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Tannery Effluent Induced Morpho-Biochemical Expressions and Chromium Accumulation in *Jatropha curcas* L. and *Pongamia pinnata* L.

(Efluen Kilang Kulit Mengaruh Pengekspresan Biokimia-Morfologi dan Pengumpulan Kromium pada *Jatropha curcas* L. dan *Pongamia pinnata* L.)

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ABSTRACT

The use of effluent from various industries by agriculture sector in developing countries may help to mitigate water scarcity and cost of fertilizers but pose considerable threats to entire ecosystem when heavy metals enter the food chain. The aim of the present study was to compare the growth and development of two biofuel tree species Jatropha curcas *L. and* Pongamia pinnata *L. when exposed to 20 and 40 mL L^{-1} of effluent discharged from a local tannery along with tap water as a control. The physico-chemical attributes assessed for quality of effluent showed higher values and significantly higher chromium (Cr) concentration than other metals. Application of effluent induced profound formation of malondialdehyde (MDA) in* P. pinnata*. With regard to growth and pigments of plant species, a significant reduction (*$p \leq 0.05$*) in root length, dry shoot biomass, leaf area, chlorophylls and carotenoids occurred in* P. pinnata *but shoot length, collar diameter and root dry biomass remained unaffected. Similarly,* J. curcas *sustained root and shoot growth, dry biomass production, collar diameter and leaf area. Increased pigment contents were found at both effluent levels. The roots of* P. pinnata *accumulated 6 and 11 times more Cr at 20 and 40 mL L^{-1}, respectively, than control. While in* J. curcas *Cr accumulation was up to 9 folds in shoots at 40 mL L^{-1}. Thus, the two species exhibited differential potential for Cr accumulation in their above and below ground tissues. The study signified the use of contaminated water for irrigation and potential of the species to act as phytoremediator to alleviate both water scarcity and metal contamination.*

Keywords: Biofuel tree species; chlorophyll pigment; Cr in plant parts; MDA content; tannery effluent

ABSTRAK

Penggunaan efluen daripada pelbagai industri dalam sektor pertanian di negara-negara membangun boleh mengatasi kekurangan air dan kos untuk baja tetapi menimbulkan ancaman kepada seluruh ekosistem apabila logam berat memasuki rantaian makanan. Kajian ini bertujuan untuk membandingkan perkembangan dan pembangunan dua spesies pokok bahan api biologi Jatropha curcas *L. dan* Pongamia pinnata *L. apabila didedahkan kepada 20 dan 40 mL L^{-1} efluen yang dilepaskan daripada yang kilang kulit tempatan bersama-sama dengan air paip sebagai kawalan. Atribut fiziko-kimia yang dinilai untuk kualiti efluen menunjukkan nilai yang lebih tinggi dan kepekatan kromium (Cr) jauh lebih tinggi daripada lain-lain logam. Penggunaan efluen mengaruh pembentukan secara mendalam untuk malondialdehid (MDA) pada* P. pinnata*. Dengan mengambil kira pertumbuhan dan pigmen spesies tumbuhan, pengurangan ketara (*$p \leq 0.05$*) pada panjang akar, biojisim pucuk kering, keluasan daun, klorofil dan karotenoid berlaku dalam* P. pinnata *tetapi panjang pucuk, diameter kolar dan biojisim pucuk kering kekal tidak terjejas. Dengan cara yang sama,* J. curcas *mengekalkan pertumbuhan akar dan pucuk, pengeluaran biojisim kering, diameter kolar dan keluasan daun. Peningkatan kandungan pigmen dilihat pada kedua-dua peringkat efluen. Akar* P. pinnata *masing-masing mengumpul 6 dan 11 kali lebih banyak Cr pada 20 dan 40 mL L^{-1}, daripada kawalan. Manakala pengumpulan Cr dalam pucuk* J. curcas *adalah sehingga 9 kali ganda pada 40 mL L^{-1}. Oleh itu, kedua-dua spesies menunjukkan potensi berbeza dalam pengumpulan Cr untuk tisu asas atas dan bawah. Kajian ini mencadangkan penggunaan air tercemar untuk pengairan dan potensi spesies untuk bertindak sebagai fitopemulih dalam mengatasi kekurangan air serta pencemaran logam.*

Kata kunci: Cr pada bahagian tumbuhan; efluen kilang kulit; kandungan MDA; pigmen klorofil; spesies pokok bahan api biologi

Introduction

Leather industry is the second most dynamic earning sector through its export which plays a pivotal role in the economy of Pakistan. It contributes about 5% GDP of the country and provides employment to more than 500,000 people (Hashmi et al. 2017). There are more than 600 tanneries and leather processing units in major cities (Gujranwala, Kasur, Sialkot, Karachi, Lahore, Multan and Peshawar) of the country. Tanning process for finished products involves the use of raw hides and skins. During this process, large amount of water and salts of different elements (ammonium, calcium, chromium, sodium and sulphate), acids, alkali, fats, liquor and many organic dyes are used (Afzal et al. 2014; Lofrano et al. 2013).

Despite wastewater treatment, the effluent discharged into drainage system by these industrial units contains excessive amount of dissolved salts, thus having high biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solid (TSS), total suspended solid (TDS), heavy metals and many other toxic substances (Dixit et al. 2015). The wastewater if used for irrigation poses potential hazard to animals, crops and human health (Dotaniya et al. 2017). The effluents containing heavy metals like Cr, Zn, As, Cd and Pb are phytotoxic and can easily be translocated through vascular tissues (Tripathi et al. 2014). Accumulation of toxic amount of metals in plant tissues results in anomalous metabolic activities that impede plant growth and development (Cheng et al. 2017).

Excessive amount of heavy metals can generate reactive oxygen species (ROS) which drastically damage biological membranes. ROS can cause oxidative stress, membrane lipid peroxidation, damage to DNA and RNA, disintegration of chloroplasts, inhibition of enzymes, oxidation of amino acids and degradation of proteins (Maleki et al. 2017; Tripathi et al. 2015).

Chromium (Cr) is 21st most abundant element of the Earth's crust. Among 129 leading pollutants, it is listed as one of the most harmful heavy metals by the United States Environmental Protection Agency (USEPA) as reported by Johnson et al. (2018). USEPA limit for Cr in potable and discharged water is 0.05 and 0.1 mg L^{-1}, respectively (de Oliveira et al. 2015). According to Sinha et al. (2018), Cr concentrations range at 0.1-0.5 mg L^{-1} in fresh water, 0.0016-0.05 mg L^{-1} in sea water and 5-3000 µg g^{-1} in soil.

Cr mainly enters into environment as Cr (III) and Cr (VI), and the latter being more toxic and highly mobile form (Gopal et al. 2014). However, wastewater from tanneries contains both forms of this metal (Dotaniya et al. 2014). Cr has recently received much attention due to its critical role in metabolic processes in human and its carcinogenic effects (Johnson et al. 2018).

The extent of damage of plants by Cr depends on its bioavailability, mobilization and subsequent accumulation in the tissues. However, it depends on several properties of soil such as pH, cation exchange capacity, redox potential, temperature and the presence of macro and micronutrients (Asfaw et al. 2017). The mechanism of Cr tolerance in plants is distinct and species specific (de Oliveria et al. 2014).

Toxic effects of Cr can be mitigated in plants via special mechanisms that alleviate metal toxicity (Sinha et al. 2018). These mechanisms involve hyper accumulation of substantial amounts of Cr in plant tissues, altered metabolic pathways and oxidative defense (Gill et al. 2015). Both antioxidant enzymes and synthesis of glutathione reductase (GR) act as ROS scavenger, metal chelator and detoxification of CrVI to CrIII by chemical and enzymatic processes (Anjum et al. 2012; Das et al. 2014). The compartmentalization of Cr by storing it to metabolically inactive organelles and its detoxification by phytochelatins are well known (Singh et al. 2013).

Several studies showed that uptake of Cr (VI) is an active process, transported by sulphate and phosphate carriers present in plants whereas accumulation of Cr (III) is passive, with zero energy requirements (de Oliveira et al. 2016). Once Cr enters the root system, it can either be stored in the roots, or further translocated to the shoots via xylem loading.

Lipid peroxidation is the key indicator of oxidative stress that leads to damage to biological membranes (Shahid et al. 2017). Malondialdehyde (MDA) is a decomposition product and its amount directly correlates with the extent of lipid peroxidation. Metal toxicity is felt via the generation of ROS which is scavenged by the action both enzymatic and non-enzymatic antioxidative defense systems (de Oliveira et al. 2016).

The evolution of mechanism for Cr uptake, translocation and acquisition of superior survival ability has been well reported for tree species which allows trees to tolerate and accumulate substantial amount of metals. Thus, such tree species can play a pivotal role in removing contaminants of heavy metals from the substrate and subsequently block them in different tissues of the plants. Several plant species such as *Acacia modesta* (Wall), *Albizzia lebbeck* L., *Melia azedarach* L., *Syzygium cumini* L. and *Terminiala arjuna* (Roxb. ex DC) have shown greater tolerance for metal stress (Cr, Ni, Mn, Zn, Cd and Pb) present in industrial effluents (Bhatti et al. 2017). Trees belonging to different species have shown their abilities for high loading rate of metals due to profuse root system, thus, can control metal toxicity in a better way than many cultivated species (Kimmatkar et al. 2015).

Among biofuel tree species, *Jatropha curcas* L. and *Pongamia pinnata* L. have recently gained considerable attention as potential bioremediators (Manzoor et al. 2015). Both species are common flora of Pakistan grown on roadsides as shelter belts and planted for shade in public parks. Responses of *J. curcas* and *P. pinnata* have widely been reported for several heavy metals (Ni, Mn, Cu, Zn, As, Cd and Pb) (Badoni et al. 2016; Bernabé-Antonio et al. 2015; Kumar et al. 2017, 2009; Marques et al. 2017; Shirbhate et al. 2012; Tulod et al. 2012), but their growth potential to cope with excessive Cr has not been addressed so far. In order to bridge this gap, our foremost objective was to report morpho-biochemical responses in *J. curcas* and *P. pinnata*, pattern of Cr bioaccumulation, and appraisal of superior survival strategy of the species under elevated Cr levels. The study also addressed the potential and efficient use of wastewater through the exploitation of the species for remediation purposes to clean up metal contaminated soils. We also analyzed physico-chemical properties of effluent and changes in soil characteristics after the application of wastewater.

MATERIALS AND METHODS

EXPERIMENTS SETUP

The study was carried out in a wire-netting greenhouse Bahauddin Zakariya University, Multan, Pakistan (30.15° N, 71.30° E) under natural conditions, with temperature at 28±5°C, 12 h day time, and 38% relative humidity. *J. curcas* and *P. pinnata* were grown under two treatments: T1 = 20 mL effluent + 80 mL tap water, and T2 = 40 mL effluent + 60 mL tap water. The plants were applied with wastewater at 20 and 40 mL L^{-1}, while control plants (T0) were irrigated with tap water. A Completely Randomized Design with three replicates was used for experiment.

SITE AND SAMPLE COLLECTION

Effluent was collected from Khawaja Tanneries (Pvt.) Ltd., Multan, Pakistan (30.12° N, 71.27° E) during peak working hours of factory when discharge contains most of waste product (Figure 1). An ample amount of effluent was collected for experimental purpose. Prior to the experiment, effluent was tested for its physico-chemical properties following the APHA (2012). One-year old plants of *J. curcas* and *P. pinnata* of uniform size were obtained from a single local nursery in Multan during March 2016. The plants were then transplanted (one each) into 18 plastic pots (h × d: 25 × 22 cm) filled with 6 kg of a loamy sand soil. After being acclimatized for 8 weeks, plants were irrigated with effluent at a regular interval of 3 months from June 2016 to March 2017. After 12 months, the plants were harvested and the soil samples from the pots were mixed well and analysed. The physico-chemical analyses of soil were carried out by methods described in United States Salinity Laboratory Staff (1954).

PLANT ANALYSIS

To determine the impact of effluent on plant growth, morphometric measurements were done for various growth parameters: Root and shoot lengths (cm), collar diameter (cm^2), dry root and shoot biomass (g) and leaf area (cm^2). The impact of effluent on collar diameter and shoot length was measured in terms of percentage increase relative to control plants.

To evaluate the effect of effluent on physiological damages, chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents were determined by method of Lichtenthaler (1987). Samples were extracted in 80% ethanol and the absorbance was measured at 663, 645 and 480 nm using spectrophotometer (U-2900 Hitachi, Japan). The plant tissues (roots and shoots) were digested in 3:1 HNO_3:$HClO_4$ (v/v) solution. The digestion was carried out at 100°C using a Microwave Digestion System (MDS 2000, Canada). Total Cr concentration was determined with Atomic Absorption Spectrometer (Agilent 200 series, AA system California, USA). A blank digestion solution was included. The detection limit was 0.01 µg/L and the recovery rate was 96-98%.

ESTIMATION OF LIPID PEROXIDATION LEVEL

Lipid peroxidation and membrane damage were determined in terms of MDA concentration in roots and shoots followed method of de Oliveira et al. (2014) with minor modifications. Root and shoot samples (each 1.0 g) were homogenized in 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at (20000 × g) for 15 min. 3 mL of 0.5% thiobarbituric acid prepared in 20% TCA was added to 0.5 mL supernatant. The mixture was heated at 95°C in a shaking water bath for 50 min. The reaction was stopped by cooling the tubes in an ice water bath. Then, the samples were centrifuged at 10000 × g for 10 min, and the absorbance of the supernatant was read at 532 and 600 nm. The MDA concentration was calculated as the difference in absorbance at 600 and 532 nm using extinction coefficient of 156 mM^{-1} cm^{-1}.

STATISTICAL ANALYSIS

Data presented as mean (± S.E.) for each parameter was subjected to Analysis of Variance (ANOVA) to find out significant difference between species and among different effluent levels. The effects of effluent levels on plant species were compared separately for each species by using a post-hoc Tukey's HSD test. The level of significance for all the tests was at 5% ($p \leq 0.05$) using IBM SPSS Statistics (Version 21).

FIGURE 1. Map of Multan, Punjab, Pakistan showing site of location of Khawaja tanneries

Results and Discussion

Physico-chemical properties of the effluent

It became evident from the analysis of the effluent (brownish in colour) that it had high alkalinity, hardness, sodium, chloride, sulphate, calcium, magnesium, BOD, COD, TDS and TSS. It also contained heavy metals (Cr, Co, As and Cd) that were above permissible limits (Table 1). A high Cr concentration (98 mL L^{-1}) was reported in effluent as compared to other metal concentrations. The BOD, COD, TDS, TSS and Cr were considered major pollution parameters to define the strength of wastewater and were widely used to measure the quality of wastewater profile (APHA 2012). This represented the both biodegradable and non-biodegradable oxidizable pollutants discharged water from a tannery but the same findings have been reported for wastewater from other industries (Chaerun et al. 2011).

Impact of tannery effluent on soil properties

We found a significant ($p \leq 0.05$) change in physico-chemical properties of soil when irrigated with tannery effluent such as soil pH, EC, available phosphorus, available potassium, organic matter contents and Cr concentration (Table 2). pH value increased from control (8) to 20 mL L^{-1} (8.4) and 40 mL L^{-1} effluent (8.8) in soil samples. A significant increase of 47% and 79% in soil EC was observed at 20 and 40 mL L^{-1} effluent level, respectively, relative to soil used as control. This increase in soil pH after effluent treatment can be due to addition of exchangeable cations such as Na which had higher values (6000 mg L^{-1}) in effluent as compared to standard limit (200 mg L^{-1}) by WHO. Increase in soil EC might be due to release of salts following decomposition of organic matter in wastewater. Similarly, high salt concentrations in tannery effluent resulted in alkaline pH and increased EC of soil.

Irrigation with wastewater with high pH and EC could potentially increase the entry of K^+ into soil clay exchangeable complex and produce alkaline hydrolysis reactions. Such changes may alter the soil structure and affect the soil biological activities. A similar pattern of increase in soil pH and EC was reported by Rani et al. (2017) and Roohi et al. (2016). The available phosphorus increased by 80% and 98%, respectively, as compared to its control upon irrigation of soil with effluent at levels of 20 and 40 mL L^{-1}. The two levels of effluent enhanced

TABLE 1. Physical-chemical analysis of effluent collected from Khawaja tannery, Multan Pakistan

Water parameters	Concentrations (mg L^{-1})	Permissible Limit (WHO)	Heavy metals	Concentrations (mg L-1)	Permissible limit (USEPA)
Color	Brownish	Colorless	Magnesium	261	150
pH	6.32	6.5 to 8.5	Potassium	8	12
Temperature (°C)	28.2	25-40°C	Phosophorus	12.1	0.05
EC (mS/cm)	126.3	400	Calcium	1250	75
Alkalinity (mmol L^{-1})	14.4	NEQS	Chromium	98	1.0
Hardness (mg L^{-1})	4200	500	Manganese	1.0	1.5
Sodium (mg L^{-1})	6000	200	Nickle	0.32	1.0
Chloride (mg L^{-1})	6352	250	Cobalt	1.1	1.0
Sulfate (mg L^{-1})	7275	250	Cupper	0.1	1.0
*BOD (mg L^{-1})	285	80	Zinc	0.2	5.0
*COD (mg L^{-1})	411	150	Arsenic	0.35	1.0
*TDS (mg L^{-1})	18936	1000	Cadmium	0.12	0.1
*DO (mg L^{-1})	2.6	4-7			
*TSS (mg L^{-1})	2181	150			

*BOD (Biological oxygen demand), COD (Chemical oxygen demand), TDS (Total dissolved solids), DO (Dissolved oxygen), TSS (Total suspended solid), USEPA (United States Environmental Protection Agency) and NEQS (National Environmental Quality Standards)

TABLE 2. Overall mean values for physico-chemical properties of soil treated with effluent. T0 (control), T1 (20 mL L^{-1}) and T2 (40 mL L^{-1}). Each soil sample is across both plant species and three replicates with ±S.E. with n=3 for each species. Different letters at the top of each column depict significant variation between effluent levels at p≤0.05 using Turkey's HSD test

Soil characteristics	T0	T1	T2
pH	8.0 ± 0.09^a	8.4 ± 0.2^b	8.8 ± 0.04^c
Ec (mS/cm)	1.9 ± 0.03^a	2.8 ± 0.02^b	3.4 ± 0.09^c
Available phosphorus (mg L^{-1})	5.0 ± 0.01^a	9.0 ± 0.12^b	9.9 ± 0.9^c
Available Potassium (mg L^{-1})	110 ± 0.00^a	150 ± 0.00^b	190 ± 1.18^c
Organic matter (%)	0.43 ± 0.00^a	0.52 ± 0.00^b	0.79 ± 0.01^c
Chromium (mg L^{-1})	0.76 ± 0.01^a	0.94 ± 0.00^b	1.02 ± 0.03^c

available potassium by 36% and 73% than its control. The observed change in the available amount of phosphorus, potassium and organic matter contents in soil corresponded with higher levels of phosphorus and potassium in the industrial effluent and are in close conformity with Manzoor et al. (2015) and Shah et al. (2010).

Soil organic matter is an indicative of soil fertility. At 20 and 40 mL L^{-1} of effluent levels, organic matter content increased by 21% and 84%, respectively. An increase in soil organic matter after effluent treatment indicated accumulation of more carbon in soil. These findings are similar to that of Mobar et al. (2015). We also found 24% and 34% increase in soil Cr when irrigated with 20 and 40 mL L^{-1} of effluent levels, respectively. This increase in soil Cr showed an affirmation with high amount of Cr (98 mL L^{-1}) present in the effluent.

IMPACT OF EFFLUENT ON MORPHOLOGICAL PARAMETERS

Jatropha curcas and *P. pinnata* showed significantly different responses ($p \leq 0.05$) for their morphological parameters - root and shoot lengths, collar diameter, leaf area and plant dry biomass (root and shoot) (Figure 2). Root length was significantly declined by 58% at 40 mL L^{-1} while a 46% reduction was observed at 20 mL L^{-1} in *P. pinnata* when compared to their respective control. The reason for decrease in root length was due to presence of various salts and other elements such as sulphide and Cr in the growth medium. Further, roots are sensitive as remained in direct contact with all these pollutants. Moreover, excessive amount of sulphide greatly restricts the uptake of cationic micronutrients such as Cu, Fe, Mn and Zn, thus affecting root development (Dotaniya et al. 2014; Rekik et al. 2017). Another reason in decline of root length in *P. pinnat*a can be due to more metabolic activities of antioxidants (peroxidases) which deposit more lignin in the cell walls that diminish plasticity of cell wall, and subsequently inhibition in its elongation as reported in some *Vigna* species under heavy metal stress (Mahmood et al. 2016). However, *J. curcas* showed 42% enhancement in root length at 20 mL L^{-1} relative to 40 mL L^{-1} (Figure 2(a)). Manzoor et al. (2015) also provided evidences that more dilutions of the effluents consequently result in reduced concentrations of many harmful substances, including heavy metals. The impact of the application of diluted effluent also resulted in better root growth of *J. curcas* though Cr is not an essential metal for plant growth, but different species respond to it in a dose dependent pattern. Generally, Cr did not cause any negative impact when present in lower quantities in the growth medium but higher doses were found to be injurious to plant tissues (Martínez-Trujillo et al. 2014). Both the species sustained their shoot length when irrigated with both the effluent levels relative to the control. Though both species showed differential shoot length, *P. pinnata* exhibited longer shoots than *J. curcas* irrespective of extent of dilution of the effluent (Figure 2(b)). However, as far as major elements such as nitrogen, phosphorus, potassium and some other minor elements (Cu, Fe, Mn and Zn) are concerned, the effluent contained an ample quantity of these elements thus had no negative impact on shoot elongation. Rao et al. (2014) also reported parallel findings in some crop plants under similar regimes. Nevertheless, different species have shown distinct responses. Drastic effects of tannery effluent were reported for sunflower (Zereen et al. 2014) while a positive impact on *Eucalyptus camaldulensis* Dehnh. was described by Shah et al. (2010).

Collar diameter did not appear to be much influenced by varying levels of tannery effluent. Thus, the trait seemed to be insensitive to effluent treatments (Figure 2(c)). No significant change in collar diameter in response to other industrial effluent was reported by Manzoor et al. (2015) in *J. curcas* and *P. pinnata*. The two species exhibited differential responses for leaf area for which 49% expansion was found in *J. curcas* at 20 mL L^{-1} as compared to 40 mL L^{-1}. However, *P. pinnata* showed 38% reduction in leaf surface at the highest level of effluent (40 mL L^{-1}) than control plants. Thus, the foliage appeared to be the most sensitive to toxic substances present in the effluent (Figure 2(d)). These findings are in parallel to other reports (Bhatti et al. 2017; Gill et al. 2015). The two species showed a marked contrast for dry biomass of root and shoot when irrigated with different effluent levels. No significant reduction was observed for dry shoot biomass in *J. curcas* but an enhancement of 80% in dry root biomass was recorded at 20 mL L^{-1} compared to its respective control (Figure 2(e) & 2(f)).

An increase in the degree of root proliferation is well reported under several kinds of stress environment and is found to be associated with more root biomass. Root biomass particularly serves as an indicator of several underlying processes under diverse conditions of the rhizosphere. Thus, more proliferation and deposition of extra wall material might contribute towards greater root biomass. However, *P. pinnata* was found to be more prone to both effluent levels as 50% reduction in dry shoot biomass than its respective control. Dry root biomass increased by 90% at 40 mL L^{-1} of effluent relative to its control. Moreover, *P. pinnata* sustained its dry root biomass at 20 mL L^{-1}. A profound decline in dry shoot biomass can be attributed to increasing concentrations of Cr that is translocated from roots to shoots (Figure 4). Afzal et al. (2014) and Zereen et al. (2014) described that increase in levels of metals in soil and their subsequent translocation adversely influences transportation, balance and distribution of essential elements in different plant parts via competitive uptake.

An overall negative impact of effluent application was observed because it contained several metal ions in excessive concentration as similar findings reported by Martínez-Trujillo et al. (2014). Nevertheless, sustainable growth in terms of biomass (root and shoot) production became evident in *J. curcas*. The effluent level 20 mL L^{-1} posed no adverse effect on *J. curcas* but the quantities of N and P present in effluent pivotally promoted macromolecule biosynthesis (proteins and DNA). Silva et

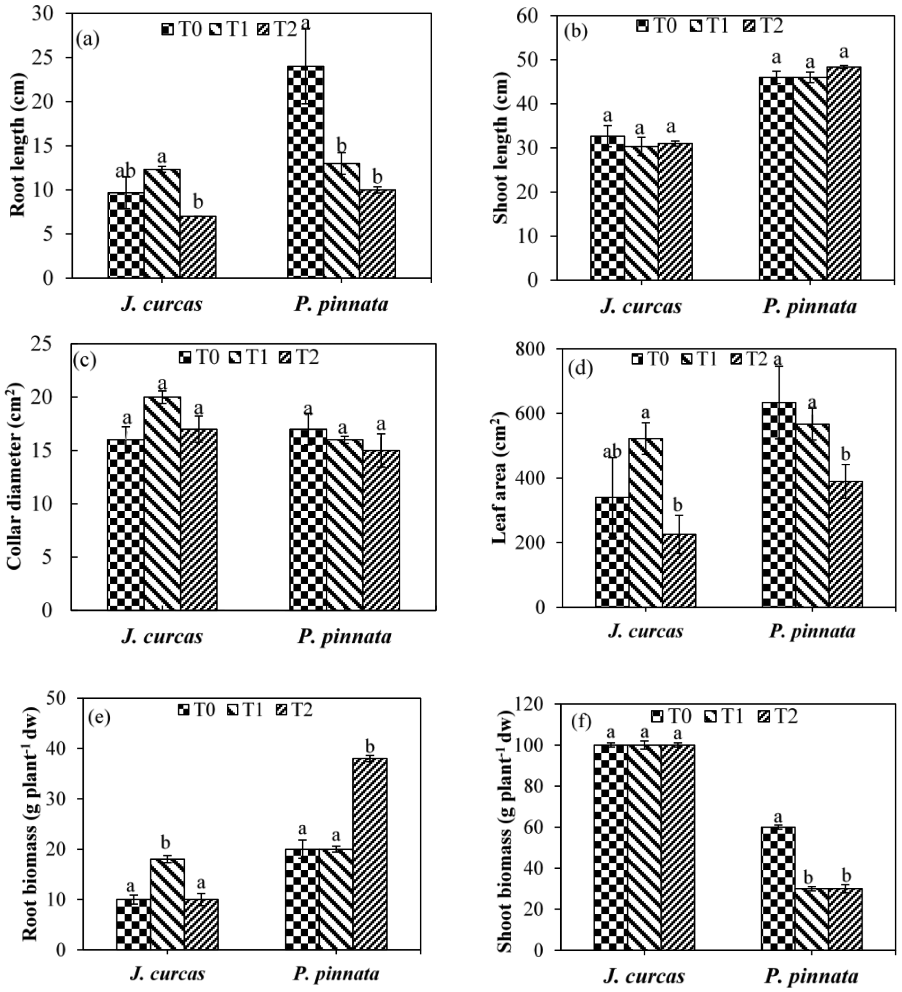

FIGURE 2. Effect of tannery effluent on morphological parameters of *J. curcas* and *P. pinnata*. (a) root length, (b) shoot length, (c) collar diameter, (d) leaf area, (e) root biomass and (f) shoot biomass. T0 (control), T1 (20 mL L^{-1}) and T2 (40 mL L^{-1}). The bars are the standard errors of means of three replicates. Different letters on the top of each column showed significant difference between the treatments at $p \leq 0.05$ using Turkey's HSD test. Treatments followed by same letters are not significantly different from each other according to Turkey's HSD test at $p \leq 0.05$

al. (2010) reported parallel findings in *Capsicum* species. Morphological predictors provide some indicators of successful survival of plants under abiotic stress, but the underlying genetic variability to cope with stress is mostly understood through protein expressions. Thus, effective means of stress mitigation are provided by such successful strategies that ultimately result in sustainable growth of plants (Rekik et al. 2017).

IMPACT OF EFFLUENT ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

The amount of chlorophyll *a*, *b* and total, carotenoids and MDA contents of the species showed significantly variable responses ($p \leq 0.05$) for chlorophylls and carotenoids production (Figure 3). *J. curcas* had 20% and 100% increase in chlorophyll *a* when irrigated with 20 and 40 mL L^{-1} of effluent, respectively. *P. pinnata* exhibited 88% reduction at the highest level of effluent (40 mL L^{-1}) (Figure 3(a)). Chlorophyll *b* was significantly increased by 9% at 20 mL L^{-1} but more drastic increase (95%) was observed at 40 mL L^{-1} in *J. curcas* (Figure 3(b)). On the other hand, the amount of chlorophyll *b* was reduced to an extent of 38% and 69% in *P. pinnata* at 20 and 40 mL L^{-1} of effluent, respectively. Overall, the pigment contents were greater in *J. curcas* that exhibited an increase of 14% and 30% at both effluent levels, correspondingly.

Again, *P. pinnata* showed its susceptibility for total chlorophyll which was profoundly declined up to 49% at 20 mL L^{-1} whereas this reduction was 81% at 40 mL L^{-1} (Figure 3(c)). With regard to carotenoids, a similar trend became evident for *J. curcas* which had an increase of 77% at 40 mL L^{-1} of effluent (Figure 3(d)) in contrast to *P. pinnata* which showed 78% reduction for carotenoids at an elevated effluent level (40 mL L^{-1}).

The chloroplast is always considered as a robust cellular entity protecting photosynthetically important pigment molecules (chlorophyll *a* and *b*), but our results clearly depicted a marked contrast between the two species, and it seems that the double membrane of the chloroplast of *P. pinnata* had different arrangement possibly because of the distinct genomes (both nuclear and chloroplast DNA) of the species and such comparisons are well reported in another plant species *Silene paradoxa* L. (Mengoni et al. 2001).

MDA production measured in root and shoot of *J. curcas* and *P. pinnata* is presented in Figure 3(e) & 3(f). Both the species exhibited higher concentrations of MDA in root and shoot as compared to the control plants. MDA contents dramatically increased by 84% in root and 67% in shoot in *J. curcas* when irrigated with 40 mL L^{-1} of effluent. However, enhanced MDA (95%) was observed for shoot at 40 mL L^{-1} in *P. pinnata*. The level of lipid peroxidation was greater in root of *J. curcas* while the reverse was true for *P. pinnata*. The results of MDA levels and pigment contents also conferred an affirmative relation between lipid peroxidation and chlorophyll biodegrading in *P. pinnata.* Besides genetic expressions, the changes in chlorophyll contents can be due to inhibitory effect of metals on chlorophyll biosynthesis, but acquisition of such response is a metal-plant specific interaction (Biswas et al. 2018). The results are in close conformity of Singh et al. (2013) and Tripathi et al. (2014) where several metal contaminates like Cr, As, Cd and Pb present in industrial effluent potentially induced lipid peroxidation, resulting in greater MDA, a potential biomarker for oxidative stress (Shahid et al. 2013).

Moreover, Cr induces more deleterious effects, including inhibition of leaf primordia, damage to chloroplast ultrastructure and decline in photosynthetic pigments (Shahid et al. 2017; Sinha et al. 2018). The greater quantity of Cr seems to induce more harmful effects in *P. pinnata* than *J. curcas*.

CR ACCUMULATION AND TRANSLOCATION

An appreciable amount of Cr (98 mL L^{-1}) than permissible limit (1.0 mL L^{-1}) was recorded in the effluent applied to plants of both species. Furthermore, Cr concentrations in plant tissues (roots and shoots) were expressed with respect to control plants (Figure 4). The amount of Cr in

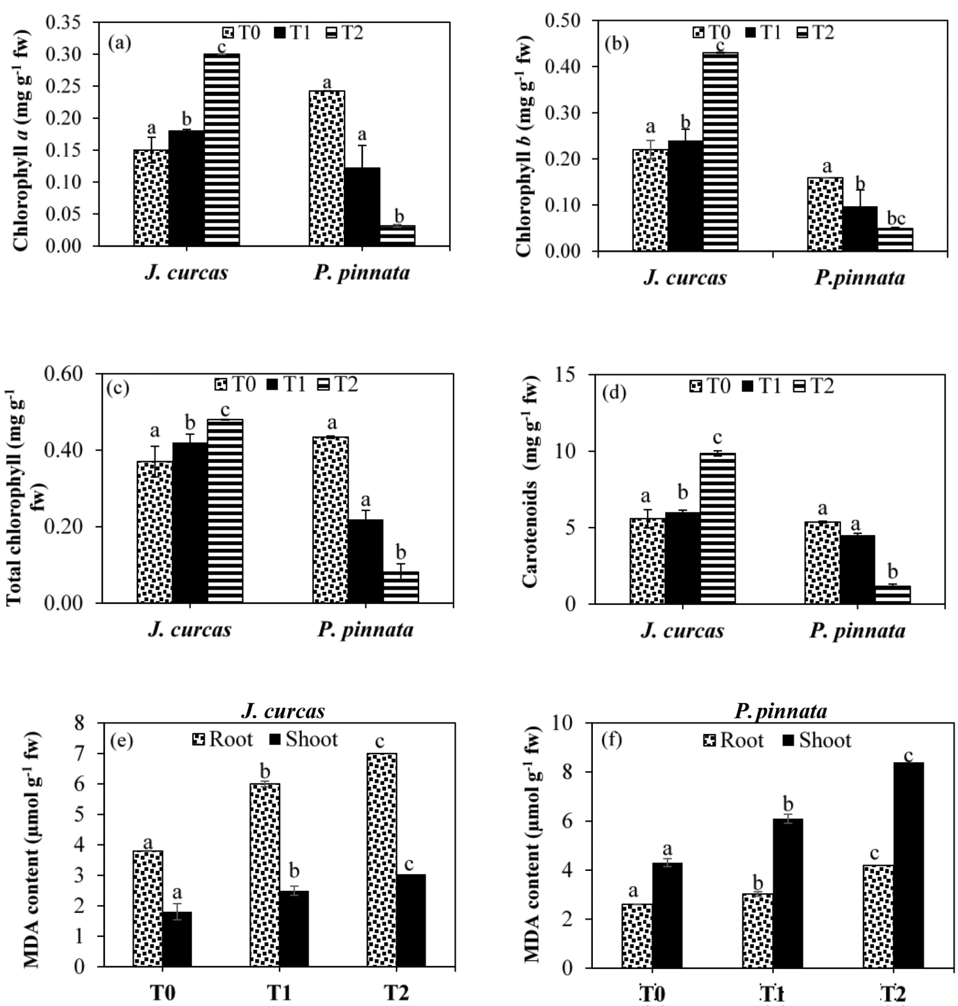

FIGURE 3. Effect of tannery effluent on biochemical parameters of *J. curcas* and *P. pinnata*. (a) chlorophyll *a*, (b) chlorophyll *b*, (c) total chlorophyll, (d) carotenoids, and (e and f) MDA content. T0 (control), T1 (20 mL L^{-1}) and T2 (40 mL L^{-1}). The bars are the standard errors of means of three replicates. Different letters on the top of each column showed significant difference between the treatments at $p \leq 0.05$ using Turkey's HSD test. Treatments followed by same letters are not significantly different from each other according to Turkey's HSD test at $p \leq 0.05$

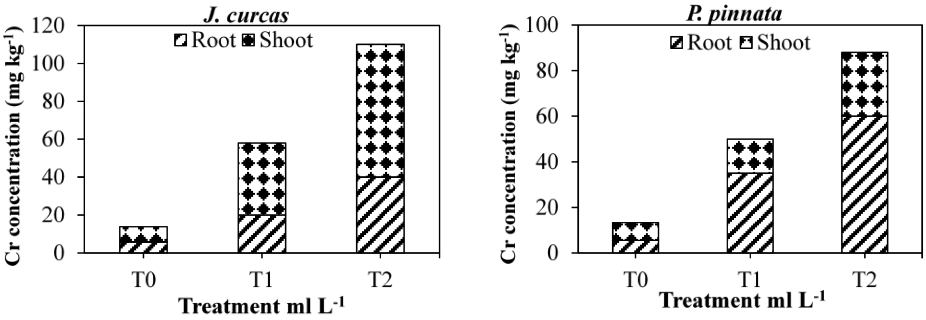

FIGURE 4. Cr concentrations in roots and shoots of *J. curcas* and *P. pinnata* irrigated with tannery effluent. T0 (control), T1 (20 mL L^{-1}) and T2 (40 mL L^{-1})

J. curcas was 3 and 5 folds in roots at 20 and 40 mL L^{-1} effluent, respectively, and 5 and 9 folds in shoots relative to their respective controls. *P. pinnata* had 11 times more Cr in roots at 40 mL L^{-1} while Cr concentration in shoots increased by only 4 folds at less diluted level of effluent. *J. curcas* showed more potential to accumulate Cr in shoots as compared to *P. pinnata,* thus the former species can be regarded as Cr accumulator. Previously it has been reported that *J. curcas* can accumulate 6000 mg kg^{-1} Cr in cell suspension (Bernabé-Antonio et al. 2015), but we noticed that *J. curcas* had 70 and 40 mg kg^{-1} Cr at above and below ground tissues, respectively. However, a lesser limit of Cr bioaccumulation (60 and 28 mg kg^{-1} in root and shoot, respectively) was observed in *P. pinnata*. The accumulation of Cr in *J. curcas* and *P. pinnata* again signifies a differential capacity of the tissues of the two species to accumulate Cr. This strategy is widely used to group different plant species as metal excluder or accumulators (de Oliveira et al. 2016). Thus, *P. pinnata* can be categorized among excluder while *J. curcas* as metal accumulator for Cr. Several plant species have been referred to as hyper accumulators as they possess the abilities to store much exceeding amount of metals in their parts than present in the medium, but hyper accumulator category cannot be established for Cr in the species. The Cr content in plant parts may cause alteration of some physiological processes due to over production of ROS, which in turn led to peroxidation of lipids and other biomolecules such proteins, enzymes and DNA (Shahid et al. 2017).

CONCLUSION

The combination of both growth parameters and bioaccumulation of Cr in plant tissues indicated differential potential of *J. curcas* and *P. pinnata* to tolerate exceeding amount of the metal. MDA production clearly signified development of oxidative stress in both the species, but robust nature of chloroplast and more biosynthesis of carotenoids (non-enzymatic antioxidant) in *J. curcas* might have provided some means of mitigating toxic effects of harmful substances present in effluent. On the other hand, *P. pinnata* showed a better threshold for shoot length, collar diameter and dry biomass of root, and it also accumulated more Cr at below ground tissue. However, it did not seem to alleviate metal stress as green pigment contents decreased without an increase in carotenoids. Differential metal translocation strategy of the two species can provide some justification to place *J. curcas* and *P. pinnata* among metal accumulator and metal excluder, respectively. Based on the performance of the two species, it can be safely concluded that *J. curcas* has better prospective for growth under irrigation of tannery effluent. This approach will allow efficient use of wastewater for growth of the species. *P. pinnata* can also serve as a good candidate, but only in situation when wastewater is loaded with moderate quantity of pollutants.

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