RESEARCH ARTICLE

Phytotranslocation of Fe by biodiesel plant *Jatropha curcas L.* grown on iron rich wasteland soil

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ABSTRACT

Experiments were conducted to evaluate the plant tolerance to Fe and its phytotranslocation by *Jatropha curcas* L. from an iron rich wasteland soil. The soil was collected from wasteland soil (WLS) of a small town Sandila, (Hardoi, U.P.) India, and three Jatropha clones were cultivated in WLS amended or not with sand or cowdung in a ratio of 3:2. The WLS had high pH, elevated electric conductivity (EC) and was rich in organic carbon and total NPK. Iron and Mn were 2-3 folds higher than that in the normal field soil. Net root and shoot elongation as well as fresh and dry biomass of the plants were only slightly affected at 100 d in WLS as compared with that grown in non-polluted soil. Tolerance index (TI) of *J. curcas* was significantly higher in cowdung amended WLS in comparison to that in WLS, or WLS amended with sand. Translocation factor (TF) from the soil to plants for Fe was significantly higher in WLS than that in the normal field soil. Bio-concentration factor (BCF) and concentration index (CI) for Fe were 0.12 to 0.37, 1.0 to 6.2 respectively. The results indicate that Jatropha plantation is suitable for phytoremediation of Fe-contaminate wasteland soils, and also that these polluted fields could be used to cultivate this important biodiesel plant species.

Key words: bio-accumulation, bio-concentration factor, concentration index, phytoremediation, tolerance index.

INTRODUCTION

Iron (Fe) is an essential micronutrient for plants and it is not toxic if available in a low quantity in rhizosphere. Fe is needed for chlorophyll biosynthesis and function, energy transfer, and for plant cell metabolism being constituent of certain enzymes and proteins, mainly that involved in N_2 fixation and plant respiration (Jeong and Connolly, 2009; Kong and Yang, 2010). However, iron can induce toxicity if available in high concentrations in soil solution as it can promote deficiency of other essential nutrients by restricting their uptake (Lin and Wun, 1994). If taken by the plants in excessive amounts Fe may also inhibit the activity of several enzymes and subsequently may lead to disturbances of the cellular metabolism (Juwarkar et al., 2008a). Iron can also be toxic when it accumulates to inappropriately high levels because free iron can participate in the Fenton reaction and generate cytotoxic hydroxyl radicals (Halliwell, and Gutteridge, 1992). Metals are non-biodegradable and persistent in the environment and can be differentially toxic to microbes (e.g., Giller et al*.,* 2009), plants (e.g., Bharti and Singh, 2003; Singh et al., 2003, 2007; Leon et al., 2006; Bauddh and Singh, 2009; Sharma et al., 2010), animals (e.g., Rainbow, 2006) and human beings (e.g., Lim and Schoenung, 2010).

Edaphic factors such as soil pH, low organic matter, soil aeration, high soil phosphorus (P), forms of nitrogen (N) applied, Fe:Zn balance, Fe:Mn balance, K:Fe balance, and Fe:Mo balance can affect the availability of Fe to the plants (Zancani et al., 2007; Abhilash et al., 2009). On the other hand, different plant species evolved the capacity to survive in soils with very high amounts of Fe and other metals.

Jatropha curcas L. (Family: *Euphorbiacae*) is a tropical plant, that can sustain harsh environment and adapt well to the semi-arid soil conditions and wasteland (Angelov et al., 1993; Foidl et al., 1996; Gubitz et al.,1999; Juwarkar et al., 2008a). The seeds contain 27-40% oil that can be processed to produce a high-quality biodiesel fuel. Genus *Jatropha* with 172 species is native to Central America and is also widely distributed in Africa and Asia (Cano-Asseleih et al., 1989; Mangkoedihardjo and Sunahmadia, 2008). Among the various *Jatropha* species, *J. curcas* is one which is widely distributed in India (Fairless, 2007). In addition to being a source of oil, *Jatropha* also provides a meal that serves as a highly nutritious and economic protein supplement in animal feed, if the toxins are removed (Achten et al., 2008).

The establishment, management and productivity of jatropha under various environmental conditions are not fully documented and the full potential of this multipurpose plant is to be realized (Openshaw, 2000). In the present work, it was investigated the Fe-tolerance and the capacity for Fe phytotranslocation and bioaccumulation of three clones of *J. curcas* L. grown in wasteland soils from Sandila (WLS), a small Indian town, which has excessive Fe and is rich in inorganic and organic matters. Effects of WLS amendment with sand and cowdung were also analyzed.

MATERIALS AND METHODS

Survey and mapping of the area: The town Sandila is located between 26º 53' and 27º 46'north latitudes and between 79º 41' and 80º 46'east longitudes (www.mapsofindia.com). The characterization of this wasteland soil and attempt to develop greenery in this wasteland has not been attempted so for as per our data bank. The wasteland soil was completely barren at the time of sampling, which was done during January, May and September 2007.

Sampling, preparation and analysis of the soil: The soil samples were collected from 0-15 cm depth randomly from 3 to 4 locations, mixed thoroughly and the representative samples of WLS were analyzed for physico-chemical characteristic. The same soil was used for planting of *J.curcas* L. (T_1). The soil (WLS) was amended with sand (T_2) or cowdung (T_3) in 3:2 ratios. A control set was maintained with garden soil from the university campus (T_0). Each soil sample was air dried for 48 h in hot air oven, ground with ball mixer and sieved through 1 mm mesh. Finally, the soil was kept in labeled polythene bags at ambient temperature for analysis. The soil was mixed with deionised water in 1:2 ratio w/v and pH as well as electric conductivity (EC) were measured using glass electrode pH meter and EC meter, respectively. Percent organic carbon, total N, total P and total K were analyzed by methods described by Kalra and Maynard (1991). The soil samples were dried in oven to constant weight and digested in conc. HNO_3 and $HClO_4$ in 5:1 (v/v) ratio at low temperature till a white residue was obtained (Fritioff and Greger, 2007). Double distilled water was used to maintain final known volume. The samples were analyzed using Atomic Absorption Spectrophotometer (VARIAN, AA240FS).

Measurement of root and shoot elongation, biomass production and estimation of Fe contents in plant parts: 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 were prepared from 1 y old plants and planted in WLS, WLS amended with 40% sand or cowdung and normal field soil (control). The root and shoot length were measured after 100 d of cultivation using meter scale. The plant parts removed carefully from the growing plants washed with deionized water and dried by blotting it on filter paper for fresh weight of roots and shoot, using single pan electrical balance. The tissues were oven dried at 70°C, until constant dry weight was achieved. The dry weight of roots and shoots were determined using single pan electrical balance. The dried plant materials were ground to less than 1 mm with a stainless steel mill, powdered and digested in microwave oven, using acid mixture (perchloric acid and nitric acid, 1:3 ratio (Fritioff and Greger, 2007). The samples were analyzed using Atomic Absorption Spectrophotometer (VARIAN, AA240FS).

Three indicators, *i.e.* phyto-tolerance, bioaccumulation and phyto-translocation, were calculated to evaluate the tolerance.

Bio-accumulation and translocation efficiency of *J.curcas* clones for Fe using the following equation.

The bioconcentration factor (BCF) is the metal uptake capacity from soil to plant tissue, which was calculated using Fe levels in plant and soil (Ghosh et al., 2005).

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Metal concentration in the plant}}{\text{Metal concentration in soil}}$$

The translocation factor (TF) was calculated from the data on the distribution of Fe in root and aerial part in the plants, grown on different type of soil to evaluate plant's ability to translocate Fe from the roots to the aerial parts (Mattina et al., 2003).

$$\text{Translocation factor (TF)} = \frac{\text{C aerial x 100}}{\text{C root}}$$

Where C = concentration of metal in μg g-1, in aerial parts i.e. stem and leaf (for each part TF was calculated separately).

The tolerance index (TI), which is the ratio between a variable measured in treated plants and that in control plants, expressed as a percentage, and was calculated considering the plant height (Kumar et al., 2008).

$$\text{Tolerance Index (TI)} = \frac{\text{Mean height of the plant growing on wasteland soil x 100}}{\text{Mean height of the plant growing on control soil}}$$

The concentration index (CI) was calculated by dividing Fe levels in the treatment plants by the Fe level in control plants (Kiekens and Camerlynck, 1982).

$$\text{Tolerance Index (TI)} = \frac{\text{Concentration of metal in treatment plant}}{\text{Concetration of metal in normal plant}}$$

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 was considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Characterization of soil samples: The four types of soil samples, control soil from the university campus (T_0), soil sampled from wasteland, WLS (T_1), the WLS amended with 40% sand (T_2) or cowdung (T_3) were analyzed before the plantation of *J. curcas* stem cuttings for various physico-chemical properties (Table 1). The pH of T_0 and T_1 was 8.03 and 8.4 respectively which indicate that it is moderately saline soil. Amendments of sand or cowdung, however, decreased the pH to 7.5. The WLS had many fold higher EC over the control soil. The EC of WLS amended with sand or cowdung increased further, which was about 8 fold higher than the garden soil. The WLS possessed about 20 fold more organic carbon (OC) over the control soil and it increased further when cowdung was amended to the soil. The WLS had more than double amount of available N over 15 fold, higher available P and about 3 fold higher available K over the control soil. The level of Fe in WLS was 3-4 times more and Mn was more than 2.0 fold higher in T_1 (Table 1) than that in T_0. Edaphic factors are known to affect plant establishment, its survival, growth and productivity on the wasteland soils (Cooke and Johnson, 2002). The presences of organic matter in soil have been reported to be beneficial for plant productivity and for structural stability of the soil (Juwarkar et al., 2006; Hati et al., 2006; Pichtel and Bradway, 2008).

Table 1. Characterization of the soils used for cultivation of *J. curcas* L. clones. Garden soil from BBAU Campus (control, T_0), Soil from industrial wasteland of Sandila (WLS, T_1), WLS amended with 40 % of sand (T_2), WLS amended with 40 % of cowdung (T_3). Data are mean ± SE (n=6).

Soil properties	Soil types			
	control soil (T_0)	WLS (T_1)	WLS + 40% sand (T_2)	WLS + 40% cowdung (T_3)
pH	8.04± 0.16	8.52± 0.25	7.54±0.12	7.61±0.09
EC (dS m $^{-1}$)	0.47±0.01	2.13±0.02	3.05±0.15	4.15±0.09
OC (%)	0.48±0.01	10.07±0.91	4.22±0.17	15.50±1.00
Available N(μgg-^{1})	24.08±2.42	55.33±3.20	35.75±3.60	68.17±3.19
Available P (μg g^{1})	4.63±0.36	81.83±3.82	25.89±1.01	43.42±3.44
Available K (μg g^{-1})	17.83±1.83	53.17±4.26	20.33±3.39	31.33±3.20
Fe (μg g^{-1})	425.83±6.24	1663.00±11.49	1200.92±9.17	1734.17±12.01
Mn (μg g^{-1})	32.15±2.29	59.23±3.22	45.25±5.26	66.12±4.54

Elongation and biomass production of *J. curcas* clones: Net elongation, fresh weight and dry biomass of the *J.curcas* clones decreased slightly in WLS containing pots, which were recovered partially when the soil was amended with 40% sand (Figure 1). The growth parameters were not only recovered but also enhanced slightly when the Jatropha clones were grown in soil containing WLS amended 40% cowdung.

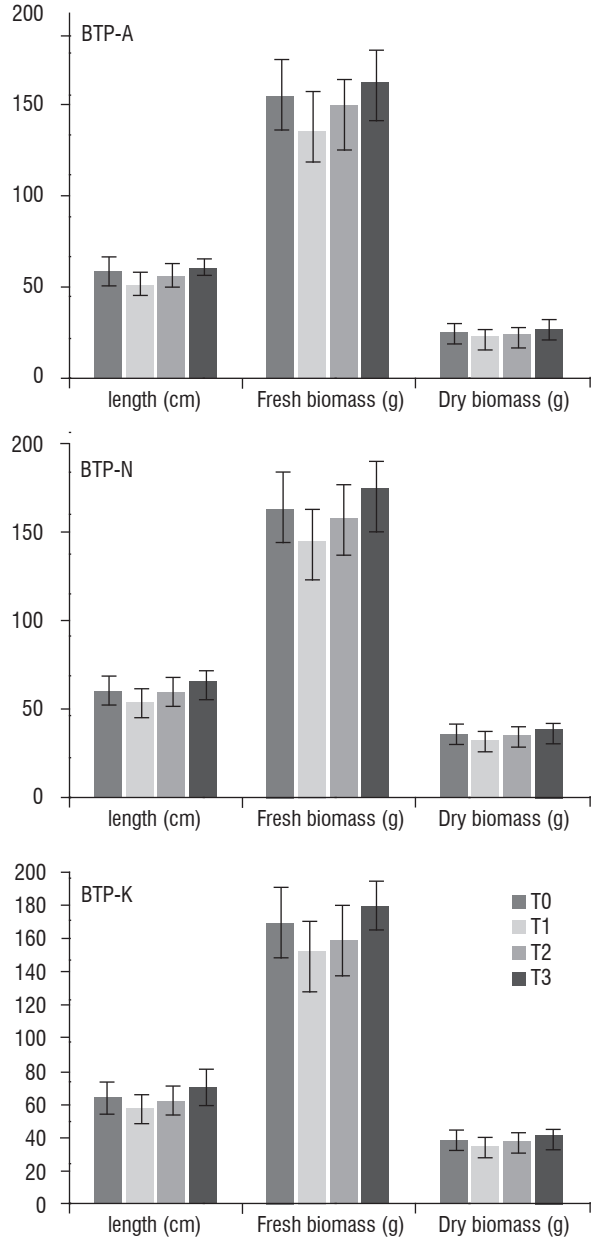

Figure 1. Plant height (cm), fresh weight and dry weight (g) of the *J. curcas* clones (BTP-A, BTP-N and BTP-K) at 100 d after plantation in different soil types *i.e.* T_0, T_1, T_2 and T_3, (as defined in Table 1).

The phytotoxicity of toxic metals and extreme infertility of the wasteland soils have been the major limiting factor for the growth of plants in many degraded ecosystems (Rotkittikhun et al., 2007; Norwood et al., 2007). A number of studies have shown that organic amendments result in successful re-vegetation of the wasteland soil (Ortiz, and Alcaniz, 2006; Kumar et al., 2008; Juwarkar et al., 2008a). Therefore applications of organic materials could enhance the biomass yield of *J. curcas*, highest yield occurred when cowdung was added to WLS (T_3).The growth performance of plants is related with the total remediation potential of plants, as the total metal accumulation capacity of plants is dependent on the plant biomass (Shukla et al., 2007).

Translocation of Iron from soil to plants: A significant amount of Fe level was detected in *J. curcas* clones grown in garden soil (control) (Table 2). The roots accumulated maximum Fe (49.25-64.94 μg g^{-1} dry wt) followed by the leaves (45.21-55.37 μg g^{-1} dry wt). The partitioning of Fe in different plant parts was similar in the wasteland soil also; however, the level of Fe in roots was 5-6 folds higher in plants grown in the iron rich wasteland soil (WLS; 301.24-319.53 μg g^{-1} dry wt). The leaves of *J.curcas* clones grown in WLS could accumulate 2-4 fold higher Fe than that in the garden soil which was genotype (clone) dependent (Table 2). The stem part of WLS grown plants accumulated about two folds higher Fe than that in the control garden soil which was largely independent to the clone type. The Fe accumulation pattern was similar in root, stem and leaves (though with a lower magnitude) in the plants grown in WLS amended with 40% cowdung or sand. Our data indicate that *J.curcas* can remove a significant amount of Fe from the soil and roots are the primary sink for accumulation of the metal. Amongst the aerial parts, leaves are the major sink. The different clones of *J.curcas* accumulated different amount of Fe and it appears that Fe accumulation by Jatropha is a genotype dependent process. The clone BTP-N was more efficient in accumulating Fe, which was closely followed by BTP-K. *J.curcas* clone BTP-A, however, accumulated lower Fe in its parts as compared to the other two clones. The data presented in Table 2 indicate that *J.curcas* L. clones, BTP-A, BTP-N and BTP-K translocate a significant amount of iron from soil in 100 d. The most effective iron translocator Jatropha clone BTP-N could translocate 0.83 μg Fe g^{-1} dry wt of aerial parts per day from normal field soil. From the WLS it could translocate 2.76 μg Fe g^{-1} dry wt of aerial parts per day.

Table 2. Iron contents in various clones of *J. curcas* L. on 100 d after cultivation. Data are mean ± SE (n=6). ANOVA significant at $p \leq 0.05$. Soil types T_0, T_1, T_2 and T_3 as defined in Table 1.

	Clones	Part of plant	Soil Type				
			T_0	T_1	T_2	T_3	C.D.
Fe μg g-1 dry weight	BTP-A	Root	49.25±2.21	301.24^a±4.45	108.2^b±2.59	175.21^b±3.56	25.32
		Stem	22.87±1.37	48.22^b±2.22	37.24^b±1.59	70.21^a±2.65	8.45
		Leaf	45.21±1.06	92.14^b±2.56	92.32^b±3.12	125.25^a±3.21	15.54
	BTP-N	Root	64.94±1.07	319.53^a±4.45	122.1^b±2.37	205.15^a±3.12	22.23
		Stem	27.94±0.80	63.26^a±1.25	41.63^b±1.01	47.47^b±1.78	5.23
		Leaf	55.37±0.68	212.56^a±2.65	108.26^b ±1.87	143.85^b ±2.12	12.32
	BTP-K	Root	50.23±0.98	310.65^a±3.86	115.91^b ±2.68	180.54^b ±3.01	20.61
		Stem	25.62±0.44	52.42^a±1.25	40.35^b±1.32	43.14±2.21	4.4
		Leaf	50.56±1.26	201.45^a±3.15	98.35^b±2.31	135.12^b±2.45	15.1

Bio-concentration factor translocation factor, tolerance index, and concentration index of Jatropha curcas for Fe: Bioconcentration factor (BCF) is the capacity of metal accumulation in relation with plant biomass. Bioconcentration of Fe was measured in the tissues of plants grown on WLS (T_1) which was significantly higher than cowdung amended soil (T_3), sand amended soils (T_2) and control soil (T_1) (Figure 1Aa). Overall bio-concentration factor (BCF) was highest (0.25) in T_1 and that was lowest (0.12) in T_2.

Translocation factor (TF) indicates the efficiency of plants to transfer metals from root to the aerial parts. The translocation factors (TF) of Fe for *J. curcas* clones (BTP-A, BTP-N and BTP-K) were higher in the plants grown in the control soil than that in the plants grown in the wasteland soil (Figure 2B). The wasteland soil (WLS) has a low translocation of Fe to shoots and the leaves in all the three clones of *J. curcas.* The amendment of sand or cowdung to WLS however, enhanced the translocation factor for Fe in *J. curcas* (Figure 2B).

The percentage of phyto-tolerance in this biodiesel source plant in control (T_0), WLS (T_1), WLS amended with sand (T_2) or cowdung (T_3) were calculated as tolerance index (TI) (Figure 2C). The tolerance index was higher in the plants grown in cowdung amended soil (T_3) as compared to the other soil types. The concentration index (CI) was the value; indicate Fe levels in the treatment plants as compared to Fe level in the control plants. The CI was higher in T_1 and T_3 soil types than that in control plants (T_0). All three clones showed almost similar values of concentration index (CI) (Figure 2D).

The statistical correlation between Fe accumulated in plants and removed from soil showed significant linear, logarithms and exponential relationship for the *J. curcas* L. clones (Figure 3). Correlation values for the BTP-A clone were linear $R^2=0.978$, logarithms $R^2=0.986$ and exponential $R^2=0.868$, those for clone BTP-N were linear $R^2=0.768$, logarithms $R^2=0.832$ and exponential $R^2=0.891$.These values were linear $R^2=0.969$, logarithms $R^2=0.998$ and exponential $R^2=0.938$ for BTP-K clone.

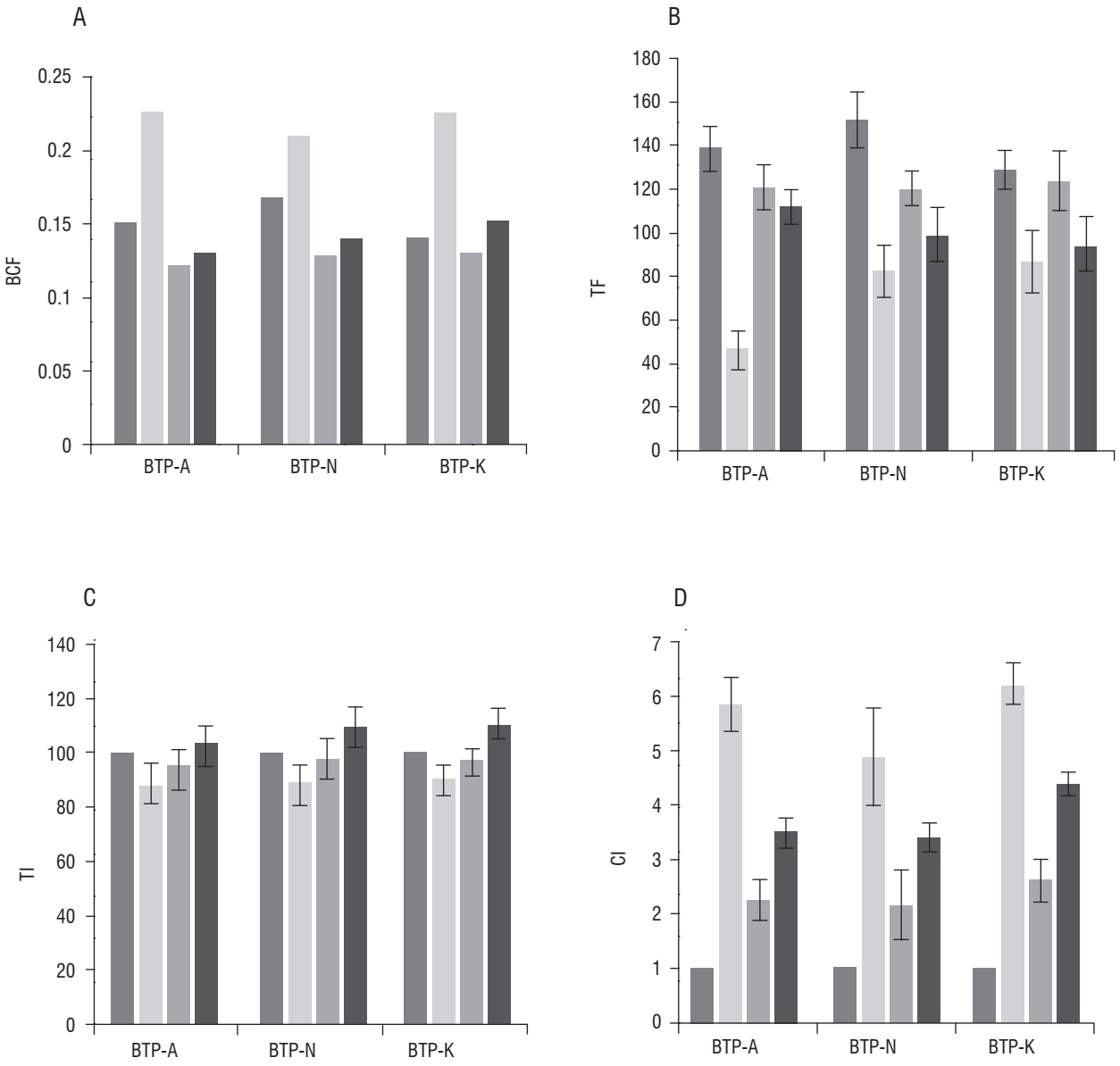

Figure 2. Bio-concentration factor (BCF) (A), translocation factor (TF) (B), tolerance index (TI) (C) and concentration index (CI) (D) of *J. curcas* clones (BTP-A, BTP-N and BTP-K) on 100 d after plantation in soil types *i.e.* T_0, T_1, T_2 and T_3 (as defined in Table 1).

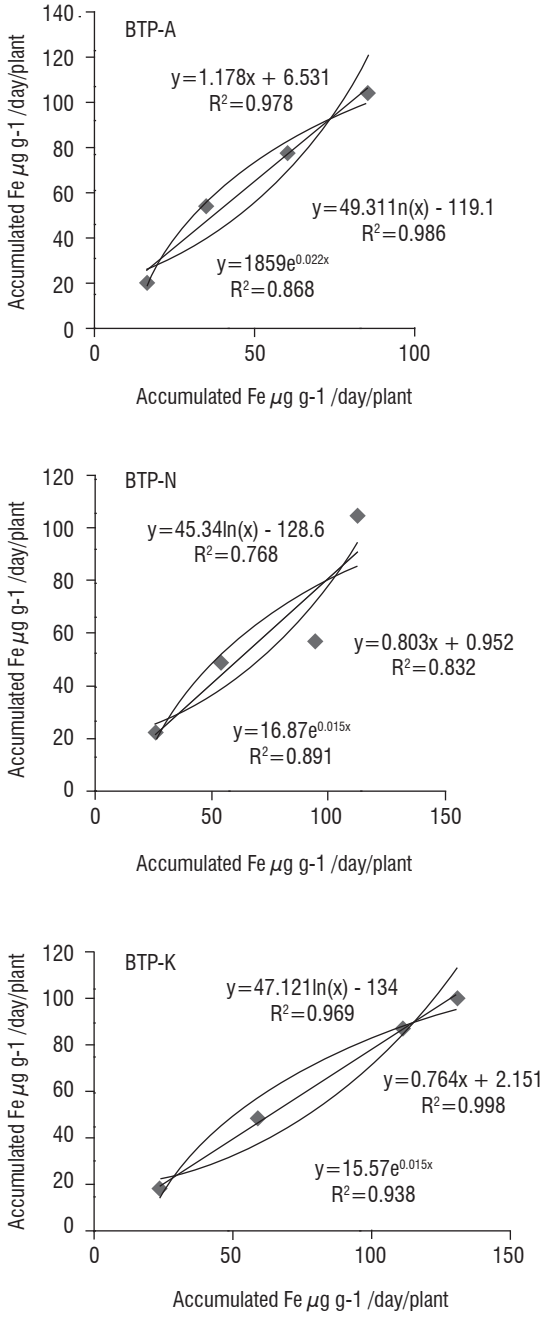

Figure 3. Correlation between Fe accumulated in plants (μg d^{-1} $plant^{-1}$) and removal from the soil (μg d^{-1}) by the *J. curcas* clones (BTP-A, BTP-N and BTP-K) at 100 d after plantation in soil types *i.e.* T_0, T_1, T_2 and T_3 (as defined in Table 1).

Iron is essential for plants, plays critical roles in important processes such as photosynthesis and respiration, and serves as a cofactor for a great number of enzymes with critical roles in DNA biosynthesis and nitrogen metabolism along with the above-mentioned processes. (Jeong and Connolly, 2009).The understanding of molecular mechanisms involved in iron uptake from the soil is relatively developed, however, the information regarding mechanisms involved to move iron into sub cellular compartments *e.g*. mitochondria, chloroplasts and vacuoles etc are yet to be elucidated. The members of Ferric Reductase Oxidase (FRO) family of ferric chelate reductases reduce ferric iron chelates to form soluble ferrous iron (Jeong and Connolly, 2009). Arabidopsis FRO2 reduces iron at the root surface so that it may be transported across the plasma membrane. Recent studies in Arabidopsis suggest that FROs may function at organellar membranes (Connolly et al., 2003). In particular, FRO7 plays an essential role in iron delivery to chloroplasts (Mukherjee et al., 2006), while two other FROs (FRO3 and FRO8) localize to the mitochondria and might therefore contribute to mitochondrial iron homeostasis (Heazlewood et al., 2004).

Plants utilize two distinct mechanisms to mobilize iron in the rhizosphere and take it up across the plasma membrane of root cells. Strategy I response, which is used by all the dicots and nongraminaceous monocots, is the set of three activities; acidification, reduction and transport at the plasma membrane of root epidermal cells following the onset of iron limitation (Romheld, 1987) whereas the Strategy II response is used by the grasses in which plants secrete high affinity ferric iron chelators termed phytosiderophores (PS) in response to iron deficiency (Jeong and Connolly, 2009). Fe (III)–PS complexes are taken up into root cells via Yellow-stripe1 (YS1) transporters that was identified in maize (Curie et al., 2001), barley (Murata et al., 2006).

Iron delivered from the root to the shoot via the xylem as a ferric iron–citrate complex (Tiffin, 1966). While the molecular mechanisms involved in iron transport into leaf cells remain unclear (Hell and Stephan, 2003). It has been reported that ferric chelate reductase activity is detectable in leaves of sunflower (Guardia and Alcantara, 1996) and *Vigna unguiculata* (Bruggemann et al., 1993). A recent study examined the fractionation of stable iron isotopes taken up from the soil in various aerial portions of bean, pea, soybean, maize, oat, wheat etc (Guelke and Von Blanckenburg, 2007). Tiffin et al. (1973) have reported that translocation of Fe from soybean cotyledons to other parts of the plants could occur, and it was suggested that a proteinase is involved in releasing Fe from the Ft protein shell. Accumulation and distribution of

metals in plant tissues are important aspects to evaluate the role of plants in reclamation of metalliferous soils (Pichtel and Bradway, 2008). In terms of stabilizing contaminated sites, a lower metal concentration in stem is preferred in order to prevent metal from entering into ecosystem.

The results show that the BCF was slightly decreased with the increasing Fe concentrations in soil. Application of cowdung in wasteland soil not only provided nutrients for plant growth, but also stabilized the metal in the soil and reduced metal toxicity to the plant. Similar results have been reported by Juwarkar et al. (2008a) using dairy sludge. Though phyto-translocation of iron in *J. curcas* was comparatively low when it is compared with other metal hyper-accumulators, being a non-food biodiesel plant it can be an ideal option to be grown in a iron rich soil. The amendment of cowdung (T_3) to WLS (T_2) improve the sustainability of the *J. curcas* in Fe wasteland soils, as reflected by increased growth performance and decreased metal accumulation.

It seems clear that much more research is required into the growing and management of *Jatropha curcas* and more information is needed on its potential for biodiesel production and for other purposes when cultivated in wasteland soils. However, the present data highlight that at least *J. curcas* L. clone BTP-N can be recommended for the cultivation in the iron rich wasteland sites, as it not only exhibited vigorous growth in the Industrial wasteland soil of Sandila but also effectively removed the excessive iron.

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