Journal of Hazardous Materials 186 (2011) 443–450

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Metal accumulation potential of wild plants in tannery effluent contaminated soil of Kasur, Pakistan: Field trials for toxic metal cleanup using *Suaeda fruticosa*

Firdaus-e Bareen*, Syeda Anjum Tahira

Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

ARTICLE INFO

Article history:
Received 27 May 2010
Received in revised form 4 November 2010
Accepted 5 November 2010
Available online 13 November 2010

Keywords:
Metal bioaccumulation
Tannery effluent contamination
Toxic metals
Suaeda fruticosa

ABSTRACT

The tannery effluent contaminated lands, adjacent to Depalpur Road, Kasur, Pakistan, have been rendered infertile due to long term effluent logging from the leather industry. The area has been colonized by twelve plant species among which *Suaeda fruticosa*, *Salvadora oleoides* and *Calatropis procera* have been found to be the most common and high biomass producing plants. *S. fruticosa* was subjected to further experimentation because of its high biomass and phytoextraction capabilities for metals. The pot and field experiments were carried out simultaneously. Pot experiments were conducted using the same field soil in column pots with stoppard bottoms to obtain the leachate. EDTA treatment caused a greater solubility of Cr in the soil pore water. In higher doses more amount of the heavy metal was leached. The increase in the amount of EDTA significantly caused a decrease in the biomass of plants without toxicity symptoms. A higher biomass of plants was observed in the field as compared to the pot experiment. The greatest amount of Na was accumulated by leaves of *S. fruticosa* followed by stem and roots. Similarly, the greatest amount of Cr was bioaccumulated by leaves of *S. fruticosa*, but followed by roots and then stem. *S. fruticosa* can be employed in rehabilitation of tannery effluent contaminated soil using small doses of EDTA.

1. Introduction

Tanning has a longstanding tradition in Kasur and it has the biggest tanning concentration in number in the country [1]. Nowadays chrome tanning is favored by the majority of the leather industry because of the speed of processing, low cost, color of leather and greater stability of the resulting leather [2]. Leather tanneries in Pakistan produce many types of waste by products, among which the toxic wastewater and solid waste are the important environmental challenges [3]. The chromium released in the chrome tanning step is especially a controversial element on account of its persistence, potential toxicity and among the few established causes of environment related cancers. Chromium is a highly toxic non-essential metal for microorganisms and plants.

Bioremediation technologies, which are soil-focused are suitable for large areas that have been contaminated with low to moderate levels of contaminants. Sustainable on-site techniques for remediation of heavy metal contaminated sites include phytoextraction, the use of plants to remove contaminants from soils, sediments or water into harvestable plant biomass.

Rehabilitation of contaminated habitats requires study of indigenous flora that can be best employed to gain back the inherent characteristics of habitats. It is because of their adaptive capability to thrive under a stressed situation. Tannery effluent logging not only destroys the structure and fertility of soil but also causes excess loading of two important toxic metals, Na and Cr. Enhanced levels of sodium in soil can prove to be toxic for plants and can also cause sodicity in adjacent soils. On the other hand, the excess amount of Cr can also be toxic to plants as well as it can reach human beings through the food chain.

Synthetic chelating agents like EDTA have been shown to increase the metal uptake capability in plants like *Brachiaria decumbens*, *Brassica campestris*, *Brassica juncea*, *Brassica rapa*, *Dianthus chinensis*, *Fagopyrum esculentum*, *Helianthus annuus*, *Hordeum vulgare*, *Phaseolus vulgaris*, *Pisum sativum*, *Sinapis alba*, *Sorghum bicolor*, *Spinacea oleracea*, *Trifolium alexandrianum*, *Triticum aestivum*, *Vigna radiata* and *Zea mays* [4–7]. Increases greater than fifty fold have been observed in lead metal accumulation in *B. rapa* [8].

The application of chelating agents in field experiments has not been widely demonstrated [6]. The findings of Kayser et al. [9] have shown that there were much higher metal tissue concentrations under greenhouse conditions as compared to the natural conditions in the field. Lead phytoextraction rates of three plants were improved by 19-, 2-, and 13-fold respectively under EDTA application in the field [10].

* Corresponding author. Tel.: +92 42 5884513.
E-mail address: fbareen@gmail.com (F.-e. Bareen).

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doi:10.1016/j.jhazmat.2010.11.022

The main objective of the present research work was to identify wild plants growing naturally in tannery effluent contaminated soil, producing high biomass and a good ability of extracting toxic metals from soil. The selected metal hyperaccumulating plant was subjected to further experimentation in soil contaminated with effluents of the leather industry so that it could be employed for on-site remediation. The well known chelating agent, EDTA was applied in different concentrations to aid the plant in the uptake of metals.

2. Materials and methods

2.1. Field observations

The most badly polluted area with degraded land surface and scanty and scrubby vegetation was selected in Kasur, Pakistan. The area is adjacent to Depalpur Road and tannery effluents have continuously been discharged there since the last many decades. In the recent past, this area was a stagnant pool having extremely polluted water with foul smell. In the year 1999, the government noticed this menace, and established the Kasur Tanneries Waste Management Agency (KTWMA) that has channelized the effluents through the properly cemented drains to the waste water treatment plant. Since then the logged water has slowly dried up and a natural population of tolerant plants has appeared on this location. By the end of the year 2005 a number of plants had invaded over the area. *Suaeda fruticosa* (L.) Forssk., a plant of most deserted and salinity-hit areas with high Na content, appeared to be the most common wild species. These surveys were carried out between October 2007 and March 2009. The natural inhabitants observed in the study area other than *S. fruticosa* included *Acacia nilotica* (L.) Del., *Alternanthera sessilis* L., *Calatropis procera* (Wild.) R. Br., *Chenopodium murale* L., *Cynodon dactylon* (L.) Pers., *Dichanthium annulatum* (Forssk.) Stapf., *Echinochloa colonum* (L.) Link, *Heliotropium eichwaldii* Steud., *Kochia indica* (L.) Schrader, *Panicum antidotale* Retz., *Rumex dentatus* L., *Salvadora oleoides* Dene, and *Trianthema portulacastrum* L.

2.2. Evaluation of natural inhabitants of polluted sites for phytoremediation potential

Contaminated areas were surveyed and the above mentioned natural inhabitants were collected from the experimental site during the years 2007–2009 and brought to the laboratory. Plant roots and shoots were separated, oven dried to a constant weight and milled. Samples were subjected to the physical (dry weight of shoot and root/plant) and chemical analyses for evaluation of their Cr and Na uptake capabilities. Digestion of the plant material was carried out with a mixture of concentrated nitric acid and perchloric acid [11].

The digested samples were subjected to estimations of Na on a flame photometer (PFP7&PFP7/C, England) and Cr on an atomic absorption spectrophotometer (AA-1275/VARIAN, Australia).

2.3. Phytoremediation of soil with S. fruticosa (greenhouse trials)

Based on the metal bioaccumulation capability and high biomass, *S. fruticosa* was found to be the best among the natural inhabitants. Therefore its seeds were used for further experiments, collected directly from the naturally growing plants in the contaminated experimental site of Kasur.

Experiments were started in March 2009. The greenhouse experiment was laid out in a completely randomized design [12]. The experiment comprised of the following four sets of treatments with five replicates each.

W: contaminated field soil without EDTA.
1 EDTA: contaminated field soil + EDTA@1 mmol kg^{-1} soil.
5 EDTA: contaminated field soil + EDTA@5 mmol kg^{-1} soil.
10 EDTA: contaminated field soil + EDTA@10 mmol kg^{-1} soil.

Plastic pots (size 15 cm diameter × 30 cm height) with stoppard bottoms were filled with contaminated soil from the experimental field adjacent to Depalpur Road, Kasur, Pakistan. The pots were arranged in a wired field area with a glass roof under natural light with air temperature ranging between 25 and 38 °C (in March to June) in the Dept. of Botany, University of the Punjab, Lahore, Pakistan.

Three seeds were sown in the central region of the pot and allowed to germinate and the healthiest plant was retained in each pot. The moisture content of the pots was maintained at pot capacity on alternate days with tap water. EDTA was applied to the pots four weeks after seed germination in the form of sprinkling solutions of 1, 5 and 10 mmol EDTA kg^{-1} soil in a closed system. The solutions of EDTA were prepared from a disodium salt of EDTA ($C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$) Merck, Germany.

The contaminated soil as such and after preparing treatments with EDTA was analyzed physico-chemically. The water extractable and total amounts of Na and Cr were analyzed to observe the affectivity of EDTA in solubilizing these metals [13]. Soluble metals, pH and ECe were determined. For estimating total amount of metals, the soil was first acid-digested using nitric acid, perchloric acid (1:4 (v/v)) method [14].

The leachate from the pot was obtained at monthly intervals. The reading taken for the first month was at the time of application of EDTA. The leachate from pots was analyzed for both metals after digestion. The plants were allowed to mature to seed formation (in four months time). Plants were uprooted carefully at maturity, washed with deionised water. Roots, shoots and leaves were separated and collected in paper bags. The detached plant parts were dried to a constant weight by placing in an oven at 60 °C for 36 h. The dried samples were milled in a grinding machine. Digestion of the powdered plant material (1 g by weight) was carried out with a mixture of concentrated nitric acid and perchloric acid (3 ml) in 25 ml of deionized water on a hot plate at 350 °C for a few hours till the solution was clear. The volume of the reduced sample was again made up to 50 ml with deionized water. The digested samples were subjected to estimations of Cr and Na on an atomic absorption spectrophotometer and a flame photometer. Prepared standards (Sigma) of the pure metals were run for preparing the standard calibration curves. Bioconcentration factors for the metals were calculated by dividing the amount of metal extracted in the plant by the amount present in the soil.

2.4. Phytoremediation of soil with S. fruticosa (field trials)

Field experiments were carried out in the same season parallel to greenhouse experiments. For these experiments the same highly contaminated fields were selected adjacent to Depalpur Road, Kasur, located opposite to KTWMA. The soil for the experiment was ploughed and experimental beds (3 × 6 m) were prepared in bare contaminated area. The experiment was laid out in a completely randomized design [12] on a pattern similar to the pot experiment. The experiment comprised of the same sets of treatments with five replicates each.

Areas of 20 cm^2 were marked in the experimental bed and ten seeds per area were sown. Thinning and weeding was done after 15 days of germination and the healthiest plant was retained in each area. Moisture was maintained at field capacity by sprinkling the ground water from the nearest supply daily. Application of EDTA was carried out in the contaminated soil after one month of sapling establishment in the form of 1, 5 and 10 mmol kg^{-1} soil doses.

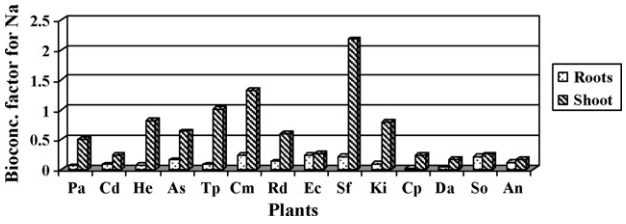

Fig. 1. Bioconcentration factors of Na in different wild plants growing at the contaminated sites of Kasur.

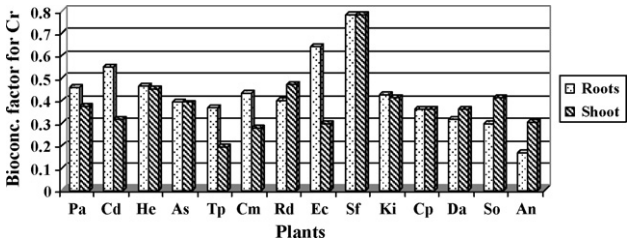

Fig. 2. Bioconcentration factors of Cr in different wild plants growing at the contaminated sites of Kasur. Pa: *Panicum antidotale*; Cd: *Cynodon dactylon*; He: *Heliotropium eichwaldii*; As: *Alternantha sessilis*; Tp: *Trianthema portulacastrum*; Cm: *Chenopodium murale*; Rd: *Rumex dentatus*; Ec: *Echinochloa colonum*; Sf: *Suaeda fruticosa*; Ki: *Kochia indica*; Cp: *Calatropis procera*; Da: *Dichanthium annulatum*; So: *Salvadora oleoides*; An: *Acacia nilotica*.

All plants were allowed to mature during four months time and were subjected to physical and chemical analyses as given above.

2.5. Statistical analyses

The statistical analysis of the data was carried out using Co-Stat Software program version 6.3 (http://www.cohort.com/Index.html). The data were analyzed for significant differences ($p < 0.05$) of main effects using a two-way ANOVA and Duncan's Multiple Range test.

3. Results and discussion

3.1. Field observations in Kasur

All observations were carried out in the contaminated soil of Kasur. Twelve plant species were of regular occurrence in the contaminated field soil. The natural community of plants appearing in soil contaminated by the leather industry was highly resistant to metal contamination and most of the plants were salinity tolerant among which *S. fruticosa* and *K. indica* are well known halophytes. *C. dactylon* and *C. procera* also represent plants of deserted, dry and extremely infertile soils.

The biomass of a halophyte *S. fruticosa* was far greater than all others (Table 1). The plant forms large patches in soil with soluble salinity and appears to be a short shrub. *C. procera* also showed a good biomass.

Identification of metal hyperaccumulator species has become an impetus for phytoremediation research. Some plants can take up, translocate, and tolerate levels of metals that would be toxic for others. *S. fruticosa* outweighed all other plants in the context of accumulation of Na (Table 2). *C. murale* also accumulated a good amount of Na (Fig. 1). The shoots showed significantly higher bioconcentration factors for metals than roots in all the plants. The shoot per root ratio was higher than or nearly 10 in *T. portulacastrum*, *H. eichwaldii*, *S. fruticosa* and *C. procera*.

The accumulation for Cr was also the highest in case of *S. fruticosa* and fairly good in case of *E. colonum* and *C. dactylon* (Table 3) corresponding to the bioconcentration factors as shown graphically in Fig. 2. In this study, amount of Cr up to 2521 mg kg^{-1} dry weight of roots and 2483 mg kg^{-1} dry weight of shoots has been observed in *S. fruticosa*. Thus, in addition to being a halophyte it can also be called a good accumulator of Cr. This also explains why *S. fruticosa* is such a successful natural inhabitant of tannery effluent contaminated soil. In all cases roots showed higher bioconcentration factors than shoots. The shoot/root ratio was higher than one in case of *R. dentatus* and *D. annulatum*, indicating a good root to shoot translocation. *S. fruticosa* and some other plants also showed quite a good root to shoot translocation of nearly one.

3.2. Contaminated soil used in experiments

The contaminated soil taken from the effluent contaminated area was analyzed without EDTA and after application of EDTA in different amounts in terms of mmol kg^{-1} of soil. The soluble content of the metals studied significantly increased with increasing amount of EDTA, especially for Cr (Table 4). The pH of soil also changed from basic to circumneutral with addition of EDTA. No significant change was observed in conductivity. The total metal content was much higher for Na as compared to Cr.

3.3. Phytoremediation of soil with S. fruticosa (greenhouse trials)

3.3.1. Effect of EDTA application on biomass of plants

EDTA application in 1, 5 and 10 mmol kg^{-1} dry weight of soil showed relatively poor growth in *S. fruticosa* and the dry weight of the plants showed a significant reduction with increasing amount of EDTA. The maximum biomass of the plants was observed in contaminated soil without EDTA treatment. The phytotoxic effect of EDTA was quite evident in all parts of plants especially in 10 mmol kg^{-1} treatment of EDTA. The maximum biomass was observed in the stems as compared to roots and leaves (Fig. 3). However, no toxicity symptoms were observed. The phytotoxic effect of EDTA has been shown by Huang et al. [15] on *Z. mays*, *P. sativum*, *H. annuus* and *Solidibago bicolor* and by Chen and Cutright [16] on sunflower without any toxicity symptoms. Whereas a significant reduction in biomass along with toxicity symptoms like wilting and necrosis was observed in *Z. mays* by Neugschwandtner et al. [17] and the decrease in biomass was directly proportional to the dose of EDTA applied as observed in the present study. Shen et al. [18] reported an insignificant decrease in dry weight of *B. rapa* while Zaier et al. [19] did not observe any significant reduction in growth of *Brassica napus* when EDTA was applied.

3.3.2. Effect of EDTA application on metal uptake

Synthetic chelating agents are well known in enhancing metal uptake in plants and uptake efficiency depends on the plants and heavy metals being studied. The efficiency of EDTA when applied to contaminated soil increased the bioavailable metals in the soil and aided in greater bioaccumulation in plants [15,20,21]. Treatment with EDTA did not show a significant impact on Na uptake in

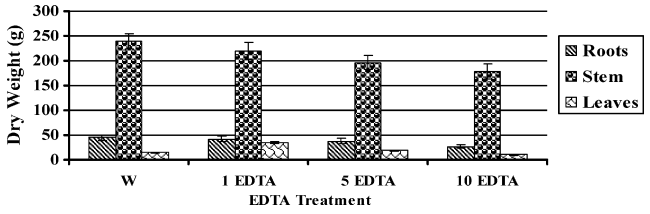

Fig. 3. Dry weight of *Suaeda fruticosa* grown in contaminated soil in pots with EDTA treatment (W = without EDTA, 1 EDTA, 5 EDTA, 10 EDTA = application of EDTA in the form of 1 mmol kg^{-1}, 5 mmol kg^{-1} and 10 mmol kg^{-1} doses respectively).

Table 1
Dry weight (g) of different wild plants growing at the contaminated sites.

Plant part	Wild plants													
	Pa	Cd	He	As	Tp	Cm	Rd	Ec	Sf	Ki	Cp	Da	So	An
Roots	1.8	1.7	4.0	1.9	1.4	2.1	1.2	1.2	86.6	3.3	18.8	1.1	64.5	–
Shoots	7.2	5.0	9.6	12.5	11.3	4.1	4.3	4.3	205.6	5.0	44.7	5.0	145.6	–
Average	4.5cd	3.4cd	6.8cd	7.2c	6.3cd	3.1cd	2.8d	2.8d	146.1a	4.2cd	31.8b	3.1cd	105a	–

Least Significant Difference (LSD) ($p = 0.05$) for plants = 3.65.
Data indicate means of five replicates. Data with the same letter represent statistically identical values ($p = 0.05$).
Pa: *Panicum antidotale*; Cd: *Cynodon dactylon*; He: *Heliotropium eichwaldii*; As: *Alternantha sessilis*; Tp: *Trianthema portulacastrum*; Cm: *Chenopodium murale*; Rd: *Rumex dentatus*; Ec: *Echinochloa colonum*; Sf: *Suaeda fruticosa*; Ki: *Kochia indica*; Cp: *Calatropis procera*; Da: *Dichanthium annulatum*; So: *Salvadora oleoides*; An: *Acacia nilotica*.

Table 2
Accumulation of Na by different wild plants (mg kg^{-1} dry wt.) growing at the contaminated sites.

Plant part	Wild plants													
	Pa	Cd	He	As	Tp	Cm	Rd	Ec	Sf	Ki	Cp	Da	So	An
Roots	3280	4791	4655	9270	4898	14 435	7706	14 275	13 213	6092	1422	1855	11 300	6660
Shoots	31 073	14 317	49 224	38 430	61 307	80 333	36 293	16 191	13 246	48 666	14 251	9964	13 500	8840
Average	17 177f	9554g	26 940d	23 850e	33 102c	47 384b	21 999e	15 233f	72 841a	27 379d	7836gh	5190	12 400f	7750gh

Least Significant Difference (LSD) ($p = 0.05$) for plants = 2502.
Data indicate means of five replicates. Data with the same letter represent statistically identical values ($p = 0.05$).

Table 3
Accumulation of Cr by different wild plants (mg kg^{-1} dry wt.) growing at the contaminated sites.

Plant part	Wild plants													
	Pa	Cd	He	As	Tp	Cm	Rd	Ec	Sf	Ki	Cp	Da	So	An
Roots	1027	1238	1045	883	825	967	894	1433	1756	954	807	713	729	409
Shoots	842	704	1020	876	436	628	1056	664	1379	925	805	813	1020	746
Average	934bc	971bc	1032b	880bc	630c	798bc	975bc	1048b	1568a	940bc	806bc	763bc	874bc	577c

Least Significant Difference (LSD) ($p = 0.05$) for plants = 326.
Data indicate means of five replicates. Data with the same letter represent statistically identical values ($p = 0.05$).

Table 4
Analysis of contaminated soil used for pot and field experiments. *Type of soil*: tannery effluent contaminated soil, *Soil texture*: silty loam.

	pH	ECe (dS m^{-1})	Amount of Na (mg kg^{-1})		Amount of Cr (mg kg^{-1})	
			Total	Soluble	Total	Soluble
W	8.46a	16.53a	835b	54 368	19.5b	2459
1 EDTA	8.10b	17.29a	1373ab	–	22.5ab	–
5 EDTA	7.66c	17.67a	1655ab	–	24.6ab	–
10 EDTA	7.65c	17.81a	2080a	–	26.5a	–
LSD	0.32	0.72	894	–	4.49	–

LSD: Least Significant Difference.
Data indicate means of five replicates. The letters following the figures indicate the similarities and differences between values pertaining to significance level. Data with the same letter represent statistically identical values ($p = 0.05$).
W: contaminated field soil without EDTA; 1 EDTA: contaminated field soil + EDTA@1 mmol kg^{-1} soil; 5 EDTA: contaminated field soil + EDTA@5 mmol kg^{-1} soil; 10 EDTA: contaminated field soil + EDTA@10 mmol kg^{-1} soil.

S. fruticosa (Table 5). The bioaccumulation of Na was high because of the inherent capability of the plant to grow in Na rich environment. The bioconcentration factor of Na was maximum for leaves in all cases (Fig. 4). On the other hand, higher EDTA doses caused a significant reduction in Na bioconcentration in roots and leaves (Table 6).

EDTA has shown to increase Pb uptake in *P. sativum* and *Z. mays* [15] in *Hemidesmus indicus* [22] and Cd and Pb in *B. rapa* [17]. Even the behavior of EDTA is metal specific. It is known to increase uptake of Cd and Ni but not Cr in the shoots of *H. annuus* [16]. According to Hernandez-Allica et al. [23] above 250 μM EDTA decreased root and shoot uptake of Cd and Zn in plants. A significant increase

Table 5
Accumulation of Na by *Suaeda fruticosa* (mg kg^{-1} dry wt.) grown in contaminated soil with EDTA applications (greenhouse trial).

Plant part	Treatment				
	W	1 EDTA	5 EDTA	10 EDTA	Average
Root	5096	6235	6638	6249	6054ab
Stems	10 235	16 882	12 362	10 238	12 429b
Leaves	69 686	74 094	69 913	62 558	69 051a
Average	28 339a	32 389b	29 637a	26 348a	

LSD ($p = 0.05$) for roots and stems and leaves = 9398, LSD ($p = 0.05$) for treatments = 10 853.
Data indicate means of five replicates. Data with the same letter represent statistically identical values ($p = 0.05$).

Table 6
Accumulation of Cr by *Suaeda fruticosa* (mg kg^{-1} dry wt.) grown in contaminated soil with EDTA applications (greenhouse trial).

Plant part	Treatment				
	W	1 EDTA	5 EDTA	10 EDTA	Average
Roots	1441	1684	1603	1597	1581a
Stems	1558	1550	1533	1513	1538a
Leaves	1518	1677	1672	1447	1578a
Average	1506a	1637a	1603a	1519a	

LSD ($p = 0.05$) for roots and stems and leaves = 247, LSD ($p = 0.05$) for treatments = 285.
Data indicate means of five replicates. Data with the same letter represent statistically identical values ($p = 0.05$).
W: contaminated field soil without EDTA; 1 EDTA: contaminated field soil + EDTA@1 mmol kg^{-1} soil; 5 EDTA: contaminated field soil + EDTA@5 mmol kg^{-1} soil; 10 EDTA: contaminated field soil + EDTA@10 mmol kg^{-1} soil.

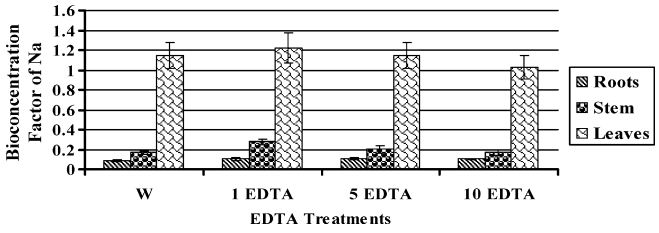

Fig. 4. Bioconcentration factor of Na in different parts of *Suaeda fruticosa* grown in contaminated soil in pots with EDTA treatment (W = without EDTA, 1 EDTA, 5 EDTA, 10 EDTA = application of EDTA in the form of 1 mmol kg^{-1}, 5 mmol kg^{-1} and 10 mmol kg^{-1} doses respectively).

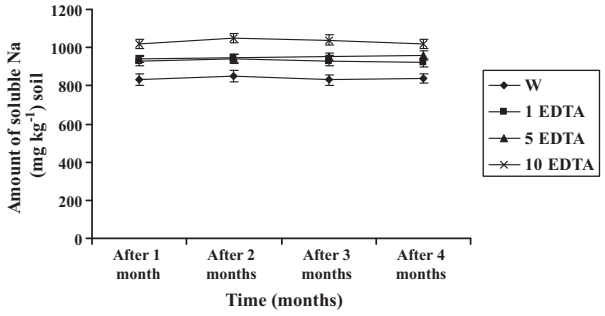

Fig. 6. Amount of Na in the leachate obtained from the pot columns at monthly intervals (W = without EDTA, 1 EDTA, 5 EDTA, 10 EDTA = application of EDTA in the form of 1 mmol kg^{-1}, 5 mmol kg^{-1} and 10 mmol kg^{-1} doses respectively).

was observed in uptake of Cr under the effect of EDTA in *S. fruticosa* in this study as well. The bioconcentration factors for Cr were higher at lower doses of EDTA (Fig. 5) and thus lower doses were found more effective for Cr. Chromium uptake by *H. annuus* was significantly enhanced under the influence of EDTA in hydroponic phytoremediation from a mixed metal solution of As, Cd, Cr, Fe and Ni [24]. Therefore, Cr is one of the metals showing greater mobility with EDTA treatment. Lim et al. [21] demonstrated a poor Cr extraction in short term experiments and found that effective Cr extraction requires a high dosage of chelating agents and a long extraction time. In this case, Cr extraction appeared more effective under low dose of EDTA although treatment time required may be longer under field conditions also depending upon the level of contamination of soil.

EDTA is thought to be a non selective extracting agent reacting with most of the trace metals like Pb, Cd, Cu, Ni, Zn, Mn, present in the soil and also alkaline earth metals like Al, Ca, Fe and Mg [25–27]. In this study, EDTA showed a difference in behavior towards the two toxic metals. Barona et al. [25] have shown that EDTA reacts with Group II, alkaline earth metals like Ca and Mg increasing the leaching risk of these metals while its reaction with Group I, alkali metals like Na is not known. Most of the work on EDTA has been focused on the phytoextraction of heavy metals and in almost all cases the phytoextraction has been enhanced [4]. Heavy metal uptake is mainly enhanced because EDTA forms a metal–chelate complex capable of being translocated easily from root to shoot. Manouchehri et al.

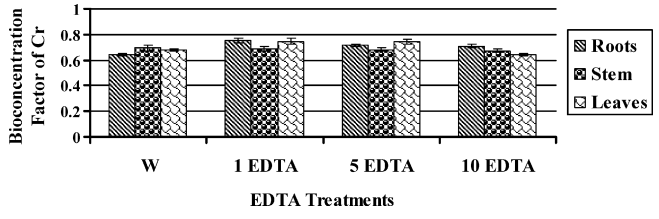

Fig. 5. Bioconcentration factor of Cr in different parts of *Suaeda fruticosa* grown in contaminated soil in pots with EDTA treatment (W = without EDTA, 1 EDTA, 5 EDTA, 10 EDTA = application of EDTA in the form of 1 mmol kg^{-1}, 5 mmol kg^{-1} and 10 mmol kg^{-1} doses respectively).

[28] have shown the reactivity of trace and major elements like Pb, Cu, Cd, Al, Fe, Ca and Mg with EDTA and observed that a concentration of 0.001 M of EDTA was enough to mobilize most of the metals. Although EDTA is non selective and reacts with all types of cations, there were differences in the solubility of the metals which depended on the soil matrix. For example among Pb, Cu and Cd, a greater amount of Pb was solubilized. Similarly among Ca, Al, Fe and Mg, Fe showed a greater solubility [28]. Solubility also depended upon the calcareous and non-calcareous nature of soil. In *S. fruticosa* EDTA was found to be more effective in mobilizing Cr as compared to Na and its extent was higher at lower dose. The non selective nature of EDTA was shown by an insignificant increase in the solubility of Na, in spite of the fact that the amount of Na was about 40 times higher in soil than Cr (Table 1).

3.3.3. *Effect of EDTA application on leaching of metals*

In the greenhouse trials the leachate from the pots was obtained once every month to observe the amount of Na and Cr that was solubilized. A greater amount of Cr was solubilized by EDTA as compared to Na (Figs. 6 and 7).

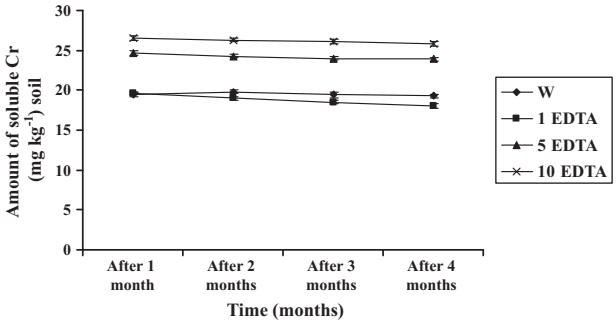

Fig. 7. Amount of Cr in the leachate obtained from the pot columns at monthly intervals (W = without EDTA, 1 EDTA, 5 EDTA, 10 EDTA = application of EDTA in the form of 1 mmol kg^{-1}, 5 mmol kg^{-1} and 10 mmol kg^{-1} doses respectively).

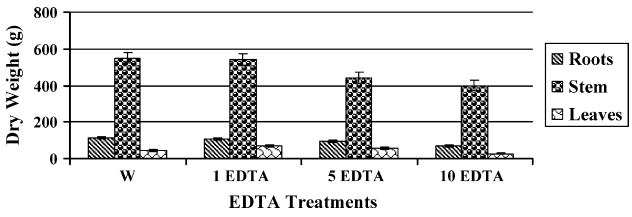

Fig. 8. Dry weight of *Suaeda fruticosa* grown in contaminated fields with EDTA treatment (W = without EDTA, 1 EDTA, 5 EDTA, 10 EDTA = application of EDTA in the form of 1 mmol kg^{-1}, 5 mmol kg^{-1} and 10 mmol kg^{-1} doses respectively).

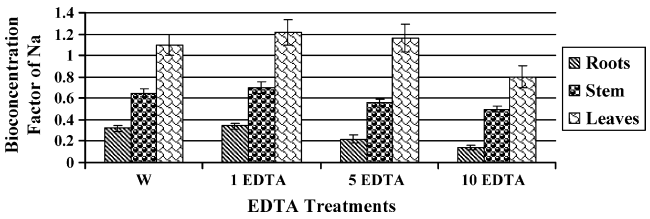

Fig. 9. Bioconcentration factor of Na in different parts of *Suaeda fruticosa* grown in contaminated fields with EDTA treatment (W = without EDTA, 1 EDTA, 5 EDTA, 10 EDTA = application of EDTA in the form of 1 mmol kg^{-1}, 5 mmol kg^{-1} and 10 mmol kg^{-1} doses respectively).

Valuable lessons are to be learnt from a decade of EDTA assisted phytoremediation research with a view to metal leaching risk [29]. Sun et al. [30] using EDTA observed the leaching behavior of four heavy metals from contaminated soil and observed Cu to be the most mobile, Zn and Cd slightly less and Pb to be the least mobile. This differential leaching behavior has also been observed for Na and Cr and Cr appeared to show greater mobility and leaching especially with increasing dosage of EDTA. Chelate doses in split applications have been suggested by Kayser et al. [9] and Shen et al. [18], who observed that though leaching of metals is reduced by split applications of chelates but they do not remain effective in metal uptake by plants. Single dose applications seem to be the most effective approach. Grčman et al. [8] have also observed that the same amount of EDTA applied in split doses was less effective in enhancing metal uptake than by a single dose. Thus, in split applications leaching of metals was probably reduced but the effectiveness of phytoextraction was also checked [8]. In the present study, application of EDTA was made in a single dose and it remained effective in mobilizing Cr within the four month period of study. However, the solubilized amount of metal decreased with time. Risk of leaching due to chelate application has been emphasized by many workers [8,31–38]. When the leaching behavior was studied for four metals in short soil-leaching columns fed with artificial rain under the impact of 5 mmol kg of soil EDTA, only 3.5%, 15.8%, 13.7% and 20.6% of Pb, Cu and Zn respectively were leached down [4]. In this study, EDTA caused mobilization of both metals to some extent but that of Cr was significantly higher in higher doses. The leaching behavior continued during the four months period especially in case of Cr and increased with increasing dose of EDTA. Application of EDTA in the field in solid form (w/w) application of 3 g kg^{-1} dry soil (3 ppm) effectively caused more metal uptake in *B. napus* without any leaching hazard [19]. Thus, the leaching hazard of metals may be minimized with the appropriate method of application and dose management of EDTA.

3.4. Phytoremediation of soil with S. fruticosa (field trials)

For any clean-up technology observed in the laboratory or under greenhouse conditions, field studies are required to confirm its feasibility. When trials were conducted under field conditions with *S. fruticosa*, a significantly higher biomass was obtained as compared to pot experiments with the same soil. As the field trials were conducted in Kasur under natural field conditions, the plants flourished at a much faster rate. The biomass of the plants was almost double that of greenhouse experiments (Fig. 8). However, the biomass of plants decreased significantly under the effect of EDTA treatment. The highest biomass was observed in case of stems as compared to roots and leaves.

Application of EDTA did not show any significant effect on Na bioaccumulation except in leaves (Table 7). On the other hand, at higher doses of EDTA, Na uptake by *S. fruticosa* was reduced (Fig. 9). As compared to the greenhouse experiment, relatively higher bioaccumulation was observed in roots and stem as well, although in all cases, it was less than leaves.

A significant effect of EDTA was observed at lower doses in mobilizing Cr into the plant (Table 8). A higher bioaccumulation of Cr was observed in roots and leaves as compared to stems (Fig. 10). Moreover, from root to shoot and further shoot to leaf translocation of Cr was significantly improved under field conditions. In the greenhouse pot experiment, it was only slightly improved in lower doses. The lower doses of EDTA were effective in Cr uptake into the plants and its translocation into the leaf. At higher doses, lesser amount of metal was translocated to leaves from roots in both the greenhouse and field experiments (Figs. 5 and 10). Although in all treatments, the highest amount of metal was observed in the roots but the metal uptake and its translocation to the aerial parts were also significantly improved in the field. The research focused on phytoremediation of metal contaminated soils deals with a greater amount of metal extraction from soil and its translocation to the aboveground parts. This end can also be achieved by EDTA application in an appropriate dose.

Field studies with chelates have been done by Kayser et al. [9], Grčman et al. [8], Liphadzi et al. [39], Anderson et al. [40], Clemente et al. [41], Xu et al. [42], Neugschwandtner et al. [17] and Zaier et al. [19]. Kayser et al. [9], observed a much higher tissue concentrations of Cd, Zn and Cu of the extracted metals in greenhouse studies as compared to under field conditions. Clemente et al. [41] observed lower Zn, Cu and Pb uptake in the field with *B. juncea*. Similarly, Neugschwandtner et al. [17] observed better results of phytoextraction of Pb and Cd under EDTA application in the greenhouse as compared to field in *Z. mays*. They attributed this to a much greater volume of soil to which the plants were exposed increasing the stress in them. On the other hand, Liphadzi et al. [39] observed a 3-fold greater uptake of Cd, Ni and Pb in sunflower with EDTA application of 1.0 g kg^{-1} under field conditions. Other scientists have shown better effectiveness of phytoextraction in field correlating with greenhouse studies e.g. of gold with *Z. mays* and *B. juncea* [40]. Zhuang et al. [10] observed 19, 2 and 13 fold greater Pb phytoextraction rates in field by *Viola baoshanensis*, *Vetiveria zizanoides* and Rumex K-1. The field grown plants of *S. fruticosa* had almost double the amount of biomass as compared to the greenhouse plants. From the morphological results of plants used in this experiment and heavy metal uptake, it can be concluded that plants for phytoex-

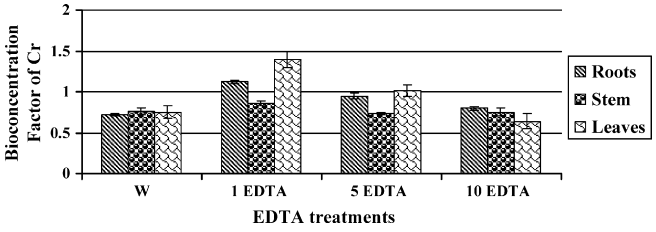

Fig. 10. Bioconcentration factor of Cr in different parts of *Suaeda fruticosa* grown in contaminated fields with EDTA treatment (W = without EDTA, 1 EDTA, 5 EDTA, 10 EDTA = application of EDTA in the form of 1 mmol kg^{-1}, 5 mmol kg^{-1} and 10 mmol kg^{-1} doses respectively).

Table 7
Accumulation of Na by different plant parts of *Suaeda fruticosa* (mg kg^{-1} dry wt.) grown in contaminated field with EDTA applications (field trial).

Treatments	W	1 EDTA	5 EDTA	10 EDTA	Average
Roots	73 524	34 338	30 468	31 489	42 455b
Stems	64 454	27 935	27 968	28 054	37 103a
Leaves	76 220	37 128	32 430	32 840	44 654b
Average	71 399b	33 134a	30 289a	30 794a	

LSD ($p = 0.05$) for roots and stems and leaves = 15 365, LSD ($p = 0.05$) for treatments = 20 442.
Data indicate means of five replicates. Data with the same letter represent statistically identical values ($p = 0.05$).

Table 8
Accumulation of Cr by different plant parts of *Suaeda fruticosa* (mg kg^{-1} dry wt.) grown in contaminated field with EDTA applications (field trial).

Treatments	W	1 EDTA	5 EDTA	10 EDTA	Average
Roots	1935	2291	2381	2521	2282a
Stems	1568	2305	2468	2483	2206a
Leaves	2089	2630	2425	2331	2369a
Average	1864a	2409b	1758a	2445b	

LSD ($p = 0.05$) for roots and stems and leaves = 260, LSD ($p = 0.05$) for treatments = 268.
Data indicate means of five replicates. Data with the same letter represent statistically identical values ($p = 0.05$).
W: contaminated field soil without EDTA; 1 EDTA: contaminated field soil + EDTA@1 mmol kg^{-1} soil; 5 EDTA: contaminated field soil + EDTA@5 mmol kg^{-1} soil; 10 EDTA: contaminated field soil + EDTA@10 mmol kg^{-1} soil.

traction should have two important characteristics, they should be able to tolerate and accumulate high levels of toxic metals and should also have a rapid growth rate. Neugschwandtner et al. [17] observed lesser growth and greater stress in plants of *Z. mays* when exposed to field conditions probably because they had used a cultivated plant in the contaminated fields. In the present study, a greater volume of available soil and natural environment has not only favored *S. fruticosa* to produce a high biomass but also extract greater amount of metals in the field under the effect of lower doses of EDTA. This is because the plant is well adapted to strive under stress conditions. So, selection from among the indigenous flora could be more useful while making phytoextraction strategies in the field as has been given by Santos et al. [5] in case of *B. decumbens*, a native plant of the area.

4. Conclusion

The two toxic metals present in the tannery effluent contaminated lands can be very effectively remediated using the indigenous fast growing halophyte *S. fruticosa*. It is the inherent character of this plant to absorb large quantities of Na whereas Cr can be mobilized using lower doses of EDTA in the field. The higher doses of EDTA could cause leaching risk of Cr. Therefore, a small dose of EDTA would not only mobilize Cr for plant uptake but would also minimize the risk of leaching.

Acknowledgement

This work is a part of a project sponsored by the Federal Environmental Management Unit (FEMU), National Drainage Program, WAPDA, Pakistan.

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