2000), black alder (*Alnus glutinosa* L.) and pine (*Pinus sylvestris* L.) grown on sewage sludge (Butkus and Baltreinaite, 2007), however, these plants may not survive in semi tropical conditions of the Gangetic plains of Uttar Pradesh.

Jatropha curcas L. (Family: Euphorbiaceae) is a potential biodiesel plant, which can survive harsh environments of semi-arid agro-climatic conditions, wastelands (Foidl *et al.*, 1996; Gubitz *et al.*, 1999; Juwarkar, 2008; Mangkoedihardjo and Sunahmadia, 2008) and grows fast with little maintenance. It can reach a height of 3-8 m (Gunaseelan, 2009). Genus *Jatropha* with 172 species having significant economic importance is native to Central America and distributed in Africa and Asia (Cano-Asseleh, 1989; Fairless, 2007). Among the various *Jatropha* species, *J. curcas*, *J. glandulifera*, *J. gossypifolia*, *J. integerrima*, *J. multifida*, *J. nana*, *J. podagrica* and *J. tanjorensis* are widely distributed in India (Achten, 2008), identified as the most suitable oil bearing plant, and has been recommended for plantation on wasteland. In this study, three clones of *J. curcas* L. have been cultivated in IWLS, with and without amendment of sand or cowdung and partitioning of the metal in aerial part of the *J. curcas* L. has been investigated in varied soil conditions. The removal and loss of Cu from the wasteland soil has also been demonstrated.

Materials and Methods

Sampling, preparation and analysis of the soil: Sandila town cover 500 ha of the contaminated wasteland, due to improper industrial practices which has damaged the soil and ecosystem of the area. It is located between 26° 53' and 27° 46' N latitudes and between 79° 41' and 80° 46' E longitudes. Certain industries *i.e.* cotton mill, vegetable oil mill, steel industry (all closed now) have discharged and safe yeast industry, milk powder factory, chemicals factory *etc.* (all operating), discharging their effluents in the water and soil of the area. The soil was completely barren at the time when sampling done during January, May and September, 2007 and 2008. The selected area was uniform in topography and was submerged with rainwater during September – November, in these years.

The soil samples randomly collected from 0-15 cm depth from 3 to 4 locations of the industrial area were mixed. A representative sample of IWLS was prepared and subjected to analysis for physico-chemical parameters. All the samples were air dried for 48 hr in hot air oven, grounded in ball mixer and sieved through 1 mm mesh, analysis of other parameters. Finally, the samples were kept in labeled polythene bags at ambient temperature before analysis. The pH and electrical conductivity (EC) 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 procedure. For the metal analysis, soil samples were dried in oven to constant weight and digested in concentrated HNO₃ and HClO₄ in 5:1 (v/v) ratio at low temperature till a white residue was obtained. Double distilled water was used to maintain final known volume (Fritioff and Greger, 2007). The samples were analyzed using VARIAN AA240FS make fast sequential atomic absorption spectrophotometer with flame (FAAS).

Cultivation, determination of root and shoot length, biomass accumulation and estimation of Cu in plants: Three clones *i.e.* BTP-A, BTP-N and BTP-K of *J. curcas* L. were obtained from Biotech Park, Lucknow. About 30 cm long stem cutting, prepared from one year old plants of each clone was planted in IWLS, (T₁) IWLS amended with 40% sand (T₂) or 40% cowdung (T₃) and normal field soil (T₀ control). The root and shoot length were recorded after 100 d of cultivation using meter scale. The plant parts were removed carefully, washed with deionized water and blotted on filter paper for determination of fresh weight of roots and shoot using single pan electrical balance. The tissues were oven dried at 70°C, till constant dry weights were recorded. The dried plant materials were ground to less than 1 mm with a stainless steel mill, powdered and digested in microwave oven (Gunaseelan, 2009). The samples were analyzed for detection of Cu using a VARIAN AA240FS make Fast Sequential Atomic Absorption Spectrophotometer with flame (FAAS).

The bioconcentration factor (BCF) is the metal uptake capacity from soil to plant tissue, which was calculated by dividing metal concentration in plant by metal concentration in soil.

The translocation factor (TF) was calculated from the data on concentration of Cu in root and aerial part (stem and leaf) divided by concentration of Cu in root and from the contents multiplied by 100. The tolerance index (TI) was the mean height of the plant growing on wasteland soil multiplied by 100 and divided by mean height of the plant growing on control soil. The concentration index (CI) was the Cu concentration of metal in treatment plant divided by Cu concentration of metal in normal plant.

Per day accumulation of metal ($\mu\text{g g}^{-1}$) from soil was calculated by multiplying total dry biomass of plant in 100 d by metal accumulated in 1 g of dry plant biomass and dividing them by total number of days (100). The total metal removed from soil ($\mu\text{g g}^{-1}$) was calculated by subtracting the amount of metal concentration in soil after planting from the amount of metal concentration in soil before planting. The losses of metal calculated by subtracting the amount of metal accumulated by plants from amount of metal removed from soil.

Statistical analysis: All treatments were replicated for six times (n=6). Results were analyzed using one-way ANOVA (SPSS statistical package and MS excel). The difference between treatments were considered significant at $p < 0.05$.

Results and Discussion

Characterization of soil samples: The four types of soil samples *i.e.* garden soil from the university campus (control T₀) wasteland soil (IWLS) collected from wasteland affected with industrial waste (T₁), the IWLS amended with 40% sand (T₂) and IWLS amended with 40% cowdung (T₃) were analyzed for various physico-chemical properties before the plantation of *J. curcas* L. stem cutting. The bulk density of T₁ was about two fold higher than that of the T₀ which increased further when IWLS was amended with 40% sand or cowdung (Table 1).

Table - 1: Characterization of various kinds of used soil for cultivation of *J. curcas* L. clones

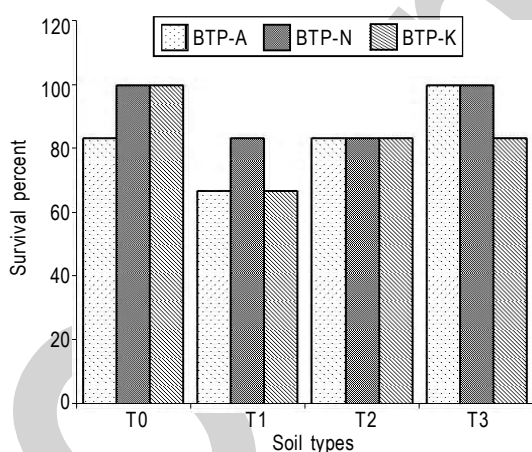
Soil properties	Soil types			
	Garden soil from BBAU, campus soil (T_0) Control	Soil from industrial wasteland of Sandila (IWLS) (T_1)	IWLS + 40% sand (T_2)	IWLS + 40% cowdung (T_3)
Bulk density	1.06±0.08	2.05±0.05	2.13±0.11	3.02±0.07
% WHC	51.91±3.37	64.17±1.83	50.50±1.64	76.17±2.64
pH	8.03±0.14	8.42±0.22	7.56±0.13	7.51±0.08
EC (dS m ⁻¹)	0.41±0.01	2.14±0.02	3.05±0.15	4.18±0.08
OC (%)	0.58±0.05	10.07±0.91	4.22±0.17	15.50±1.00
Total N ($\mu\text{g g}^{-1}$)	152.33±8.21	490.17±17.67	226.17±7.03	1251.67±22.73
Available N ($\mu\text{g g}^{-1}$)	27.08±2.42	55.33±3.20	35.75±3.60	68.17±3.19
Total P ($\mu\text{g g}^{-1}$)	177.67±8.31	625.00±15.84	257.42±10.13	519.00±30.10
Available P ($\mu\text{g g}^{-1}$)	5.62±0.36	81.83±3.82	25.89±1.01	43.42±3.44
Total K ($\mu\text{g g}^{-1}$)	183.00±6.39	968.67±34.30	261.67±11.69	263.33±11.69
Available K ($\mu\text{g g}^{-1}$)	20.83±1.83	53.17±4.26	20.33±3.39	31.33±3.20
Cu ($\mu\text{g g}^{-1}$)	22.00±0.86	71.20±3.77	60.07±1.68	80.17±4.49
Mn ($\mu\text{g g}^{-1}$)	30.15±2.28	67.23±3.32	42.25±5.26	56.22±4.64

Values are mean of six replicates ± SE

Table - 2: Copper ($\mu\text{g g}^{-1}$ d.wt.) in plant parts in 100 d old plant of *J. curcas* L. clones (BTP-A, BTP-N and BTP-K)

Soil type	Cu ($\mu\text{g g}^{-1}$ d.wt.)								
	BTP-A			BTP-N			BTP-K		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
T_0	4.21	3.01	3.99	5.61	4.19	5.04	5.21	3.51	4.02
T_1	16.87 ^a	12.21 ^a	14.2 ^a	20.99 ^a	12.54 ^a	16.73 ^a	18.24 ^a	11.24 ^a	15.21 ^a
T_2	13.31 ^a	9.23 ^a	12.35 ^b	18.34 ^a	8.70 ^a	9.15 ^b	14.12 ^b	8.12 ^b	11.21 ^b
T_3	16.24 ^b	10.35 ^a	12.58 ^b	15.65 ^a	12.15 ^b	10.80 ^a	17.35 ^a	10.23 ^a	12.21 ^a
C.D.	2.13	1.86	2.45	1.85	0.89	1.66	1.65	1.56	1.54

Values are mean of six replicates ± SE. ANOVA significant at $p \leq 0.05$. Soil types *i.* (T_0, T_1, T_2 and T_3).

**Fig. 1:** Percentage survival of three different clones of *J. curcas* L. (BTP-A, BTP-N and BTP-K) on 100 d plantation in soil types (T_0, T_1, T_2 and T_3)

The bulk density of T_3 was, however, three fold higher than that of the T_0 . Percent water holding capacity (WHC) of T_1 as well as T_3 was significantly higher than that of the T_0 . On the other hand T_2 showed a lower WHC than that of T_0 . The pH of T_1 was slightly

sodic/saline (8.4) than T_0 (8.03). Amendment of sand or cowdung, however, brought the pH down (7.5) which became more favorable for plant growth. The EC of T_3 or T_4 increased further which was about 8 fold higher than T_0 and T_3 . The T_1 possessed about 20 fold more organic carbon (OC), which was yielded further when cowdung was amended to the soil. The T_1 had more than double amount of available N, about 3-4 fold higher total P and over 15 fold higher available P, 4-5 fold higher total K and about 2.5 fold available K) over T_0 (Table 1). The total N was 3 fold higher in T_1 , about 2 fold higher in T_2 and about 8 fold higher in T_3 . Level of these nutrients was reduced per unit of soil in T_2 however; the total and available N increased about significantly in T_3 . The level of P and K were though, lower in the amended soil as compared to T_1 but still higher than that in T_0 soil. The level of Cu in T_1 was 3-5 times higher than that in T_0 , and that Mn was about 1.5-2 fold higher in T_1 (Table 1). The wasteland of Sandila (the experimental site), which cover around 500 ha agricultural soil contaminated with industrial effluents contained very high level of Cu, Zn, NPK and organic carbon as compared to the normal field soil of the area (Table 1). Higher levels of inorganic and organic contaminants are common feature of many similar

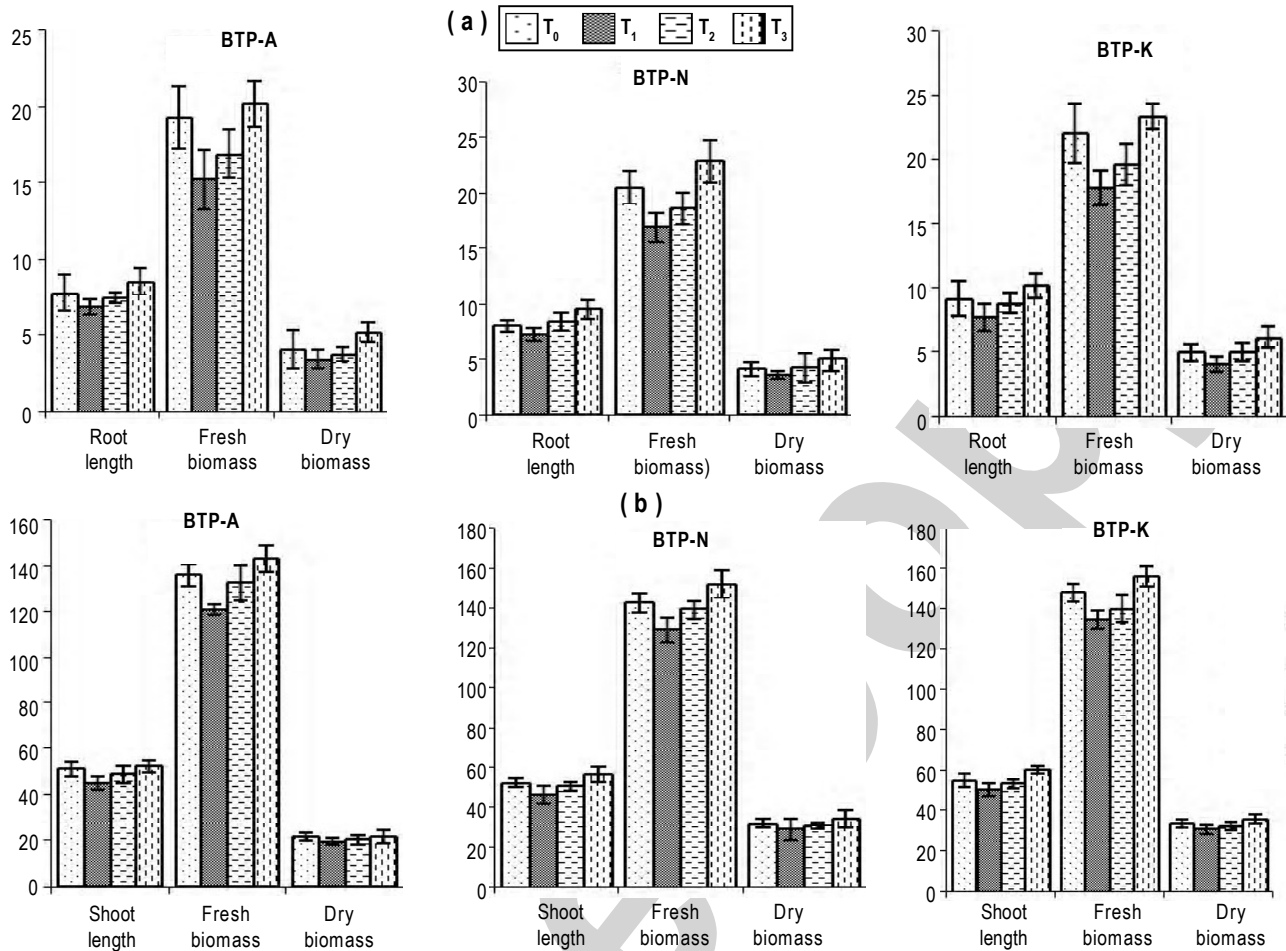


Fig. 2: (a) Root length (cm), Root fresh biomass (g) and Root dry biomass (g). (b) Shoot length (cm), Shoot fresh biomass (g) and Shoot dry biomass (g) of *J. curcas* L. clones (BTP-A, BTP-N and BTP-K) on 100 d, planted in soil (T₀, T₁, T₂ and T₃)

industrial wasteland sites at various places too (Sinha *et al.*, 2006; Gubitz *et al.*, 1999).

Survival of plant: The percent survival of stem cuttings of *J. curcas* L. clones was generally decreased in T₁ alone or T₂ (Fig 1). Among the three clones examined, BTP-A could survive up to 80% only in T₀, which was again decreased up to 70% in T₁. When T₁ amended with sand, the survival rate of BTP-A was almost similar to that in T₀ soil (Fig 1). The survival of this clone was, however, increased up to 100%, in T₃ was amended to IWLS. The survival of the other two clones of *J. curcas* L. i.e. BTP-N and BTP-K was, however, 100% in T₀ soil. The T₁ reduced the survival of these clones also in which BTP-K was found more sensitive than BTP-N. The survival of BTP-N decreased by about 25% in T₁ or T₂ soil. An amendment of 40% cowdung in IWLS, however, maintained a 100% survival of *J. curcas* L., BTP-N (Fig 1).

The BTP-K clone of *J. curcas* L., however, appeared more sensitive to T₁ for its survival as compared to the other two clones. The

percentage survival rate of *J. curcas* L. clones in T₀ and T₃ were near about 100% where as minimum survival rate (70%) was found in T₁. Due to addition of 40% cowdung, T₃ had sufficient nutrients, which are required for plant growth. Mangkoedihardjo and Sunahmadia have reported that cutting of *J. curcas* from the middle and base part showed 80% survival in the normal soil condition. Plants are variably tolerant to such toxic contaminants present in the wasteland soil and since soil of the wasteland of Sandila lack significant vegetation during most of the year, no indigenous plant species could get identified through the bio-prospecting of the area, which can be used for the removal of the toxic substances. *J. curcas*, the bio-diesel plant have been reported to survive in the wasteland and remediate certain soil pollutants (Juwarkar *et al.*, 2008; Cano-Asseleh *et al.*, 1989). All the three clones of *J. curcas* i.e. BTP-A, BTP-N and BTP-K, could survive in the waste land soil of Sandila, however, the survival rate was better when this contaminated soil was amended with 40% cowdung. The survival rate of BTP-N was better in T₁, and in both the amendments T₂ and T₃ as compared to the other clones of *J. curcas*.

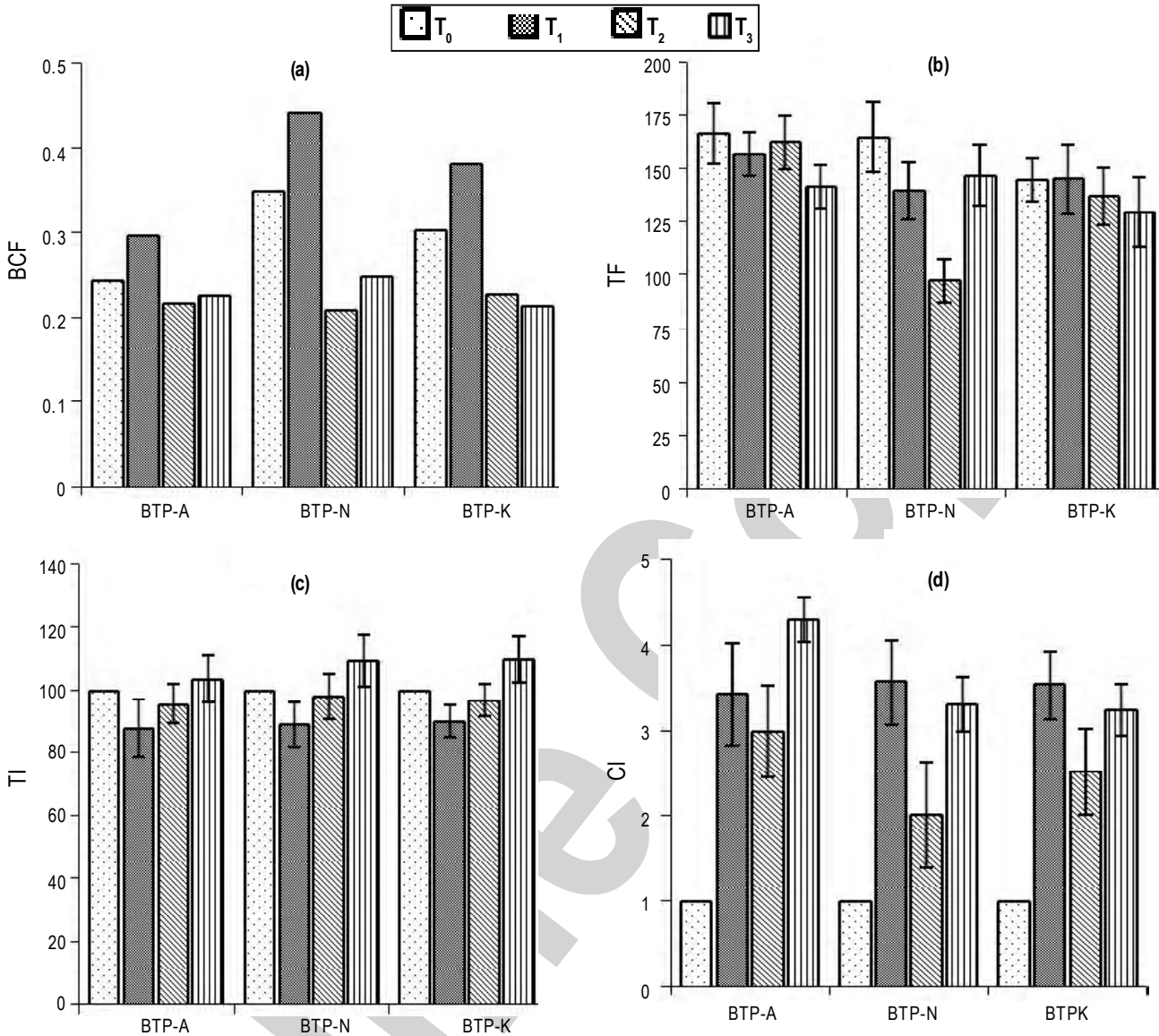


Fig. 3: (a) Bio-concentration factor (BCF), (b) Translocation factor (TF), (c) Tolerance index (TI), (d) Concentration index (CI) of *J. curcas* clones (BTP-A, BTP-N and BTP-K) on 100 d, planted in soil types (T₀, T₁, T₂ and T₃)

Table - 3: Removal of copper (Cu d⁻¹) from different soil type (T₀, T₁, T₂ and T₃) using *J. curcas* L. clones (BTP-A, BTP-N and BTP-K)

Soil type	BTP-A			BTP-N			BTP-K		
	µg Cu removed d ⁻¹ from 3 kg	µg Cu accumulated µg d ⁻¹ plant	Loss of Cu from soil due to leaching	µg Cu removed d ⁻¹ from 3 kg	µg Cu accumulated µg d ⁻¹ plant	Loss of Cu from soil due to leaching	µg Cu removed d ⁻¹ from 3 kg	µg Cu accumulated µg d ⁻¹ plant	Loss of Cu from soil due to leaching
T ₀	1.33	1.09	0.24	1.78	1.45	0.34	1.92	1.58	0.34
T ₁	6.58 ^a	4.90 ^a	1.68 ^a	6.76 ^a	5.22 ^a	1.54 ^a	6.15 ^a	4.41 ^a	1.75 ^a
T ₂	4.13 ^b	3.27 ^a	0.86 ^b	4.94 ^b	4.15 ^a	0.79 ^b	3.71 ^b	3.14 ^b	0.57 ^b
T ₃	5.82 ^a	4.41 ^a	1.41 ^a	6.62 ^a	5.24 ^a	1.38 ^a	5.98 ^a	4.52 ^a	1.46 ^a
CD	1.21	1.98	0.85	1.02	1.54	0.75	2.14	1.36	0.59

Values are mean of six replicates ± SE. ANOVA significant at p < 0.05, Soil types *i*. (T₀, T₁, T₂ and T₃).

Root and shoot length and biomass production: The net elongation of root, its fresh and dry biomass (Fig. 2a) and that of shoot (Fig. 2b) in all three clones of *J. curcas* used in this study decreased in T_1 at 100 d after planting. The T_2 soil recovered this decrease significantly.

The T_3 soil not only recovered the decrease in root and shoot elongation and biomass accumulation but even improved it significantly over T_0 soil. This indicates the potentials of survival and growth of *J. curcas* L. clones in T_3 (Fig. 2a,b). Elongation of root and shoot was slightly inhibited in T_1 , however, it was recovered significantly in T_3 . A similar response was observed in T_1 and T_3 for the fresh and dry biomass of the *J. curcas* L. clones. Previous studies revealed that Cu cause severe phytotoxic symptoms in the roots of *Betula*, *Vigna mungo* (Bibi *et al.*, 2006). Shoot elongation, fresh and dry biomass decreased significantly with increasing Cu concentration in *Triticum turgidum durum* (Aurelia *et al.*, 2008). In our study root elongation, fresh and dry biomass was more Cu sensitive in comparison to shoot elongation and shoots fresh and dry biomass (Fig. 2a,b). Similar results have been observed in durum wheat (Michaud *et al.*, 2007).

Translocation of Cu from soil to the plants: Though, significant amount of Cu varied from 3.0 to 5.6 $\mu\text{g g}^{-1}$ d. wt. was estimated in root, stem and leaf of various clones of *J. curcas* L. grown in the garden soil, its level increased by about 4 fold in the plant parts when grown in T_1 (Table 2).

Root accumulated higher amount of the metal followed by the leaf and stem. The maximum accumulation of Cu was found in BTP-N which has showed a better survival potential in T_1 . The other clone of *J. curcas* L. i.e. BTP-K had Cu contents in between the clones BTP-A and BTP-N. Translocation of Cu from soil to plant was however, slightly lower in T_2 and T_3 in the given period.

Bioconcentration factor (BCF) is the capacity of metal accumulation in relation with plant biomass. Bioconcentration of Cu was measured in the tissues of plants grown on T_1 which was significantly higher than T_3 , T_2 and T_0 (Fig. 3a). Overall bioconcentration factor (BCF) was highest (0.45) in T_1 and that was lowest (0.19) in T_2 .

The TF indicates the efficiency of plants to transfer metals from root to the aerial parts. The translocation factors (TF) of Cu for *J. curcas* clones (BTP-A, BTP-N and BTP-K) were higher in the plants grown in T_0 soil than that in the plants grown in the contaminated soil (Fig. 3b). The T_3 soil had a low translocation of Cu to shoots and the leaves in all the three clones of *J. curcas*.

The percentage of phyto-tolerance in this bio diesel plant in T_0 , T_1 , T_2 or T_3 were calculated as TI (Fig. 3c). The TI was higher in the plants grown in T_3 as compared to the other soil types. The CI was the value; indicate Cu levels in the treatment plants as compared to Cu level in the control plants. The CI was

higher in T_1 and T_3 soil types than that in T_0 . All three clones showed almost similar values of CI (Fig. 3d). The CI of Cu ranged from 2.2 to 4.5 in this plant species. Higher CI was found in T_1 as compared to T_2 or T_3 . BTP-A showed better CI as compared to the BTP-N and BTP-K clone.

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; Kopitke and Menzies, 2006) even for field-grown cereals (Michaud *et al.*, 2007).

Removal and losses of Cu from the soil: Table 3 represents Cu removed, accumulated, lost due to the leaching, and other activities from soil in $\mu\text{g d}^{-1}\text{plant}^{-1}$ by *J. curcas* L. clones. Copper removed off in $\mu\text{g d}^{-1}$ in clones of *J. curcas* L. were better in T_3 , where as slightly less in T_2 as compared to T_0 soil. The accumulation of Cu in the plants were same in correspondence with the removal of metal from the soil. The losses of Cu due to leaching and other activities were higher in T_1 and T_3 . Copper removed from soil in $\mu\text{g d}^{-1}$ of *J. curcas* L. clones, Cu accumulated $\text{d}^{-1}\text{plant}^{-1}$ of *J. curcas* L. clones and loss of Cu from soil due to leaching in $\mu\text{g d}^{-1}\text{plant}^{-1}$ were ranged from 1.33 to 6.67, 1.09 to 5.24 and 0.24 to 1.46 $\mu\text{g g}^{-1}$, respectively.

In this study, *J. curcas* removed excessive Cu from the contaminated soil which was, maximum in BTP-N. The $\mu\text{g d}^{-1}$ removal of Cu from the soil was better in the contaminated soil without any amendment as compared to the other treatments; however, the survival efficiency and plant growth was better in T_3 soil. The applied cowdung and other organic matter in the contaminated soil not only provide nutrients for the plant growth, but also stabilize heavy metals in the soil and reduce metal toxicity to the plants (Juwarkar *et al.*, 2008; Jamil *et al.*, 2009; Yadav *et al.*, 2009). The role of organic matter amendments, such as fermented compost, which contain high proportion of humid organic matter has been reported for decreasing the bioavailability of heavy metal of the soil, (Juwarkar *et al.*, 2008) thus permitting 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 plants in remediation of metaliferous soils. In term 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; Baudh and Singh, 2009, 2011). Our results indicate that *J. curcas* L. clones (bio-diesel plant) can be recommended for the cultivation in the industrial wasteland soil (study site) at Sandila (Hardoi) which could be helpful in removal of excessive copper effectively in a phased manner.

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