

Jatropha curcas L. and *Pongamia pinnata* L. Exhibited Differential Growth and Bioaccumulation Pattern Irrigated with Wastewater

(*Jatropha curcas* L. dan *Pongamia pinnata* L. Mempamerkan Pertumbuhan Pembezaan dan Corak Bioakumulasi yang Diairkan dengan Air Buangan)

RAFIA ABID*, SEEMA MAHMOOD, SAMAN ZAHRA, SHAZIA GHAFFAR, MAHWISH MALIK & SIBGHA NOREEN

ABSTRACT

Pakistan currently faces an acute shortage of water, which has increasingly been devastating for the past few decades. In order to mitigate water scarcity, agriculture sector of the country started using wastewater discharged from various industries. The present study aims to assess the impact of fertilizer industry effluent on *Jatropha curcas* L. and *Pongamia pinnata* L., which are popular biofuel tree species. Initially, one-year-old saplings were acclimatized in pots, then wastewater was applied in diluted concentrations of effluent using 20 and 40 mL L⁻¹ to the treatment group while control plants were irrigated with tap water. The physico-chemical properties of the effluent showed high values 179 mg L⁻¹ for biological oxygen demand (BOD), 257 mg L⁻¹ for chemical oxygen demand (COD) and 1200 mg L⁻¹ for total dissolved solid (TDS), respectively. Surprisingly, high concentrations of arsenic (15 µg L⁻¹) and cadmium (0.78 mg L⁻¹) were present but chromium (Cr) concentration was found within permissible limit to WHO. The levels applied caused a significant ($p \leq 0.05$) increase in plant growth and biomass. The extent of membrane damage assessed via malondialdehyde (MDA) production was also greater in the roots of *P. pinnata* while reverse was true for shoots of *J. curcas*. A more profound ($p \leq 0.05$) reduction in photosynthetic pigments and carotenoids was observed in *P. pinnata* at concentrated level of effluent. Overall, the study signifies a 2-folds potential of biofuel tree species for efficient reuse of wastewater, as well as for remediation of metals from wastewater and soil.

Keywords: Heavy metals; *Jatropha curcas*; lipid peroxidation; photosynthetic pigments; *Pongamia pinnata*

ABSTRAK

Pakistan baru-baru ini menghadapi kekurangan air yang teruk dan ia semakin teruk sejak beberapa dekad yang lalu. Bagi mengatasi kekurangan air, sektor pertanian di negara ini mula menggunakan air buangan yang dikeluarkan daripada pelbagai industri. Kajian ini bertujuan untuk menilai kesan efluen industri baja pada *Jatropha curcas* L. dan *Pongamia pinnata* L. yang merupakan spesies pokok bahan api biologi yang terkenal. Pada mulanya, anak pokok berusia satu tahun diaklimat dalam pasu, kemudian air sisa limbah digunakan dalam kepekatan efluen yang cair dengan menggunakan 20 dan 40 mL L⁻¹ pada kelompok rawatan manakala tanaman kawalan diairi dengan air paip. Sifat fisiko-kimia efluen menunjukkan nilai yang tinggi masing-masing sebanyak 179 mg L⁻¹ untuk permintaan oksigen biologi (BOD), 257 mg L⁻¹ untuk permintaan oksigen kimia (COD) dan 1200 mg L⁻¹ untuk jumlah pepejal terlarut (TDS). Anehnya, kepekatan arsenik (15 µg L⁻¹) dan kadmium (0,78 mg L⁻¹) yang tinggi hadir tetapi kepekatan kromium (Cr) dijumpai dalam had yang dibenarkan kepada WHO. Tahap yang digunakan menyebabkan peningkatan ($p \leq 0.05$) pertumbuhan tanaman dan biojisim yang ketara. Tahap kerosakan membran yang dinilai melalui pengeluaran malondialdehid (MDA) juga lebih besar pada akar *P. pinnata* manakala sebaliknya berlaku untuk pucuk *J. curcas*. Pengurangan pigmen fotosintesis dan karotenoid yang lebih mendalam ($p \leq 0.05$) diperhatikan pada *P. pinnata* pada tahap efluen yang pekat. Secara keseluruhan, kajian ini menunjukkan potensi spesies pokok bahan api biologi sebanyak 2 kali ganda untuk penggunaan semula air buangan yang berkesan, serta untuk pemulihan logam dari air buangan dan tanah.

Kata kunci: *Jatropha curcas*; logam berat; peroksidasi lipid; pigmen fotosintesis; *Pongamia pinnata*

INTRODUCTION

Water is the most valuable resource on planet earth. Like many other densely populated countries of the world, Pakistan is also facing the challenge of water scarcity for

crop production. Pakistan has been declared as the most water scarce countries of the world (Arif et al. 2018). In order to combat acute water shortage and high cost of fertilizers, the cooperative farmers have started to use

industrial wastewater for crop production. In addition, the use of chemical fertilizers becomes necessary owing to deficiency of various essential nutrients in the soils of the country. Most arable soils are mostly calcareous and are deficient in macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) as well as micronutrients such as boron (B) and zinc (Zn) (Rehim et al. 2016). Nutrient balance in soil is maintained by integrated nutrient approaches such as crop rotation, green manuring, microbes/biofilterizers but preference has always been given to the use of chemical fertilizers owing to subsidized cost, particularly in the absence of any kind of tax both for industry and consumer (Iqbal et al. 2015).

The global market for chemical fertilizers is expected to be 151.8 billion USD and major investments for their production have been done in Pakistan. Despite substantial earnings by this sector, all production units are major contributors of various types of contaminants that are thrown into the environment without following EPA standards (Abid et al. 2019; Manzoor et al. 2015). Almost all industrial effluents are heavily loaded with several contaminants having high biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solid (TDS), total suspended solid (TSS) and heavy metals (Younas et al. 2017) like arsenic (As), cadmium (Cd), and chromium (Cr) which are of serious concern (Ronzan et al. 2017). The PAK-EPA surveys reported more alarming figures for As in ground water and found 12-488 ($\mu\text{g L}^{-1}$) As concentration in drinking water in Tehsil Mailsi, Punjab. These amounts are far greater than the permissible limit ($10 \mu\text{g L}^{-1}$) by WHO (Rasool et al. 2017). Afzal et al. (2014) reported enhanced concentrations of Cd (37 mg kg^{-1}) and Cr (2443 mg kg^{-1}) in soils of Kasur, Punjab, Pakistan where industrial wastewater was used for irrigation.

The As, Cd, and Cr are toxic metals of co-existence in nature (Ronzan et al. 2017). As is ranked at 1st, Cd at 7th and hexavalent Cr (VI) at 17th positions among the hazardous pollutants by ATSDR (2017). The As, Cd and Cr VI are carcinogenic and neurotoxic, and cause respiratory depression in humans (ATSDR 2017). The major dilemma associated with heavy metals is their persistence, non-biodegradability and bioaccumulation in plants (Chang et al. 2014) causing considerable decline in crop productivity leading to food security issues (Shahid et al. 2017) and risk to humans through food chain contamination (Zare et al. 2018).

Mitigation of soil contamination involves some expensive techniques such as chemical amendment, physical, and mechanical methods. However, in the last two decades, low cost and environmentally friendly techniques for removal of heavy metals from ecosystem called phytoremediation/bioremediation have received considerable attention (Dadea et al. 2017). Using woody plants/species such as *Ailanthus altissima*, *Betula pendula*,

Robinia pseudoacacia on contaminated soil showed great prospective for phytoremediation due to their high growth rate, high transpiration rate, deep root systems, high biomass and capacity to accumulate metals in their shoots (Constán-Nava et al. 2010; Dadea et al. 2017). Though metal tolerance has been reported from a wide range of wild and cultivated plant species including trees, shrubs and herbs that have potential to extract metal from soil (Luo et al. 2016) but these reports clearly demonstrate that herbaceous cultivated species have greater sensitivity for toxic levels of metals than woody shrubs and trees (Wang et al. 2016). Nevertheless, for both tolerance and sensitivity, the underlying physiological and biochemical mechanisms govern the ultimate expression of the plants. This involves inhibition of chloroplast biosynthesis, disturbance of electron transport chain, photosynthesis, water uptake, oxidation of amino acids, damage to biological membranes and DNA and RNA via oxidative stress (Ronzan et al. 2017).

Although extensive work has been carried out to explore the potential of trees regarding As, Cd and Cr contamination through tolerance mechanism (Dadea et al. 2017; Hussain et al. 2017), but a little effort has been done so far to discover the responses of long-lived biofuel species such as *Jatropha curcas* and *Pongamia pinnata*. Keeping in view the growing concerns on As, Cd, and Cr and the exploitation of some biofuel species for remediation purposes, the aim of the present study was to assess the growth and biochemical responses of the two biofuel yielding species and the pattern of metal accumulation in their tissues in addition to changes in physico-chemical properties of soil after the application of effluent from a fertilizer factory containing substantial amount of As, Cd, and Cr, and then to evaluate their phytoremediation potential.

MATERIALS AND METHODS

SAMPLE COLLECTION AND EXPERIMENTAL DESIGN

The effluent (35 L) was collected from Pakarab Fertilizer (Pvt.) Ltd., Multan, Punjab, Pakistan during peak working hours of the factory. The effluents were drained into main Kacha canal through water courses and drainage system without any pretreatment. Prior to the growth experiment, the effluent was analyzed for physico-chemical parameters such as pH, temperature, electrical conductivity (EC), biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solid (TDS), dissolved oxygen (DO), total suspended solid (TSS) and sulphates following the standard protocols by American Public Health Association (APHA 2012). Total As, Cd, and Cr concentration in the effluent was determined with Atomic Absorption Spectrometer (AAS) Agilent 200 Series, AA

System California, USA. In March 2016, one-year-old uniform saplings of *J. curcas* and *P. pinnata* obtained from a local nursery were transplanted into plastic pots (h × d: 25 × 22 cm) filled with 6 kg of a sandy loam soil.

The experiment with three replicates for each treatment was placed in a Complete Randomized Design (CRD) in a wire netting greenhouse with 28±2 °C, 12 h day time, and 38% relative humidity. The effluent dilutions (20 and 40 mL L⁻¹) were applied as treatments, control pots were irrigated with tap water. After acclimatization of plants for 8 weeks, each pot/plant was irrigated with 2 L for each treatment after regular interval of 3 months from June 2016 to June 2017. Effluent was applied three times in year. Water loss was compensated by gentle sprinkling of tap water when required.

After 12 months exposure of effluent, the plants were harvested and soil samples were collected from rhizospheric zone. Physico-chemical analysis of soil (pH, EC, total nitrogen %, available potassium, available phosphorus and soil texture) was carried out using laboratory manual (US Salinity Laboratory Staff 1954). Wet double acid digestion for soil and plants was carried out with 4:1 HNO₃:HClO₄ (v/v) following Allen et al. (1986) at 100 °C by using Microwave Digestion System (MDS 2000, Canada). Total As, Cd, and Cr contents in soil and plant tissues were determined with Atomic Absorptions Spectrometer (Agilent 200 Series, AA System California, USA). The minimum detection limit was 0.01, 0.0001 and 0.1 ppm for As, Cd, and Cr, respectively, and the recovery rate was 98% for As, 96% for Cd and 101% for Cr. The range of detection limit was 0.01-2 ppm for As, 0.0001-10 ppm for Cd and 0.01-100 ppm for Cr. The accuracy of the analytical method was confirmed by the determination of the content by AA standards for As, Cd and Cr (Camlab, U.K.) to estimate their total amounts in plant tissues. The AsIII concentration was determined by the use of arsenic speciation cartridge (Waters SPE cartridge) for wastewater samples and AsV concentration was calculated by subtracting AsIII from total As following Mathews et al. (2010) through SPE cartridge for speciation of AsV and AsIII.

MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS

Plant growth attributes such as root and shoot elongation, dry biomass of root and shoot, leaf area, number of leaves per plant and collar diameter were measured after the application of effluent. The standard protocols were used for each parameter for consistent measurements (Abid et al. 2019). Photosynthetic pigments (chlorophyll a, b and total) and total carotenoids (Cx+c) were determined as described by Lichtenthaler (1987).

ESTIMATION OF LIPID PEROXIDATION LEVELS IN PLANT TISSUES

The concentration of MDA in root and shoot was measured by following de Oliveira et al. (2014) with minor modifications. The MDA concentration was calculated as the difference in absorbance at 600 and 532 nm using extinction coefficient of 156 mmol⁻¹ cm⁻¹.

STATISTICAL ANALYSIS

Data presented as means of three replicates (±S.E) for each parameter was subjected to Two Way Analysis of Variance (ANOVA) to elucidate the effect of effluent (12 month treatment) on the growth of species. Mean values were compared separately by using a post-hoc Tukey's HSD test. The level of significance for all parameters was set at p≤0.05 using statistical software (SAS Version 13.1 Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

After 12 months of exposure with 20 and 40 mL L⁻¹ of effluent, morpho-biochemical parameters, MDA concentration, As, Cd, and Cr concentration were measured in *J. curcas* and *P. pinnata* relative to their respective control plants. Physico-chemical properties of the effluent and its impact on soil characteristics were also assessed.

PHYSICO-CHEMICAL PROPERTIES OF EFFLUENT

Analysis of physico-chemical properties of effluent revealed light yellowish colour and high temperature of the effluent (Table 1). The concentrations of EC, BOD, COD, TDS, TSS, total As, Cd, P, and N were higher than permissible limit by World Health Organization (WHO) for irrigation water (Table 1). However, DO, chloride (Cl), potassium (K), sodium (Na) and sulphate (SO₄²⁻) were found to be below while pH, arsenite (AsIII), arsenate (AsV), and chromium (Cr) were within safe limit of WHO. The cumulative results for BOD, COD, TDS, TSS, total As, and Cd served as major pollution indicators to depict strength of effluent as per quality profile of wastewater (APHA 2012). These predictors represented both biodegradable and non-biodegradable oxidizable pollutants in the wastewater discharge from the factory.

Chemical characterization of the effluent from various industrial units have been carried out by other workers (Arif et al. 2016; Biswas et al. 2018; Roohi et al. 2016). Their reports conferred consistent results for quality parameters of wastewaters. The characterization of the colour is an important parameter because the strength of coloration strongly suggests impurities present

in wastewater and its treatment for reuse. The yellowish colour of effluent has been reported to contain more dissolved salts and other substances. Our findings are in parallel to Afzal et al. (2014) and Imtiazuddin et al. (2014) describing strong coloration of wastewater from tannery and textile industries. The industrial wastewater discharged with high temperature than permissible limit set by WHO can be a potential cause for thermal pollution of water bodies that can lead to increase BOD and decrease DO by the aquatic organism for oxygen. Our result on temperature is parallel to the findings of Patel et al. (2015) who reported similar increase in temperature of effluent discharged from other textile units. The higher EC alters the chelating properties of wastewater system, which create conditions for free metal availability to flora and fauna (Younas et al. 2017). More AsV contents had been detected than AsIII. Shakoor et al. (2015) found more AsV concentration and attributed it to continuous pumping of groundwater that oxidize metal elements present in subsurface sediment and aquifers. In our study, As pollution levels in the effluents

exhibited a similar pattern as that of groundwater that is more concentration of AsV than AsIII, since the source of water used by industrial process is groundwater. The data regarding As speciation in wastewater has not been reported so far, but Brahman et al. (2014) and Shakoor et al. (2015) carried out As speciation in the groundwater of Punjab and Sindh Provinces of Pakistan. Our results are in parallel to Shakoor et al. (2015) for more AsV contents than AsIII. However, our findings are contrary to Brahman et al. (2014) who reported more concentration of AsIII than AsV in groundwater in District Tharparker, Sindh. Thus, parallel existence of both forms of As (III and V) in groundwater as well as in effluent discharged from fertilizer factory can be established from this study. However, N and P in the fertilizer effluent found can be beneficial for plant growth as reported by Kumar et al. (2017b). But on the other hand, more N and P have been described to produce nitrophos (Younas et al. 2017). Thus, dilution of effluent reduced its toxicity level.

TABLE 1. Physical-chemical properties of effluent collected from Fertilizer Factory, Multan, Pakistan

Water parameters	Values	Permissible limit (WHO)
Physico-chemical analysis		
Color	Light yellowish	Colorless
pH	7.6	6.5 to 8.5
Temperature (°C)	30	<30 °C
EC (mS/cm)	1.324	0.4
BOD (mg L ⁻¹)	179	80
COD (mg L ⁻¹)	257	10
DO (mg L ⁻¹)	2.6	4-7
TDS (mg L ⁻¹)	1200	1000
TSS (mg L ⁻¹)	274	150
Cl ⁻	14	250
K ⁺ (mg L ⁻¹)	5.5	12
Na ⁺ (mg L ⁻¹)	129	200
Total N (mg L ⁻¹)	59	2.5
Total P (mg L ⁻¹)	0.97	0.05
SO ₄ ²⁻ (mg L ⁻¹)	240	500
Metals		
Total As (µg L ⁻¹)	15	10 (µg L ⁻¹)
AsIII (µg L ⁻¹)	5	10 (µg L ⁻¹)
AsV (µg L ⁻¹)	10	10 (µg L ⁻¹)
Cd (mg L ⁻¹)	0.78	0.003
Cr (mg L ⁻¹)	0.04	0.05
Hg (mg L ⁻¹)	BDL	
Pb (mg L ⁻¹)	BDL	

EC (Electrical Conductivity), BOD (Biological oxygen demand), COD (Chemical oxygen demand), TDS (Total dissolved solids), DO (Dissolved oxygen) and TSS (Total suspended solid) and WHO (World Health Organization)

PHYSICAL-CHEMICAL PROPERTIES OF SOIL AFTER
FERTILIZER EFFLUENT APPLICATION

The changes in soil properties after the application of effluent provided were observed (Table 2). The pH of soil was found to be alkaline ranging at 8.4-9.4. The high pH of soil after effluent treatment is an indication of more accumulation of cations such as P, Ca, and Mg that changed the pH. Our findings are similar to that of Hossain et al. (2015) who found changes in soil pH after irrigation with industrial effluent. More alkaline pH of the soil can be attributed to various salts present in the effluent. A significant increase in soil EC, 16-93% for *J. curcas*, was noticed at 20 and 40 mL L⁻¹ of effluent, respectively. For *P. pinnata* 82-183% increase in soil EC was observed at respective levels of effluent. Increase in soil EC signifies increase in soil salinity. Higher soil salinity is an indication of more ions present in the effluent that contributed increase in soil EC. The increase in soil EC may also be attributed to pH modification and nutrients availability in soil after the length of exposure (Table 2). Our results are in lines with Ahmad et al. (2012) who reported more increase in soil EC treated with industrial effluent. Irrigation with wastewater having high pH and EC could potentially increase soil exchangeable complexes and alkaline hydrolysis reactions (Chaerun et al. 2011). All such changes also alter soil structure and can affect the soil microflora.

In addition to soil pH and EC, soil organic matter (OM) is an indicative of soil fertility and appeared to be of particular interest for study carried out for heavy metals sorption in soil as it affects the mobility of heavy metals by forming insoluble and soluble organic complexes (Alamgir 2016). At elevated level of effluent, OM content increased 138-182% in soil samples collected from *P. pinnata* and *J. curcas* as compared to their respective controls. An increase in soil OM might be due to long term

application of effluent that resulted decay in plant parts and nematode decay that served as the source of carbon. Our findings are comparable to Mobar et al. (2015) who reported more OM in soil when source of irrigation was textile effluent. Maurya (2017) also observed similar increase in soil OM content in sandy loam soil with alkaline pH. OM improves water retention capacity of sandy and sandy loamy soil and also provide suitable conditions to enhance soil microflora. The soil used for this study was also sandy loam with alkaline pH thus exhibited the above properties. Further, sandy soil poses lesser restriction and is ease for root penetration that results in extensive development of underground tissue system while loamy soil is advantageous because of their higher nutrient retention properties, owing to more clay particles which possess negative charges and bind macronutrients with positive charge. This binding increases the fertility of the soil, thus it is considered as the best medium for plant growth. Issa et al. (2009) also provided similar arguments for varied types of soil textures. Our results depicted that available P, K, and total N (%) increased in soil when irrigated with 20 and 40 mL L⁻¹ effluent as compared to control plant soil samples. However, a maximum increase (10.1%) in N at 40 mL L⁻¹ effluent level and available K and P (K; 260 and P; 2.73 mg kg⁻¹) at 20 mL L⁻¹ effluent level. The observed change in available amount of P and K and total N contents in soil corresponded with higher levels of K, N and P in the industrial effluent is in close conformity with Arif et al. (2016) and Rani et al. (2017) who reported more K, N and P contents in soil irrigated with different dilutions of industrial effluent. Furthermore, these studies documented that increase in soil pH and EC promotes the more concentrations of K, P and N, and these findings are consistent with the results of this study and more pH and EC values after the application of fertilizer wastewater.

TABLE 2. Changes in soil properties and metal concentrations (As, Cd and Cr) under varying levels of effluent from a fertilizer factory

Soil parameters	<i>J. curcas</i>			<i>P. pinnata</i>		
	T0	T1	T2	T0	T1	T2
pH	7.5±0.09 ^a	8.90±0.09 ^b	9.20±0.07 ^c	7.00 ±0.06 ^a	8.40±0.06 ^a	9.4 ±0.12 ^b
EC (mS/cm)	2.70±0.04 ^a	3.13±0.08 ^b	5.21±0.07 ^c	1.73±0.01 ^a	3.14±0.03 ^b	4.90±0.1 ^c
Organic matter (%)	0.66±0.03 ^a	1.39±0.06 ^b	1.86±0.09 ^{ab}	0.96±0.02 ^a	1.09±0.02 ^b	2.28±0.02 ^c
Total N (%)	0.29±0.0 ^a	9.70±0.02 ^b	8.90±0.01 ^c	0.94±0.03 ^a	9.60±0.02 ^b	10.1±0.03 ^b
Available K (mg kg ⁻¹)	100±4.04 ^a	260±7.2 ^b	140±6.00 ^c	120±3.02 ^a	120±5.57 ^a	140±5.57.7 ^b
Available P (mg kg ⁻¹)	0.26±0.01 ^a	2.73±0.09 ^b	0.14±0.02 ^c	0.96±0.02 ^a	0.16±0.02 ^b	0.20±0.02 ^b
As (mg kg ⁻¹)	1.20±0.01 ^a	13.90±0.01 ^b	13.80±0.01 ^b	14.40±0.01 ^a	13.80±0.01 ^b	13.60±0.01 ^b
Cd (mg kg ⁻¹)	0.66±0.01 ^a	1.25±0.01 ^b	0.85±0.01 ^{ac}	0.94±0.01 ^a	0.81±0.01 ^a	1.03±0.02 ^b
Cr (mg kg ⁻¹)	0.88±0.01 ^a	0.92±0.02 ^b	0.84±0.01 ^a	0.63±0.01 ^a	0.72±0.02 ^b	0.79±0.01 ^b

Control (T0), 20 mL L⁻¹ (T1) and 40 mL L⁻¹ (T2). Values are expressed as mean±S.E with n=3. Different letters at the top of each column depict significant variation between effluent levels at p≤0.05 using Turkey's HSD test

The concentrations of As (13.80 mg kg⁻¹), Cd (1.25 mg kg⁻¹) and Cr (0.92 mg kg⁻¹) were found greater in soil samples of *J. curcas* at more diluted level as compared to control plant soil samples. While *P. pinnata* had higher concentration for Cd (1.03 mg kg⁻¹) and Cr (0.79 mg kg⁻¹) after the application of less diluted level than control soil samples (Table 2). Higher concentrations of metals in the soil might be due to irrigation of soil with wastewater. Furthermore, these metals are naturally occurred in soil and are non-biodegradable and remain persistent in the soil. Parallel findings reported by Rekik et al. (2017) who found more concentrations of As, Cd and Cr in soil after the long term application of industrial effluents.

MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS IN *J. curcas* AND *P. pinnata*

After the application of effluent, changes were observed for morpho-biochemical expressions of *J. curcas* and *P. pinnata*. Both species exhibited a significant ($p \leq 0.05$) increase in root length, shoot length, collar diameter, root and shoot biomass, number of leaves, and leaf area at 20 mL L⁻¹ (Figure 1). Root length was more pronounced in *J. curcas* which showed 70% increase then 21% enhancement in *P. pinnata*. *J. curcas* exhibited 17% reduction in root length when treated with 40 mL L⁻¹ effluent (Figure 1(a) and 1(b)). However, shoot elongation increased 30% and 36% in *J. curcas* and *P. pinnata*, respectively. While shoot dry biomass increased 58% in *J. curcas* and only 22% elongation in root dry biomass for more diluted level than their respective control (Figure 1(g) and 1(h)). Both species also sustained growth at an elevated level (40 mL L⁻¹) of effluent but only *J. curcas* showed significant increase for dry root biomass extent and root collar diameter relative to the control plants (Figure 1(c) and 1(h)). Number of leaves per plant increased 2-3 folds in both species at less diluted level (40 mL L⁻¹) relative to their respective control (Figure 1(f)). At 40 mL L⁻¹, leaf surface also expanded 32 and 33% in *J. curcas* and *P. pinnata* in comparison to their control plant counterparts (Figure 1(e)).

Enhanced growth, dry biomass production and greater leaf area indicate the sustainable growth of the species under effluent application that contains many essential macro and micronutrients, including NPK (Table 1; Figure 1(g) and 1(h)). Nitrogen being important component of proteins, cellular material, DNA and RNA might have contributed towards growth and biomass enhancement of the species. Thus, as such many principal nutrients have resulted positive impact on health and vigor of the plant particularly for foliage attributes (Kumar et al. 2017b). Similarly, P and K have a beneficial influence because of

the pivotal role for many metabolic activities of plants. The positive impact of NPK was also well documented for *J. curcas* and *P. pinnata* where application of fertilizers caused greater biomass production of the species (Kumar et al. 2017b; Manzoor et al. 2015).

Although no significant reduction in chlorophyll b was found in *J. curcas* at more diluted level of the effluent, however a higher concentration of effluent caused a profound reduction in chlorophyll a, b, and total in addition to carotenoids in both species (Figure 2). The presence of ions including heavy metals in the effluent might have substituted Mg²⁺ ions in the chlorophyll molecules. Metal ions are able to decrease the activity of -SH group of enzymes that result in formation of heavy metal complexes with chlorophyll, thus decreased biosynthesis of chlorophyll and other pigments. An early senescence of leaves with chlorosis induced by metal stress is well documented for decline in photosynthetic pigments (Rekik et al. 2017; Shah et al. 2010). Nevertheless, the robust nature plastids and their structural modifications as orientation and self-replicating properties make them more resistant to environmental stresses including heavy metal.

Effluent impacted the TBARS concentration in roots and shoots of the species than their respective controls. TBARS production in terms of malondialdehyde (MDA) is used as measure of oxidative stress through ROS generation and is dependent on the extent of stress. An increase in MDA by 4 and 2.5 folds in roots, and 3 and 7 folds in shoots of *J. curcas* and *P. pinnata* were recorded at 40 mL L⁻¹ effluent than control plants that signified generation of ROS (Abid et al. 2019). The increase in MDA concentrations indicates that plants can tolerate stress caused by effluent and heavy metals due to the activation of enzymatic and non-enzymatic anti oxidative defense systems of plants which cater for oxidative stress in the plants (Kandziora-Ciupa et al. 2016).

METAL ACCUMULATION POTENTIAL OF *J. curcas* AND *P. pinnata*

The concentrations of As, Cd, and Cr were determined in plant parts after the application of effluent in varying dilutions (Table 3). The ability of plants to translocate metals from below ground tissues to aerial parts is due to translocation factor (Baker 1981). A consistent pattern of arsenic (As) accumulation was recorded in both species, and it was significantly ($p \leq 0.05$) higher in the shoot (Table 4). However, *J. curcas* showed greater As concentration (8.48 mg kg⁻¹) as compared to *P. pinnata* (8.18 mg kg⁻¹) in shoots at elevated level of

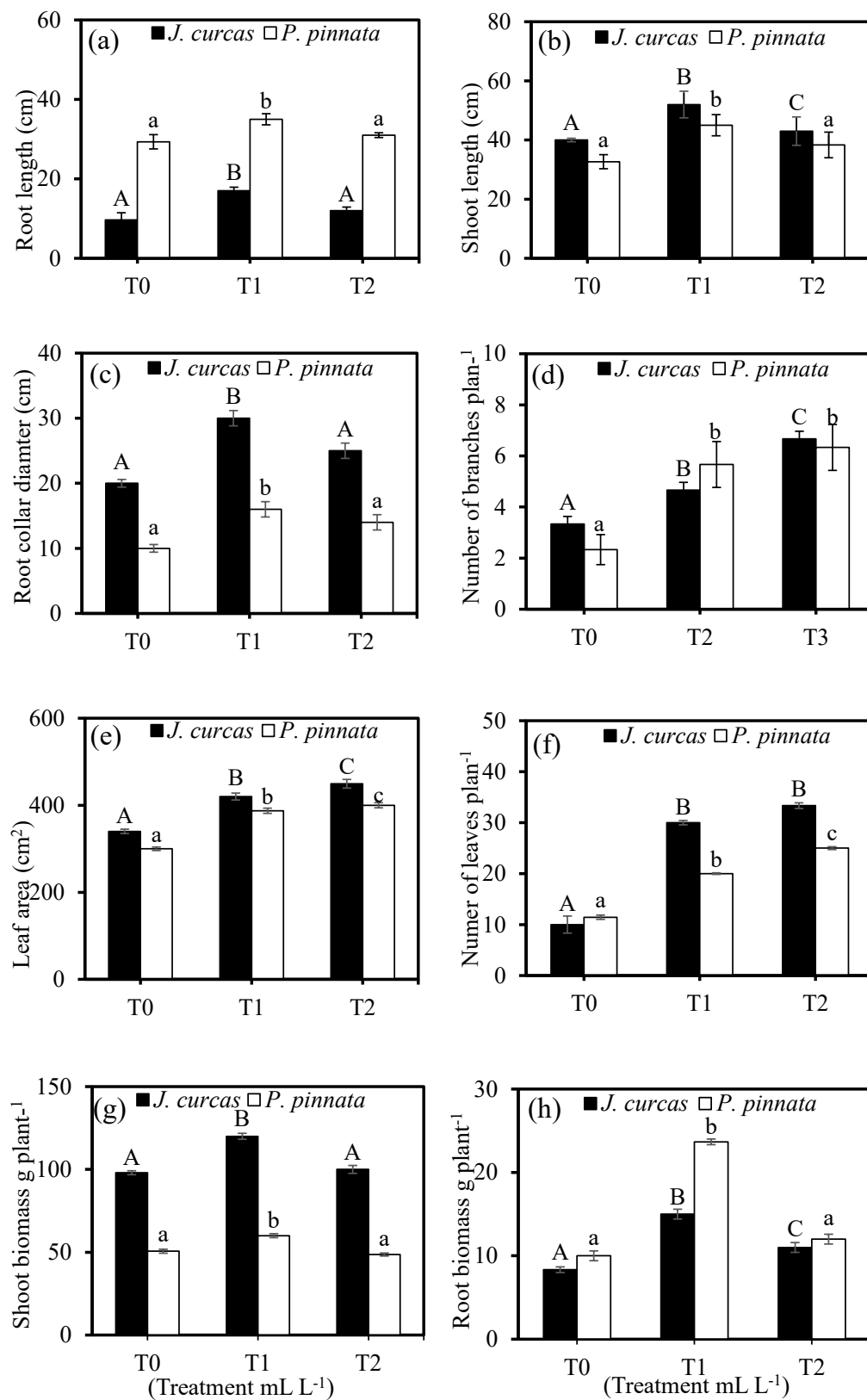


FIGURE 1. Morphological parameters of *J. curcas* and *P. pinnata*, irrigated with effluent levels; control (T0), 20 mL L⁻¹ (T1) and 40 mL L⁻¹ (T2). Data presents mean±S.E. dw=dry weight. Mean comparisons for each plant species were done separately and indicated by uppercase and lower case letters. Different letters at the top of each column depict significant variation between effluent levels at p≤0.05 using Turkey's HSD test

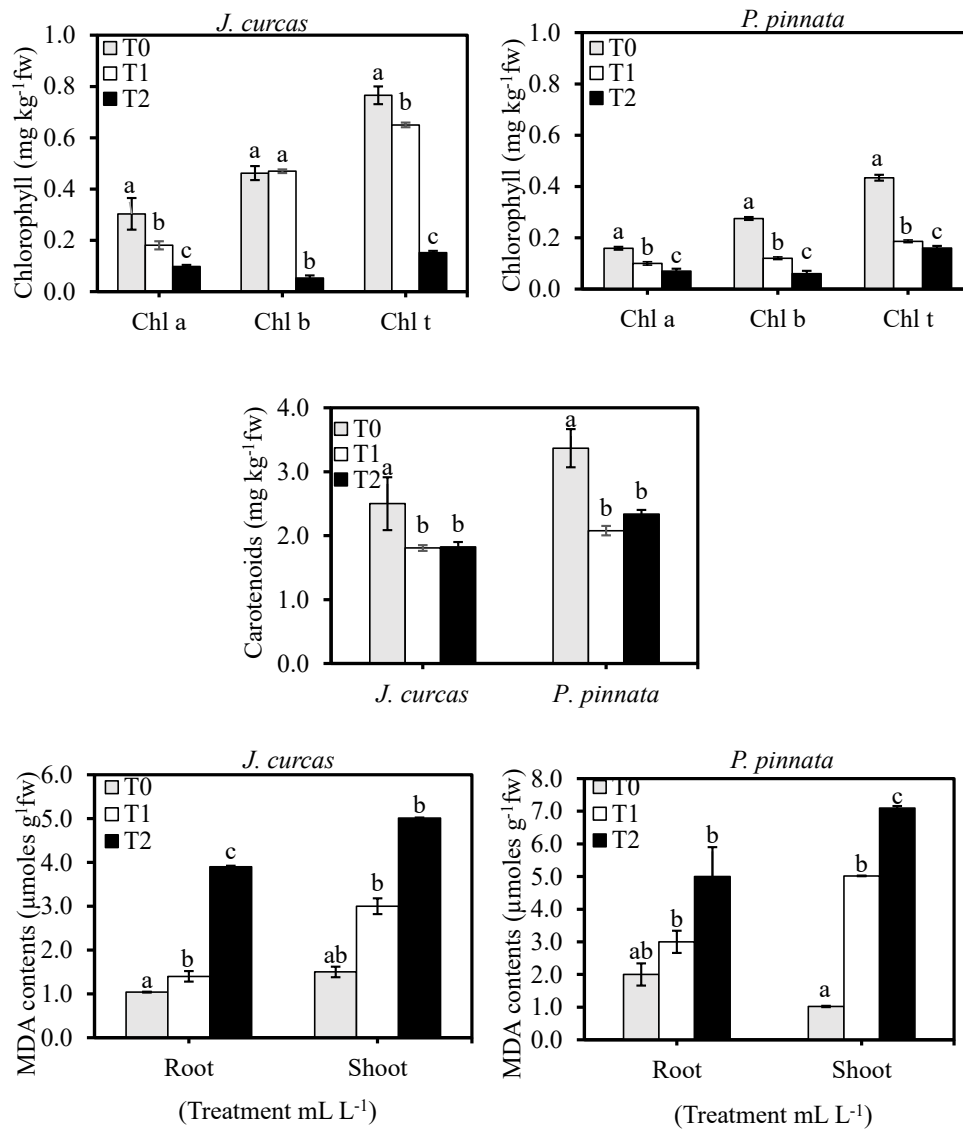


FIGURE 2. Biochemical parameters of *J. curcas* and *P. pinnata*, irrigated with effluent levels; control (T0), 20 mL L⁻¹ (T1) and 40 mL L⁻¹ (T2). Data presents mean± standard error, fw =fresh weight. Different letters at the top of each column depict significant variation between effluent levels at p≤0.05 using Turkey's HSD test

effluent. A similar trend was also observed in roots where accumulation of As was greater (6.10 mg kg⁻¹) in roots of *J. curcas* than *P. pinnata* (4.20 mg kg⁻¹) at the effluent level. On the basis of translocation factor, *P. pinnata* showed more potential for As translocation from root to shoot (Table 4). Our findings are parallel to Kumar et al. (2017a) who reported much greater accumulation of As in the above ground parts (3663 mg kg⁻¹) than roots (3233 mg kg⁻¹) in *P. pinnata* and regarded it as As accumulator species. Study

regarding As uptake and accumulation in *J. curcas* is not available, but *Pteris vittata* as an As hyperaccumulator is well reported. Though fern has different vascular anatomy, the translocation pattern of more As in shoots (4973 mg kg⁻¹) than roots (698 mg kg⁻¹) were reported (Lessl et al. 2015). However, their findings should be compared with great cautions. Nevertheless, the greater quantity of metal in above ground parts of both the species can be ascribed to phosphate transporter. In another fern study, more As was

found to be transported to aboveground tissues via xylem loading, thus significant quantity of metals was found in the shoots (de Oliveira et al. 2018).

Translocation of Cd to the shoots was higher for *J. curcas* (7.07 mg kg⁻¹) than *P. pinnata* (2.72 mg kg⁻¹) at elevated level of fertilizer wastewater (Tables 3 & 4). Our findings are in parallel to Nan et al. (2013) who found higher Cd concentration in aboveground part (14 mg kg⁻¹) than roots (4 mg kg⁻¹) of *Polygonum hydropiper* when irrigated with river water receiving effluents of various industries. Nevertheless, greater amount of Cd in aboveground part have also been reported in other plants (Ahmad et al. 2015; Huang et al. 2011; Ismail et al. 2014).

Jatropha curcas and *P. pinnata* had contrasting behaviors for Cr translocation when irrigated with both levels of fertilizer effluent (Table 4). *J. curcas* exhibited a marked contrast to Cr that remained substantially higher (39%) in the shoots whereas *P. pinnata* showed more deposition of Cr (23%) in roots than shoots at less diluted level of effluent. The accumulation of Cr in *J. curcas* and *P. pinnata* again signified a differential capacity of the tissues of the two species to accumulate Cr. These findings are in parallel to Malik et al. (2010) who reported more Cr translocation from roots (18.5 mg kg⁻¹) to shoots (49.7 mg kg⁻¹) in *Malvestrum coromandalinum* grown in various zones receiving industrial wastewaters. Our findings are also in parallel to Bernabé-Antonio et al. (2015) who

reported more Cr translocation from roots to shoots in *J. curcas*. However, more Cr in the roots of *P. pinnata* can be ascribed to its immobilization in underground tissues (de Oliveira et al. 2016).

The pattern of As, Cd, and Cr translocation in *J. curcas* followed a similar trend for metal accumulation and translocation into aboveground tissues thereby suggesting the phytoextraction potential of the species. It is highly likely that the species acquired an active mechanism for the translocation of these metals for their transport. Presumably, sulphate, phosphate, and iron might serve as common transporters for As and Cr (de Oliveira et al. 2018), but, Zn/Fe regulator-like protein (ZIP) played a role for translocation for Cd (Zare et al. 2018). In addition, the variability of the state of the metal also profoundly influence either accumulation in roots or further translocation to shoots. *P. pinnata* appeared to be a metal tolerant species through phytostabilization for Cr and metal extractor for As and Cd. Both the species are non-edible and potential biofuel trees and have differential metal accumulation strategy, thus they can be a choice for As, Cd, and Cr contaminated soils or in situation where irrigation water contain these metals. Conclusively, both the species have potential for phytoremediation which is either acquired through phytostabilization or phytoextraction, and thus serve as valuable future biofuel energy plants.

TABLE 3. Metal concentration in *J. curcas* and *P. pinnata* in plant tissue under varying levels of effluent from a fertilizer factory

Treatment (mL L ⁻¹)	Metal concentrations (mg kg ⁻¹ dw)					
	As		Cd		Cr	
	Root	Shoot	Root	Shoot	Root	Shoot
<i>J. curcas</i>						
T0	3.00±0.21 ^a	3.41±0.04 ^a	1.0±0.01 ^a	2.35±0.02 ^a	1.16±0.04 ^a	1.00±0.01 ^a
T1	4.92±0.2 ^b	8.90±0.02 ^b	3.03±0.31 ^b	5.96±0.00 ^b	1.32±0.01 ^b	1.85±0.03 ^b
T2	6.10±0.06 ^c	8.48±0.01 ^c	4.81±0.04 ^c	7.07±0.01 ^c	1.22±0.01 ^b	1.69±0.01 ^c
<i>P. pinnata</i>						
T0	3.13±0.19 ^a	3.22±0.1 ^a	1.02±0.04 ^a	1.89±0.01 ^a	1.0±0.021 ^a	0.87±0.04 ^a
T1	4.60±0.24 ^b	8.60±0.02 ^b	1.36±0.02 ^b	2.30±0.01 ^b	1.42±0.01 ^a	1.27±0.03 ^b
T2	4.20±0.21 ^b	8.18±0.03 ^c	2.47±0.03 ^c	2.77±0.09 ^c	2.20±0.33 ^b	1.70±0.02 ^c

Control (T0), 20 mL L⁻¹ (T1) and 40 mL L⁻¹ (T2). Values are expressed as mean±S.E with n=3. DW=Fresh weight. Different letters at the top of each column depict significant variation between effluent levels at p≤0.05 using Turkey's HSD test

TABLE 4. Translocation factor (TF) of As, Cd, and Cr in *J. curcas* and *P. pinnata* under varying levels of effluent from a fertilizer factory

Treatment (mL L ⁻¹)	As	Cd	Cr
	TF	TF	TF
<i>J. curcas</i>			
T0	1.13	2.35	0.86
T1	1.80	1.96	1.40
T2	1.39	1.46	1.38
<i>P. pinnata</i>			
T0	1.02	1.85	0.87
T1	1.86	1.69	0.89
T2	1.94	1.12	0.77

Translocation factor; Ratio of shoot and root metal concentrations. Translocation factor of metal excluder species is TF<1 whereas metal accumulator species has TF>1 (Baker 1981)

CONCLUSION

Positive impact of the effluent was observed in *J. curcas* and *P. pinnata* for the growth parameters studied. However, some adverse effect of effluent was noticed on chlorophyll and carotenoid at higher doses. Nevertheless, the innate tolerance for the heavy metals and potential of species for growth make them a successful candidate for phytoremediation through phytoextraction or phytostabilization. These biofuel species can be grown by using heavily contaminated industrial effluents to save irrigation water and its cost that resolve the water scarcity problem. Both the species can be a choice for phytoremediation programs as well as a source of eco-friendly fuel for future.

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Institute of Pure and Applied Biology
 Bahauddin Zakariya University
 Multan-60800
 Pakistan

*Corresponding author; email: profrafia@gmail.com

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