Current Research Journal of Biological Sciences 5(1): 36-41, 2013
DOI:10.19026/crjbs.5.5470
ISSN: 2041-076X, e-ISSN: 2041-0778

Submitted: January 01, 2013 Accepted: January 18, 2013 Published: January 20, 2013

Research Article

Phytoremediation of Heavy Metals Contamination in Industrial Waste Water by *Euphorbia prostrata*

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Abstract: Contamination of freshwater resources continues due to domestic effluents, industrial discharges and use of chemicals in agriculture. Sugar manufacturing industry produces a large amount of wastewater that contains different chemicals and various heavy metals. Heavy metals are toxic to human and also cause water and soil pollution. The objectives of this study were: to find a cost-effective phyto-remediation and biosorption method to reduce heavy metal contamination, i.e., Cd, Cr and Pb by evaluating the potential of *Euphorbia prostrata*, an indigenous plant species, in *in situ* and *ex situ* experiments. *Euphorbia prostrata* were grown on different concentrations (100%, 50% and 0%) of wastewater in controlled conditions of 23 °C with 12 hr. dark and light cycle for 45 days and compared with underground extracted tap water collected from the same area, as control. Three harvests were taken after 15, 30 and 45 days of plant germination. Plants grown on wastewater show reduced growth on fresh and dry weight basis (80% and 50%), respectively as compared to plants grown on control. In *phyto-remediation* experiment, plants germinated on wastewater in field accumulated higher amount of heavy metals (20-55%) over a period of 45 days. Whereas, in *biosorption* experiment, biomass collected from plants germinated in lab adsorbed higher amount of heavy metals (>70%). The results suggest *Euphorbia prostrate* as a promising plant for both phyto-remediation and *biosorption* of heavy metals.

Keywords: Biosorption, *Euphorbia prostrata,* heavy metals, phytro-remediation, wastewater contamination

INTRODUCTION

Wastewater is any water that has been adversely affected in quality by anthropogenic influence. It comprises liquid waste discharged by domestic residences, commercial properties, industry and/or agriculture and can encompass a wide range of potential contaminants and concentrations. In the most common usage, it refers to the municipal wastewater that contains a broad spectrum of contaminants resulting from the mixing of wastewaters from different sources (Alves *et al*., 1993). Heavy metal is referred to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations, i.e., Arsenic (As), Cadmium (Cd) Chromium (Cr), mercury (Hg) and lead (Pb) etc. Trace amount of heavy metals is essential for human metabolism, i.e., chromium is essential for metabolism of fatty acids, glucose and protein. However, at higher concentrations they can lead to poisoning. Heavy metal ions are used in various industries due to their technological importance and may become part of waste water released from these industries and hence could cause toxic effects through food chain (Baker *et al*., 1994; Soltan and Rasheed, 2003). The release of heavy metals in biologically available forms by human activity could destroy or change both natural and man-made ecosystems. Heavy metal poisoning could result from drinking contaminated water, presence in ambient air near emission sources, or through food intake (Muchuweti *et al*., 2006). Various techniques employing chemical treatment (Ajmal *et al*., 2000; Wase and Forster, 1997), biosorption and bioremediation (Alkorta and Garbisu, 2001; Friis, 1998) and physical removal (Ajmal *et al*., 2003; Bishnoi *et al*., 2004) have been reported to remediate heavy metals in wastewater. However, most chemical and physical engineering technologies fail to remove heavy metals from effluents completely. Hence, developing cost effective and environmentally friendly technologies for the remediation of heavy metals from polluted soils and wastewaters is a topic of global interest. Naturally-occurring biological tools are being substituted as an alternative in pollution control program due to their harmless nature (Raskin *et al*., 1997).

In order to avoid health hazards, it is essential to remove heavy metals from wastewater. Various

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techniques based on physical and chemical removal have been developed (Alvarez and Sanchez, 2003; Sanchez *et al*., 1999), however, a complete removal of heavy metals from effluents is failed. As an alternate, naturally occurring biological tool called *Bioremediation,* is being substituted in pollution control programs, globally. It includes the use of microorganisms, plants and their products to remove contaminants. The use of plants or Phyto-bioremediation is one of the most important techniques to remove heavy metals especially from wastewater. The use of specially selected and engineered pollutant accumulating plants for environmental cleanup is an emerging technology called as Phyto-bioremediation (Raskin and Ensley, 2000; Tyler *et al*., 1989). Therefore, the development of cost effective and environmentally friendly technologies for the remediation of heavy metals from polluted soils and wastewaters is a topic of global interest. The value of metal-accumulating plants to wetland remediation has been recently realized and can be used as *in-situ* or *ex-situ* technique (Cheng *et al*., 2002; Rai *et al*., 1995). Similarly, several terrestrial plants that have been identified in the last two decades as highly effective in absorbing and accumulating various toxic heavy metals are being evaluated for their role in the phytoremediation of soils and water polluted with heavy metals and trace elements (Fritioff and Greger, 2003; Baker *et al*., 1994; Dunbabin and Bowmer, 1992). Plants such as sunflower, Indian mustard, tobacco, rye, spinach and corn have been studies for their ability to reduce lead from effluent; sunflower has greater ability to remove lead from effluents (Raskin *et al*., 1997). *Euphorbia prostrata* belongs to family *Euphorbiaceae* and is commonly known as prostrate sand-mat. It is native to West Indies and some parts of South Africa but now it is widely distributed all over the world and grow as roadside weed (Reeves, 2003; Reeves and Baker, 2000). It is an annual herb producing slender prostrate stem up to 20 cm long.

In Pakistan, Sugar manufacturing industry produces a large amount of wastewater that contains different chemicals and various heavy metals. Such heavy metals are not only toxic to human but also cause water and soil pollution. The objectives of this study were: to find a cost-effective phyto-remediation and biosorption method to reduce heavy metal contamination. For this purpose, both field experiment and indoor pot experiments with were set up and were used to study heavy metals (Cd, Cr and Pb) uptake from wastewater produced from sugar-industry by using an indigenous plant species *Euphorbia prostrata*.

MATERIALS AND METHODS

Material:

Water and waste water sampling: To check the ability of *Euphorbia prostrata* for reduction of heavy metals from wastewater of sugar mills of Layyh a study

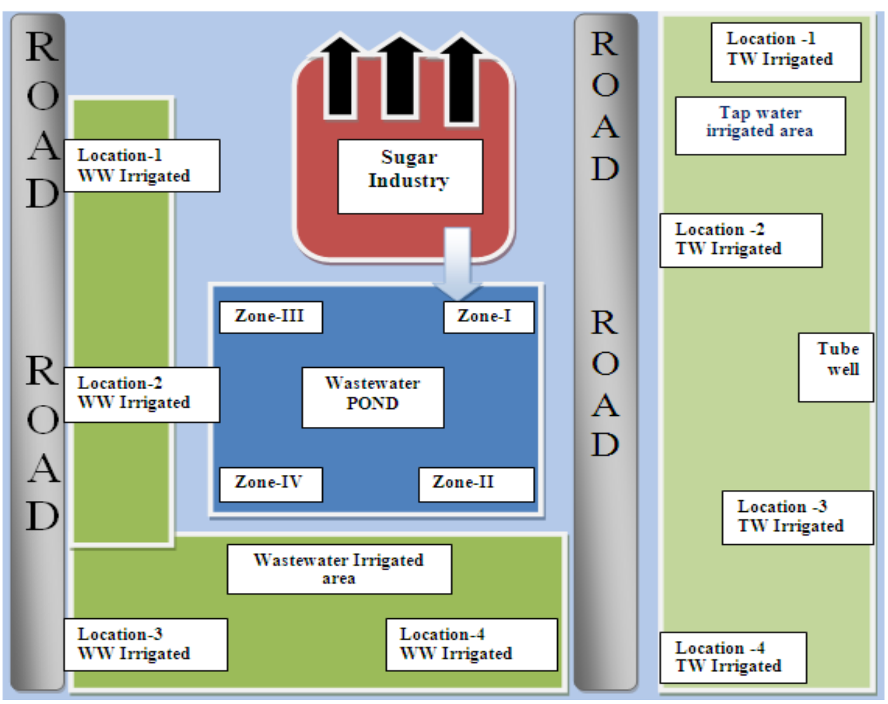

Fig. 1: Map of sampling location and sugar plant

was conducted in district layyah. Samples of waste waterwere collected in sterile plastic bottles from Wastewater Pond divided in 4 zones. Tap water samples were collected from tubewell and running taps fed by tube well in the area (Fig. 1). Samples were subject to physic-chemical analysis, i.e., pH, EC, turbidity and Electrical conductivity, on site. Samples were transported to laboratory in cooler and stored at 4C till further heavy metal analysis (Benjamin *et al*., 2007; Singer *et al*., 2002).

Plant samples: A site irrigated by tap water was selected and after removing old plants new germinating plants were allowed to grow and four (4) samples of plants were collected three times after regular intervals of fifteen days (in total 45 days). Another site irrigated by waste water from sugar industry was selected and four (4) samples of plants were collected in similar fashion (Jones *et al*., 1991) (Fig. 1).

Chemicals and equipments: All chemicals were of analytical grade procured from Merck Inc. Germany. Spectronic 20 (Spectronic, USA) and . Atomic Absorption Spectrophotometer (AAS) (Hitachi A-1800, Japan) were used to determine amount of heavy metals.

Methods:

Water and waste water sampling: To check the ability of *Euphorbia prostrata* for reduction of heavy metals from wastewater of sugar mills of Layyh a study was conducted in district layyah. Samples of waste water were collected in sterile plastic bottles from Wastewater Pond divided in 4 zones. Tap water samples were collected from tubewell and running taps fed by tube well in the area (Fig. 1). Samples were subject to physic-chemical analysis, i.e., pH, EC, turbidity and Electrical conductivity, on site. Samples were transported to laboratory in cooler and stored at 4C till further heavy metal analysis (Benjamin *et al*., 2007; Singer *et al*., 2002).

Plant Sampling from Field: Two sites were selected for collection of plants one irrigated by wastewater and other irrigated by tube well water (Fig. 1). Plants were collected, washed with distilled water, oven dried (105 °C); crushed and store in air tight bottles for further analysis (Jones and Case, 1990). Similar plants were also grown in lab on both tap and waste water.

Germination of plants in Laboratory: Seeds of plant *Euphorbia prostrata* were collected from old plants in field irrigated by tap water. Seeds were grown in pots and the pots arrangement was comprised of two treatments in a Randomized Complete Block Design (RCBD). Twelve pots with plants in treatment-1 (T1) were irrigated by tap water and in treatment-2 (T2) 12 potted plants were irrigated by waste water of sugar industry. Three harvests were (over a period of 45 days) from each treatment were collected as above and plants were taken for chemical analysis (Jones *et al*., 1991).

Chemical Analysis: Water samples were subject to Atomic Absorption Spectroscopy (AAS) for heavy metals contents (Raskin *et al*., 1997; Sanchez *et al*., 1999). For plant experiment, digestion of plants was carried out in HNO_3 and $HClO_4$, and AAS spectrophotometer method was adopted to determine amount of heavy metal (Jones and Case, 1990).

RESULTS AND DISCUSSION

Cadmium uptake by plants: Results in Table 1 and Fig. 2 show that after 15 days of germination, plants irrigated by wastewater in field showed a greater accumulation of cadmium (0.5 µg) as compared to control (Plants irrigated by tube well water germinated in lab) that witnessed no accumulation of cadmium. After 30 days of germination, plants irrigated by wastewater in field show greater accumulation of cadmium (0.88 µg) whereas, plants irrigated by tube well water germinated in lab showed no accumulation of cadmium. Similarly, after 45 days of germination, plants irrigated with wastewater in field showed greater accumulation of cadmium (1.97 µg). However, the plants irrigated by tube well water germinated in controlled atmosphere did not accumulate cadmium,

Table 1: Amount of cadmium uptake by plants (micrograms)

	15 days old plants				30 days old plants				45 days old plants			
Sample no.	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
S1	N.P*	0.92	0.36	0.50	N.P*	0.14	0.79	0.80	N.P*	2.13	1.1	1.68
S2	N.P*	0.09	0.36	0.39	N.P*	0.21	0.83	0.98	N.P*	1.80	1.10	1.97
S3	N.P*	0.57	0.37	0.67	N.P*	0.23	0.80	0.77	N.P*	1.91	1.10	2.2
S4	N.P*	0.59	0.47	0.41	N.P*	0.16	0.65		N.P*	2.60	1.17	2.0
Uptake		0.53	0.40	0.50		0.18	0.77	0.88		2.11	1.12	1.97
mean±SD)	N.P*	±	±	±	N.P*	±	±	±	N.P*	±	±	±

S1 = Plants collected from location-1: S2 = Plants collected from location 2; S3 = Plants collected from location 3: S4 = Plants collected from location 4; C = Control: NP* = Not found: * All values are Mean±Standard deviation

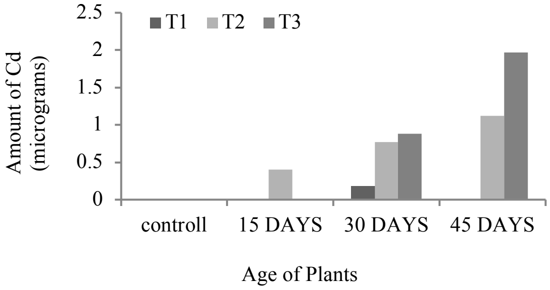

Fig. 2: Uptake of cadmium by *Euphorbia prostrata* (micrograms) C = Plants germinated in lab on tap water. T1= plants collected from field irrigated by tap water; T2 =plants germinated in lab on waste water. T3=plants collected from field irrigated by waste water

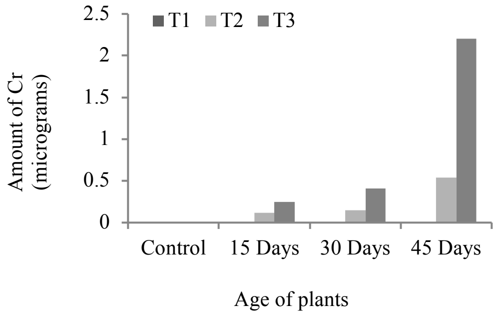

Fig. 3: Uptake of chromium by *Euphorbia prostrata* (micrograms) C = Plants germinated in lab on tap water. T1 = plants collected from field irrigated by tap water; T2 = plants germinated in lab on waste water. T3 = plants collected from field irrigated by waste water

irrespective of the number of days of treatment. These results proved that wastewater had significantly affected the accumulation of cadmium in plants.

Previously, Sharma (2010) conducted a study on accumulation of heavy metals from waste water and concluded similar results that these vegetables collected from polluted areas accumulate heavy metals in their body. It was also reported previously that sugar cane grown in polluted contaminated areas accumulate heavy metals in their various parts of body (Reeves and Baker, 2000; Rath *et al*., 2010).

Chromium uptake by plants: According to the results, after 15 days of germination, plants irrigated by wastewater in field accumulated chromium to 0.25 µg, whereas, plants germinated in lab on wastewater accumulate 0.12 µg chromium during same period (Table 2 and Fig. 3). However, Plants irrigated by tube well water germinated in lab as well as in field show no accumulation of chromium. The same pattern followed after 30 days of germination as plants irrigated by wastewater in field show greater accumulation of chromium (0.41 µg) while plants germinated in lab on wastewater accumulated 0.15 µg chromium. Furthermore, after 45 days of germination, plants irrigated by wastewater in field show greater accumulation of chromium, which is 2.20 µg. Plants germinated in lab on wastewater accumulate 0.54 µg chromium (Table 2 and Fig. 3).

As control, plants irrigated by tube well water germinated in lab as well as in field showed no accumulation of chromium. These results show that

Table 2: Uptake of chromium by plants (micrograms)

	15 days old plants				30 days old plants				45 days old plants			
Sample no.	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
S1	N.P*	N.P*	0.08	0.02	N.P*	N.P*	0.34	0.06	N.P*	N.P*	0.50	2.0
S2	N.P*	N.P*	0.16	0.56	N.P*	N.P*	0.09	0.60	N.P*	N.P*	0.55	1.94
S3	N.P*	N.P*	0.12	0.22	N.P*	N.P*	0.13	0.41	N.P*	N.P*	0.53	3.03
S4	N.P*	N.P*	0.10	0.23	N.P*	N.P*	0.04	0.60	N.P*	N.P*	056	1.84
Uptake			0.12				0.15	0.41			0.54	2.20
(mean±SD)	--	--	±	0.25 ±	---	---	±	±	---	---	±	±

S1 = Plants collected from location-1: S2 = Plants collected from location 2; S3 = Plants collected from location 3: S4 = Plants collected from location 4; C = Control: NP* = Not found: * All values are Mean±Standard deviation

Table 3: Uptake of lead in plants (micrograms)

	15 days old plants				30 days old plants				45 days old plants			
Sample no.	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
S1	N.P*	N.P*	N.P*	0.20	N.P*	N.P*	N.P*	0.66	N.P*	N.P*	0.31	0.56
S2	N.P*	N.P*	N.P*	0.38	N.P*	N.P*	N.P*	0.86	N.P*	N.P*	0.45	1.03
S3	N.P*	N.P*	N.P*	0.25	N.P*	N.P*	N.P*	0.04	N.P*	N.P*	0.51	1.6
S4	N.P*	N.P*	N.P*	0.03	N.P*	N.P*	N.P*	0.46	N.P*	N.P*	0.19	0.41
Uptake				0.21				0.50			0.36	0.90
(Mean±SD)	--	--	--	±	---	---	---	±	---	---	±	±

S1= Plants collected from location-1: S2 = Plants collected from location 2; S3 = Plants collected from location 3: S4 = Plants collected from location 4; C = Control: NP* = Not found: * All values are Mean ± Standard deviation

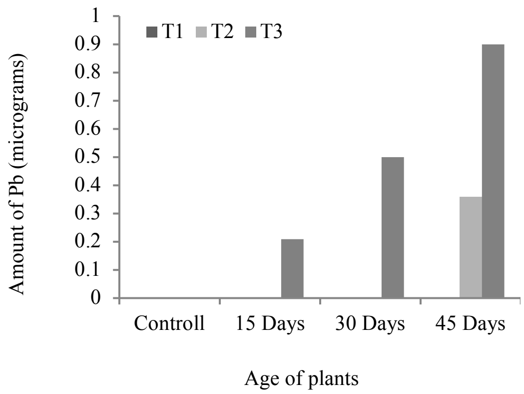

Fig. 4: Uptake of lead by *Euphorbia prostrata* (micrograms)

wastewater significantly affect the accumulation of chromium in plants. Similar results of chromium uptake accumulated by *Euphorbia helioscopia* to 0.2 mg/kg has been reported previously (Khan *et al*., 2008; Reeves, 2003), however the current data reports a greater accumulation by *Euphorbia prostrate* in both field and under controlled conditions.

Lead (pb) uptake by plants: After 15 days of germinating plants irrigated by wastewater in field showed greater accumulation of lead (0.21 µg) (Table 3 and Fig. 4). However, plants germinated in lab on wastewater accumulated no lead similar to plants irrigated by tube well water germinated in lab as well. After 30 days of germination, plants irrigated by wastewater in field showed a greater accumulation of lead (0.50 µg)). Plants germinated in lab on wastewater did not accumulate lead. Plants irrigated by tube well water germinated in lab and in field however did not show any lead accumulation due to absence in water (control). Similarly, after 45 days of germination plants irrigated by wastewater in field witnessed the greater accumulation of lead, which was above 0.90µg. Plants germinated in lab on wastewater accumulate 0.36 µg of lead. Plants irrigated by tube well water germinated in lab as well as in field show no accumulation of lead. More lead is accumulated by *Euphorbia helioscopia* which is 2.1 mg/kg that is much greater than from unpolluted area, as reported previously (Khan *et al*., 2008). Although uptake of heavy metals has been reported for *Elsholtzia haichowensis* and *Commelina communis* in China and have been found metal tolerant without compromise on growth (Tang *et al*., 2001), the results of current study also reports the efficiency of indigenous plants to absorb metals from affected soil more efficiently.

CONCLUSION

Heavy metals affect plants, animals and humans in different ways. The different techniques are available to reduce amount of heavy metals from wastewater but all these chemical techniques have limitations and drawbacks. *Phyto-remediation* and *Biosorption* techniques could be cost-effective methods for heavy metal uptake and removal from contaminated sites. Furthermore, biosorption and phytoremediation have been reported as appropriate techniques without causing any environmental impact. Current study reports that wastewater of sugar industry contain various heavy metals in different concentration found above permissible level. The use of *Euphorbia prostrata* and removal efficacy through biosorption technique by using biomass could be a promising techniques to reduce amount of heavy metals. Further studies with other heavy metals contaminated wastewater are under progress and a comparative analysis and potential is under investigation.

ACKNOWLEDGMENT

This research study would not be possible without the support and funding through FFHP Project of HEC. The authors also wish to thank the *Institute of Pure And Applied Biology, Bahauddin Zakariya Univeristy* (BZU) Multan, Pakistan, for their cooperation in setting up the experiments in their Biology department.

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