GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Estimating genetic potential of biofuel forest hardwoods to withstand metal toxicity in industrial effluent under dry tropical conditions

S.A. Manzoor[1], S.N. Mirza[2], M. Zubair[1], W. Nouman[1], S.B. Hussain[3], S. Mehmood[4], A. Irshad[5], N. Sarwar[6], A. Ammar[5], M.F. Iqbal[7], A. Asim[8], M.U. Chattha[9], M.B. Chattha[10], A. Zafar[5] and R. Abid[4]

[1]Department of Forestry, Range and Wildlife Management,
Bahaudddin Zakariya University, Multan, Punjab, Pakistan
[2]Department of Forestry & Range Management,
Pir Mehar Ali Shah-Arid Agriculture University, Rawalpindi, Punjab, Pakistan
[3]Department of Plant Breeding & Genetics,
Bahaudddin Zakariya University, Multan, Punjab, Pakistan
[4]Institute of Pure and Applied Biology, Bahauddin Zakariya University,
Multan, Punjab, Pakistan
[5]Regional Agricultural Research Institute Bahawalpur, Punjab, Pakistan
[6]Department of Agronomy,
Bahauddin Zakariya University, Multan, Punjab, Pakistan
[7]Department of Soil Science, Bahauddin Zakariya University,
Multan, Punjab, Pakistan
[8]Department of Horticulture, Bahauddin Zakariya Univeristy, Multan,
Punjab, Pakistan
[9]Department of Agronomy, University of Agriculture Faisalabad,
Punjab, Pakistan
[10]Institute of Agricultural Sciences, Punjab University, Lahore, Pakistan

Corresponding author: S.A. Manzoor
E-mail: amir.kzd@gmail.com

Genet. Mol. Res. 14 (3): 9543-9554 (2015)
Received November 25, 2014
Accepted April 13, 2015
Published August 14, 2015
DOI http://dx.doi.org/10.4238/2015.August.14.17

ABSTRACT. Biofuel tree species are recognized as a promising alternative source of fuel to conventional forms. Additionally, these tree species are also effective in accumulating toxic heavy metals present in some industrial effluents. In developing countries such as Pakistan, the use of biofuel tree species is gaining popularity not only for harvesting economical and environmentally friendly biofuel, but also to sequester poisonous heavy metals from industrial wastewater. This study was aimed at evaluating the genetic potential of two biofuel species, namely, *Jatropha curcas* and *Pongamia pinnata*, to grow when irrigated with industrial effluent from the Pak-Arab Fertilizer Factory Multan, Southern Punjab, Pakistan. The growth performances of one-year-old seedlings of both species were compared in soil with adverse physiochemical properties. It was found that *J. curcas* was better able to withstand the toxicity of the heavy metals present in the fertilizer factory effluent. *J. curcas* showed maximum gain in height, diameter, and biomass production in soil irrigated with 75% concentrated industrial effluent. In contrast, *P. pinnata* showed a significant reduction in growth in soil irrigated with more than 50% concentrated industrial effluent, indicating that this species is less tolerant to higher toxicity levels of industrial effluent. This study identifies *J. curcas* as a promising biofuel tree species that can be grown using industrial wastewater.

Key words: Biofuels; Phytoremediation; Industrial effluent; *Jatropha curcas*

INTRODUCTION

Rapid industrialization can have adverse environmental impacts because of excessive production and poor disposal of industrial effluents (Nasrullah et al., 2006). According to the report of the Pakistan Council of Research in Water Resources (PCRWR, 2004), only 1% of the industrial effluent from Pakistani industries is treated before being discharged into rivers or other drains; consequently, an excess concentration of arsenic and fluoride has been reported in the water supplies of six major cities. The use of industrial wastewater for growing trees and agricultural crops has been suggested to be a cost-effective way to nurture plants as it eliminates the need for commercial fertilizers (Marecos do Monte et al., 1989). However, when soil is exposed to such wastewater on a consistent basis, it causes drastic changes to the soil properties, such as pH, electrical conductivity (EC), and nutrient availability (Russell et al., 1988). These changes to the physical and chemical properties of the soil may eventually pose a threat for sustainable land use. Nevertheless, with regard to combating the increasingly serious issue of industrial effluent and heavy metal toxicity, phytoremediation is considered as a very useful green technology which offers a promising alternative for cleaning wastewater. The technology is friendly to the environment, cost-effective, and aesthetically pleasing. It is also equally effective for removing both inorganic and organic pollutants from water and soil (Yu et al., 2007). Plants that have the ability to sequester heavy metals in their harvestable biomass are termed hyperaccumulators (Gardea-Torresdey et al., 2004). Employing plants to remediate waste water is a long-standing practice; for example, Baumann (1885) reported some plant species that have the ability to accumulate heavy metals in their leaves. Subsequently, a number of other researchers have demonstrated that a variety of plant species can be used to remediate waste water and contaminated soil (Kaushik et al., 2005).

In a list of the major contributors to pollution in the large cities of Pakistan, Sandhu (1993) identified the Pak-Arab Fertilizer Factory to be the largest contributor to pollution in Multan, one of the most populous cities of Punjab Province. The factory releases toxic effluent into the main canal of Multan through a kacha channel without any check. This release of untreated waste threatens around one million acres of highly productive agricultural land. The current study is aimed at evaluating bioremediation of this pollution through use of biofuel tree species. Here, we examine the growth of two species, namely, *Jatropha curcas* and *Pongamia pinnata* on soil irrigated with industrial effluent from the Pak Arab Fertilizer Factory to determine whether the toxic effluent can be used to grow these valuable tree species. These biofuel tree species have been selected since they are known bioremediators and are highly valuable in terms of their biomass production. In this study, the effluent from the Pak Arab Fertilizer factory in Multan was characterized and its impact on growth of the biofuel tree species and the physiochemical properties of the soil were assessed.

MATERIAL AND METHODS

The research was carried out at the nursery area of the Department of Forestry, & Range Management, Faculty of Agricultural Science and Technology, Bahauddin Zakariya University, Multan.

The biofuel tree species *J. curcas* and *P. pinnata* were grown under four concentrations of Pak-Arab Fertilizer Factory Effluent (25, 50, 75, and 100%); control trees were irrigated with tap water. A completely randomized design, with three replicates, was used for this experiment.

Samples of industrial effluent for irrigation were collected from the Pak Arab Fertilizer Factory located on Khanewal Road, Multan during the peak working hours of the factory. Prior to starting the experiment, a sample of the effluent was tested by the Water Testing Laboratory of the Department of Agricultural Engineering, Bahauddin Zakariya University, Multan for its physiochemical composition using the standard procedures described by the US Salinity Laboratory Staff (1954). The following parameters were measured in the effluent sample: temperature, color, pH, EC, biological oxygen demand, chemical oxygen demand, dissolved oxygen, total suspended solids, and heavy metals.

One-year-old seedlings of *J. curcas* and *P. pinnata* were obtained from a single provenance from the ‘Faiz-e-Aam’ Nursery, Multan in the first week of March 2012 and were transplanted into pots filled with 6 kg of well drained loamy soil in the same week. The transplanted seedlings were then irrigated with different concentrations of industrial effluent (0, 25, 50, 75, and 100%) from March 2012 to October 2012. Approximately 2 L of liquid were applied to each pot at each irrigation. The irrigation frequency differed from once per day to once per six days, depending on weather conditions.

Soil samples were collected from the top 10-15 cm of the soil layer since most nutrient uptake by plants occurs within this zone of the soil (Allen, 1989). The soil samples were taken to the ‘Soil and Water Testing Laboratory For Research’, Multan for analysis using the methods described by the US Salinity Laboratory Staff (1954). The following parameters were measured: soil EC, pH, organic matter content, available nitrogen, available potassium, saturation percentage, and soil texture.

In order to gauge the effect of different concentrations of industrial effluent on the growth performance of the biofuel seedlings, root and shoot lengths, fresh and dry root mass, fresh and dry shoot mass, leaf area, number of branches, number of leaves, and collar diameter were measured.

Although the seedlings were all one-year-old at the start of the experiment they showed a little variation in height and collar diameter. These differences among seedlings might introduce a source of error if absolute changes in shoot height and collar diameter were compared at the end of the experiment. For this reason, the effect of industrial effluent on collar diameter and shoot length was gauged in terms of percentage increase using following formulae:

Percentage Increase in Shoot Length =

$$\frac{\text{Shoot Length at the end of experiment} - \text{Shoot Length at the start of experiment}}{\text{Shoot Length at the start of experiment}} \text{x } 100$$

Percentage Increase in Collar Diameter =

$$\frac{\text{Collar Dia. at the end of experiment} - \text{Collar Dia. at the start of experiment}}{\text{Collar Dia. at the start of experiment}} \text{x } 100$$

Statistical analysis

The data were subjected to statistical analysis using Statistix 8.1 and comparison of means was carried out by applying the LSD test (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

Effect of fertilizer factory effluent on seedling growth

The physiochemical characteristics of the fertilizer factory effluent are shown in Table 1.

Table 1. Physiochemical characterization of fertilizer factory effluent (mg/L).

	Physiochemical characterization of fertilizer factory effluent (mg/L)
Color	Yellowish
Odor	Ammonia
Temperature (°C)	25°C
pH	7.5
EC (µmos/cm)	9.7
Dissolved solids	390
Suspended solids	160
Dissolved oxygen	2.09
Biological oxygen demand	36
Chemical oxygen demand	315
Total nitrogen	55
Sulphate	22
Chloride	72
Calcium	80
Magenesium	65
Cadmium	0.11

Irrigation of the two tree species with this effluent resulted in reduced growth in shoot length (Figure 1) and collar diameter (Figure 2) at the higher concentrations of industrial effluent.

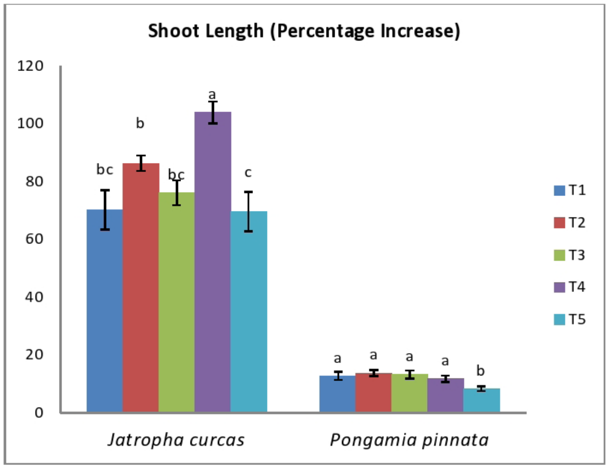

Figure 1. Shoot Length (% increase) in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at P ≤ 5.

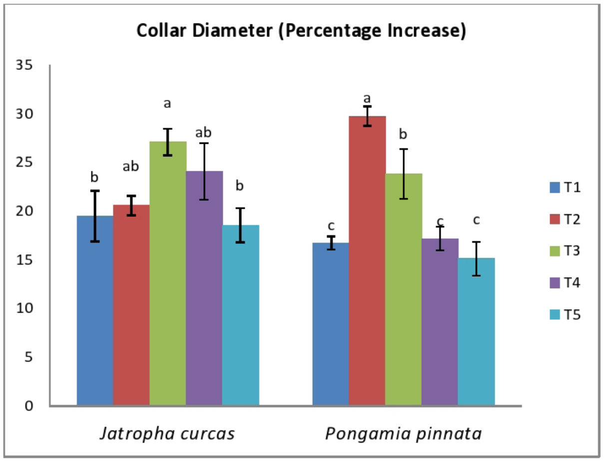

Figure 2. Collar Diameter (% increase) in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at P ≤ 5.

Compared to controls, *J. curcas* seedlings showed a 47% increase in shoot length in 75% effluent and a 39.4% increase in collar diameter in 50% effluent; both metrics showed a substantial decrease in 100% effluent. Similar trends were witnessed in case of *P. pinnata*; the rate of increase in shoot length increased in 10% effluent (7.4% greater mean value than control) but showed a 53% decrease in 100% effluent. The analyses also revealed an increase in number of leaves compared to control of 27 in 75% effluent (Figure 3), in leaf area of 23 in 75% effluent (Figure 4) and of 10% in number of branches in 50% effluent (Figure 5) in *J. curcas* seedlings.

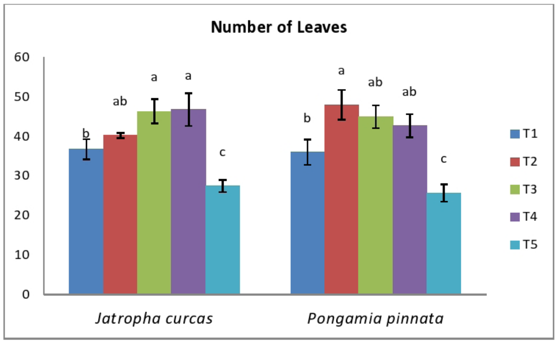

Figure 3. Number of leaves in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at P ≤ 5.

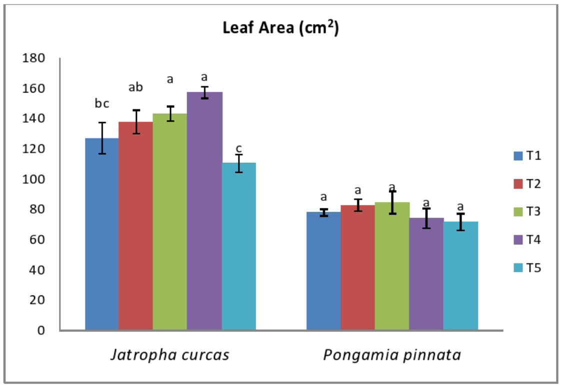

Figure 4. Leaf Area in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at P ≤ 5.

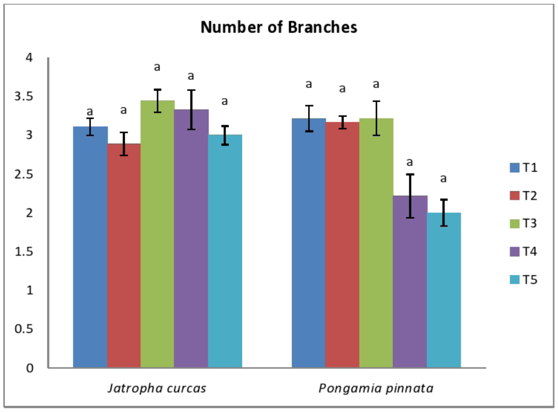

Figure 5. Number of branches in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at P ≤ 5.

However, irrigation with 100% effluent caused a decrease of 34, 15, and 12% in these parameters, respectively, compared to control. For *P. pinnata* seedlings, number of leaves increased by 33 in 10% effluent, leaf area increased by 8.5 in 50% effluent and the number of branches increased by 3 in 50% effluent compared to control. However, irrigation with 100% resulted in decreases in these parameters of 40, 9, and 60%, respectively, compared to control. Moreover, root lengths in both species increased when plants were irrigated with lower concentrations of industrial effluent (Figure 6).

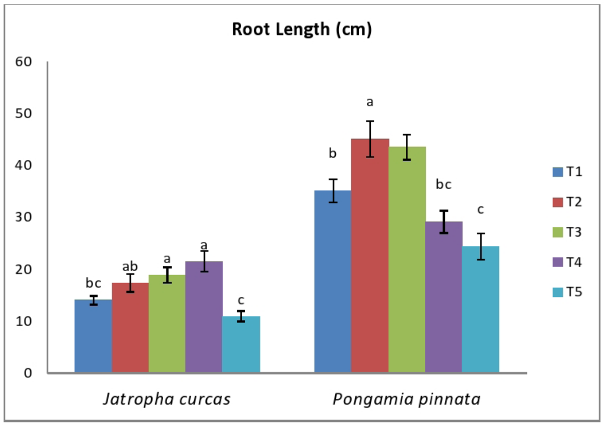

Figure 6. Root Length in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at P ≤ 5.

Effect of fertilizer factory effluent on plant biomass

After irrigation with 75% effluent, *J. curcas* seedlings showed a 28% increase in fresh shoot weight (Figure 7) and a 68% increase in fresh root weight (Figure 8) compared to the control.

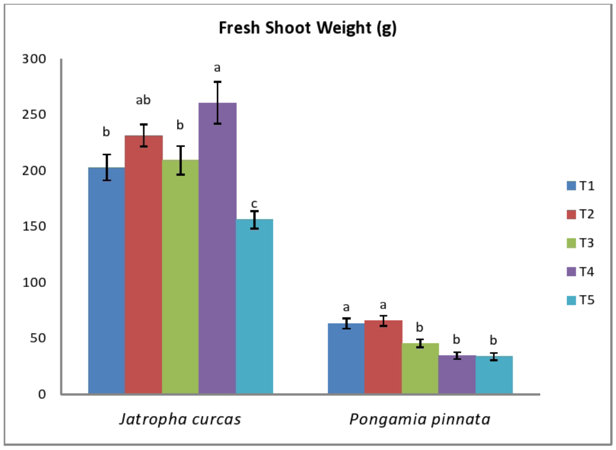

Figure 7. Fresh Shoot Weight in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at P ≤ 5.

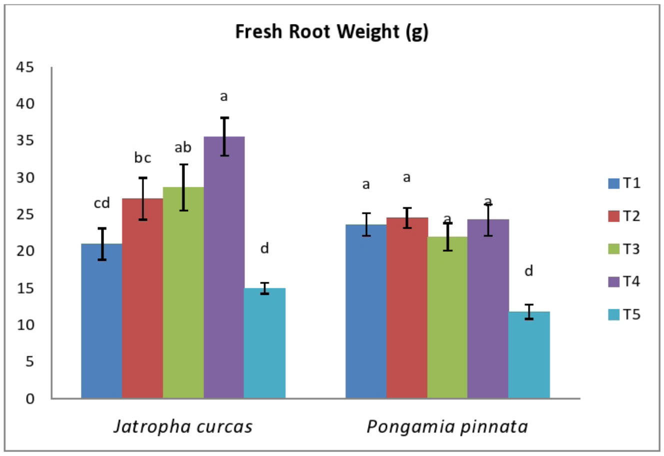

Figure 8. Fresh Root Weight in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at P ≤ 5.

However, these two metrics were 30.2 and 40.1% lower than controls in the 100% effluent treatment. Dry weights of shoots and roots increased by 46 and 41%, respectively, compared to control in the 75% effluent treatment. However, after irrigation with 100% effluent, dried weights of shoots and roots were 6 and 50% lower compared to control.

For *P. pinnata* seedlings treated with different concentrations of industrial effluent, increases of 3.1 and 3.9% were found for fresh shoot and root weight compared to control after 10% effluent treatment. Dried root and shoot weights increased by 16.1% (Figure 9) and 10.3% (Figure 10) compared to control in the 50% effluent treatment. After irrigation with 100% effluent, dried root and shoot weights were 20 and 180% lower, respectively, than control.

DISCUSSION

At the highest levels of effluent, the reduction in shoot growth and collar diameter was the result of the decrease in chlorophyll content induced by the physiological stress of high concentrations of heavy metals. The likely reason for the enhanced growth of the seedlings at low and medium concentrations of effluent was that the level of dilution reduced the concentration of toxic metals to a tolerable range for seedlings and plants, which then were able to avoid heavy metal stress but at the same time make use of the minerals and other nutrients present in the effluent (Skórzyńska-Polit and Baszynski, 1995). Another cause of a decrease in shoot length at the most concentrated levels of industrial effluent is that heavy metals, when transported to above ground parts of the plant, disturb cellular metabolism in the shoots (Shanker, 2003).

In comparison with shoots, the roots of plants are more sensitive to the stress-inducing effects and toxicity of heavy metals in industrial wastewater since they are first to come into direct contact with the wastewater (Öncel et al., 2000). In the present study, a similar response was found in *J. curcas* and *P. pinnata* plants where root lengths were reduced by 28.3 and 44.1%, respectively, in 100% effluent compared to the control. Vajpayee et al. (2001) reported that the heavy metals present in industrial wastewater can hamper the activity of different enzymes in roots and, thereby, impair root growth. The reduction in root length in the present study might also be attributable to the accumulation of toxic metals in the root mass, which might retard mitotic activity in the meristematic zones of roots to cause a serious decrease in the root growth. The maximum increases in root lengths were observed at lower concentrations of effluent: the highest rate of growth was at 50% effluent for *J. curcas* and 10% effluent for *P. pinnata*. Possibly, heavy metals such as magnesium and cadmium are not toxic at these lower concentrations and, thus, roots are able to make use of the higher levels of nitrogen, phosphorus and potassium in the fertilizer factory effluent (Zou et al., 2008) Figure 6.

The increase in fresh biomass compared to controls in plants grown under low effluent concentration conditions might be due to increased activities of enzymatic and non-enzymatic antioxidants (Chandra et al., 2007). However, the trend for lower dry weights in seedlings at higher concentrations was observed in both species. This might be due to the increasing concentration of metal elements and sodicity in the wastewater (Shah et al., 2008), which might adversely affect enzymatic activities through a series of physiological and biochemical changes in the plants (Singh and Bhati, 2003) and ultimately lead to a decrease in water potential and plant growth (Gardea-Torresdey et al., 2004; Peralta et al., 2000). Reduction in biomass production at higher effluent concentrations in wastewater could have been the result of changes in absorption and translocation of nutrients and biochemical functions due to ion stress (Singh and Bhati, 2003).

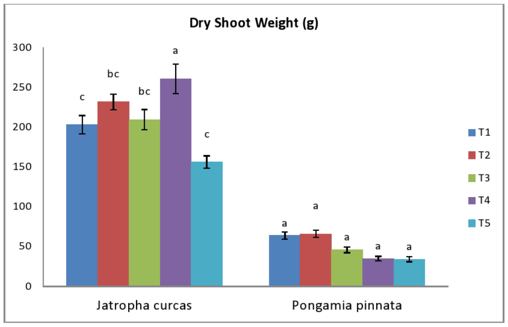

Figure 9. Dry Shoot Weight in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at $P \leq 5$.

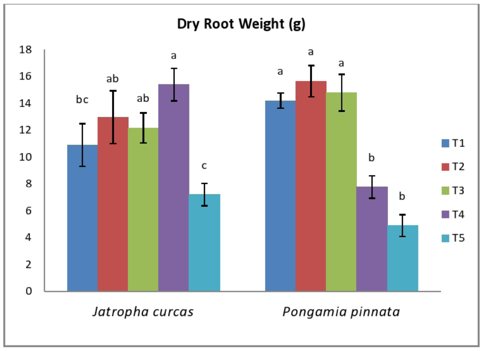

Figure 10. Dry Root Weight in *Jatropha curcas* and *Pongamia pinnata* seedlings as treated by industrial effluent. Letters show statistical significance of treatments at $P \leq 5$.

Effect of fertilizer factory effluent on physiochemical properties of soil

We identified changes in soil EC after irrigation with varying concentrations of industrial effluent (Table 2). These changes were likely due to the presence of different types of salts in the industrial effluent (Bishnoi et al., 1993). The presence of salts may also be the reason for an increase in soil pH (Stewart et al., 1986). The EC of effluent-irrigated soil samples might have increased because of the high EC of the effluent. This is in line with the reports of Rusan et al. (2007) and Jahantigh (2008). Thus, higher concentrations of cations, including K and Na, in the effluent might have led to increases in EC and exchangeable Na and K in soils irrigated with wastewater (Khai et al., 2008). An increase in organic matter content, which is one possible cause of the increase in soil pH, might be attributed to the high nitrogen levels in the fertilizer factory effluent (Rusan et al., 2007). These results are in agreement with the findings of Rattan et al. *(*2005) and Debosz et al. (2002). Our results have also shown that the available K and P in the soil was increased after irrigation with effluent (Table 2). This change is likely due to the higher levels of P and K in fertilizer factory effluent. The presence of minerals in the effluent may also be the reason for the increased chloride levels in the soil (Najafi and Nasr, 2009). These results are in agreement with those described in the reports of Narwal et al. (1993), Brar and Arora (1997), and Olaniya (1998).

Table 2. Effects of industrial effluent on phsicochemical properties of soil.

Treatment	*Jatropha curcas*	*Pongamia pinnata*	*Jatropha curcas*	*Pongamia pinnata*
	Soil EC (dS/m)		Soil pH	
T_1	2.261 ± 0.066^{a}	2.600 ± 0.057^{e}	8.383 ± 0.044^{c}	8.000 ± 0.057^{b}
T_2	2.531 ± 0.033^{b}	2.866 ± 0.033^{d}	8.416 ± 0.083^{c}	8.066 ± 0.033^{b}
T_3	3.000 ± 0.05^{c}	3.266 ± 0.033^{c}	8.483 ± 0.092^{bc}	8.333 ± 0.033^{a}
T_4	3.362 ± 0.08^{b}	3.710 ± 0.1^{b}	8.733 ± 0.088^{a}	8.400 ± 0.1^{a}
T_5	3.833 ± 0.02^{a}	4.000 ± 0.017^{a}	8.666 ± 0.09^{ab}	8.333 ± 0.019^{a}
	Organic matter (%)		Available phosphorus (ppm)	
	Jatropha curcas	*Pongamia pinnata*	*Jatropha curcas*	*Pongamia pinnata*
T_1	0.68 ± 0.08^{d}	0.687 ± 0.008^{c}	8.667 ± 0.166^{ab}	7.332 ± 0.166^{b}
T_2	0.713 ± 0.003^{bc}	0.693 ± 0.003^{bc}	8.13 ± 0.185^{c}	7.162 ± 0.2^{b}
T_3	0.723 ± 0.003^{b}	0.711 ± 0.005^{b}	8.166 ± 0.166^{bc}	7.1692 ± 0.169^{b}
T_4	0.766 ± 0.003^{a}	0.733 ± 0.006^{a}	8.812 ± 0.152^{a}	8.331 ± 0.23^{a}
T_5	0.773 ± 0.009^{a}	0.740 ± 0.007^{a}	7.333 ± 0.09^{d}	6.632 ± 0.26^{c}
	Available potassium (ppm)		Texture	
	Jatropha curcas	*Pongamia pinnata*	*Jatropha curcas*	*Pongamia pinnata*
T_1	260 ± 9.8^{d}	260 ± 15.5^{c}	Sandy Loam	Sandy Loam
T_2	283.333 ± 6.8^{c}	270 ± 12.3^{b}	Sandy Loam	Sandy Loam
T_3	290 ± 13.2^{bc}	270.000 ± 14.2^{b}	Sandy Loam	Sandy Loam
T_4	303.333 ± 22.1^{a}	286.677 ± 14.98^{a}	Sandy Loam	Sandy Loam

T_1 = Tap water (control); T_2 = 25% industrial effluent; T_3 = 50% industrial effluent; T_4 = 75% industrial effluent; T_5 = 100% industrial effluent. Values with different letters are significantly different from each other at $P \leq 0.05$.

Both biofuel tree species studied here showed promising results as bioremediators when grown in soils irrigated with diluted fertilizer factory effluent. However, neither species flourished if irrigated with 100% effluent. A comparison of the two species indicated that *J. curcas* has a greater ability to withstand the toxicity of industrial effluent as it showed better growth performance after irrigation with 50 and 75% effluent. In contrast, *P. pinnata* showed a decrease in growth performance if treated with wastewater contain more than 50% effluent. The peak values for different parameters were substantially greater compared to control in *J. curcas,* but increased to a lesser extent in *P. pinnata*.

REFERENCES

Allen SE (1989). Chemical analysis of ecological materials. 2nd edn. Blackwell Scientific Publication, Oxford.

Baumann A (1885). Das Verhalten von Zinksatzen gegen Pflanzen und in Boden. Landwirtsch. *Vers.-Statn.* 31: 1-53.

Bishnoi NR, Dua A, Gupta VK and Sawhney SK (1993). Effect of chromium on seed germination, seedling growth and yield of peas. *Agric. Ecosyst. Environ*. 47: 47-57.

Brar MS and Arora CL (1997). Concentration of microelements and pollutant elements in cauliflower (*Brassica olesacea* var. *Botrytis*). *Ind. J. Agric. Sci*. 67: 141-143.

Chandra SS, Chaturvedi PK and Misra V (2007). Toxic effect of arsenate and cadmium alone and in combination on giant duckweed (*Spirodela polyrrhiza* L.) in response to its accumulation. *Environ. Toxicol.* 22: 539-549.

Debosz K, Petersen SO, Kure LK and Ambus P (2002). Evaluating effects of sewage sludge and household compost on soil physical, chemical and microbiological properties. *Appl. Soil Ecol*. 19: 237-248.

Gardea-Torresdey JL, Peralta-Videa JR, Montes M, de la Rosa G, et al. (2004). Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L.: impact on plant growth and uptake of nutritional elements. *Bioresource Technol*. 92: 229-235.

Jahantigh M (2008). Impact of recycled wastewater irrigation on soil chemical properties in an arid region. *Pak. J. Biol. Sci*. 11: 2264-2268.

Kaushik P, Garg VK and Singh B (2005). Effect of textile wastewaters on growth performance of wheat cultivars. *Bioresource Technol.* 96: 1189-1193.

Khai NM, Tuan PT, Vinh CN and Oborn I (2008). Effects of using wastewater as nutrient sources on soil chemical properties in periurban agricultural systems. *VNU J. Sci. Earth Sci.* 24: 87-95.

Marecos do Monte H, Silva e Sousa M and Silva Neves A (1989). Effects on soil and crops of irrigation with primary and secondary effluents. *Water Sci. Technol.* 21: 427-434.

Najafi P and Nasr Sh (2009). Comparison effects of wastewater on soil chemical properties in three irrigation methods. *Res. Crops* 10: 277-280.

Narwal RP, Gupta AP, Singh A and Karwasra SPS (1993). Composition of some city waste waters and their effect on soil characteristics. *Annals Biol*. 9: 239-245.

Nasrullah NR, Bibi H, Iqbal M and Durrani MI (2006). Pollutionl load in industrial effluent and ground water of Gadoon Amazai industrial estate (GAIE) Swabi, NWFP. *J. Agric. Biol. Sci*. 1: 18-24.

Olaniya MS (1998). Heavy metal pollution of agricultural soil and vegetation due to application of municipal solid waste-A case study. *Int. J. Environm. Health* 40: 160-168.

Öncel I, Keleş Y and Űstůn AS (2000). Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environ. Poll.* 107: 315-320.

Pakistan Council of Research in Water Resources (2004). Water quality status. Third report. 2003-2004. Pakistan Council of Research in Water Resources, Islamabad, Pakistan.

Peralta JR, Gardea-Torresdey JL, Tiemann KJ, Gómez E, et al. (2000). Study of the effects of heavy metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*) grown in solid media. *Proc. Conf. Hazardous Waste Res*. 135-140.

Rattan RK, Datta SP, Chhonkar PK, Suribabu K, et al., (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater - a case study. *Agric. Ecosyst. Environm*. 109: 310-322.

Rusan M, Hinnawi S and Rousan L (2007). Long term effect soil and plant quality parameters. *Desalination* 215: 143-152.

Russell JM, Cooper RN and Donnison AM (1988). Irrigation of high-nitrogen containing wastes to pasture. In: Alternative waste treatment systems (Bhamidimarri R, ed.). Elsevier Applied Science, London, 45-54.

Sandhu GR (1993). Sustainable agriculture. A Pakistan national conservation strategy sector paper 2, IUCN Pakistan.

Shah FR, Ahmad N, Masood KR and Zahid DM (2008). The influence of cadmium and chromium on the biomass production of shisham (*Dalbergia sissoo* roxb.) seedlings. *Pak. J. Bot.* 40: 1341-1348.

Shanker AK (2003). Physiological, biochemical and molecular aspects of chromium toxicity and tolerance in selected crops and tree species. PhD thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Singh G and Bhati M (2003). Mineral accumulation, growth, and physiological functions in *Dalbergia sissoo* seedlings irrigated with different wastewaters. *J. Environ. Sci. Health Part A - Toxic/Hazardous Substances Environm. Eng*. 38: 2679-2695.

Stewart HTL, Allender E, Sandell P and Kube P (1986). Irrigation of tree plantations with recycled water - I. Research developments and case studies. *Aust. Forest*. 49: 81-88.

Skórzyńska-Polit E and Baszynski T (1995). Photochemical activity of primary leaves in cadmium stressed *Phaseolus coccineus* depends on their growth stages. *Acta Soc. Bot. Pol*. 64: 273-279.

Steel RG and Torrie JH (1991). Principles and procedures of statistics. 2nd edn. McGraw Hill, New York.

United States Salinity Laboratory Staff (1954). Diagnosis and improvement of saline and alkali soils. USDA Handbook 60, Washington, D.C.

Vajpayee P, Rai UN, Ali MB, Tripathi RD, et al. (2001). Chromium induced physiological changes in *Vallisneria spiralis* L and its role in phytoremediation of tannery wastewater. *Bull. Environ. Contam. Toxicol.* 67: 246-256.

Yu XZ, Gu JD and Huang SZ (2007). Hexavalent chromium induced stress and metabolic responses in hybrid willows. *Ecotoxicology* 16: 299-309.

Zou J, Xu P, Lu X, Jiang W, et al. (2008). Accumulation of cadmium in three sunflower (*Helianthus Annuus* L.) cultivars. *Pak. J. Bot.* 40: 759-765.