BIOREMEDIATION POTENTIAL OF SOME LOCAL GRASSES OF KARACHI CITY

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خلاصہ

موجودہ تحقیقی کام صنعتی علاقہ شرافی گوٹھ کراچی میں عام طور پر اگنے والی چار اقسام کی گھاس کھبل، ویٹیور، ہاتھی گھاس اور جنگل چاول گھاس پر کیا گیا ہے۔ سب سے زیادہ وزن تازہ اور خشک ہاتھی گھاس میں مشاہدہ کیا گیا جبکہ کچھ اقسام کی جڑوں اور ڈالیوں میں بھی پایا گیا ہے۔ ویٹیور گھاس میں لیڈ دھات (0.8 mg/kg) کی مقدار سب سے زیادہ پائی گئی جبکہ ہاتھی گھاس میں زنک (2.41 mg/kg) آلودہ مٹی میں کھبل گھاس میں کاپر (2.44 mg/kg) اور کیڈمیم (0.3 mg/kg) ملی جلی مٹی میں پائے گئے ہیں۔ جنگلی گھاس میں تمام دھاتوں کی مناسب مقدار پائی گئی ہے۔

Abstract

The study was conducted to explore bioremediation potential of four commonly growing grass species *e.g.* Khabal (*Cynodon dactylon*) (Linnaeus) Persoon, Vetiver (*Vetiveria zizanioides*) (Linnaeus) Nash, Elephant (*Pennisetum purpureum*) Schumach. and jungle rice (*Echinochloa colona*) (Linnaeus) Link, common inhabitants of industrial disposal point, Sharafi Goth, Karachi, Pakistan. The highest fresh and dry weight recorded in Elephant grass (*Pennisetum purpureum*) *i.e.* 360g and 257g respectively. While the maximum shoot (147 cm) and root lengths (249 & 217 cm) were also revealed by the same species. The highest value of Lead (0.8 mg/kg) was observed in Vetiver (*Vetiveria zizanioides*), while Elephant (*Pennisetum purpureum*) showed the maximum bioaccumulation of Zinc (2.41 mg/kg) was observed in contaminated soil. However, high uptake level of Copper (2.44 mg/kg) and Cadmium (0.31 mg/kg) was observed from the mixed soil of Khabal (*Cynodon dactylon*). Whereas, Jungle rice (*Echinochloa colona*) exhibited remarkably low remediation capability.
Keywords: Phytoremediation, Local Grass Species (Khabal, Vetiver, Elephant and Jungle rice grass), Heavy metals, Bioaccumulation Factor**.**

Introduction

In Pakistan, untreated industrial waste discharge is the worse and threatening source of soil, water and sediment pollution (Waseem *et al*., 2014). There are approximately 6000 different industries which are the main source of soil pollution in Karachi such as oil refineries, chemical, pharmaceutical, tanneries, metal, petrochemical and textile industries which produce a massive discharge (Abumaizar and Smith, 1999; Kashif *et al*., 2009). It is reported that approximately 300 million gallons/day of untreated industrial waste is discharged directly into the Lyari and Malir River (Hall, 2000). Multiple studies have been carried out on untreated Korangi industrial discharge that reflects, the high concentration of a hazardous substance in soil not only deteriorate the ecosystem but also affect the quality of life (Abumaizar and Smith, 1999; McBride, 1989; Burd *et al*., 2000).

The main source of inorganic contamination in the soil is the use of sludge, municipal compost, fertilizer, pesticides, exudates, emissions from municipal waste incinerate and residue from smelting industries (Alloway, 2013). These types of dumping sites are the major threat to both terrestrial and aquatic ecosystem resulting in ecotoxicological effects (Daud *et al*., 2017). It mainly disrupts the optimal range of essential elements *i.e.* EC (Electrical Conductivity), CEC (Cation Exchange Capacity), pH, salinity, sodicity, and SAR (sodium adsorption ratio) which is required for the survival of organisms (Wuana and Okieimen, 2011). Ultimately, it also affects the plant growth, contaminates the food chain and also a negative impact on human health (Järup, 2003).

This kind of pollutants are removed from the environment by several methods however, these procedures involve high cost ultimately become less attractive to the industrialist (Dermont *et al*., 2008). On the other hand, some plant species can do this job with less cost and less involvement of labor, by the process known as phytoremediation. Then process of phytoremediation not only remove toxic contaminants from soil and environment but also produce biomass which can be used for biofuel and biofumigation process, will be a source of extra income (Szczygłowska *et al.,* 2011). Phytoremediation is 10 times less expensive process as compare to conventional methods (Willey, 2006). So, the importance of phytoremediation emphasized that it requires further research to explore the new hyperaccumulator species. It has been reported that growth potential of local species on contaminated site responds better in stress condition rather than the plant introduced from a different environment (Salazar and Pignata, 2014).

The present study was carried out to identify the remittable species in industrially contaminated soil, further its phytoremediation potential and also investigated the effect of various heavy metal ions on their growth.

Materials and Methods

To determined the concentration of multiple heavy metals in biomass. A pot experiment was carried out in a greenhouse to evaluate the phytoremediation potential of four wild native grass species (Khabal, vetiver, Elephant and Jungle rice) were tested to cope with Pb, Zn, Cu, and Cd toxicity.

Study Area: Contaminated soil and fresh specimens of wild native grass species were collected from Industrial disposal point (Sharafi Goth), adjacent to the Korangi industrial area, Karachi, Pakistan (Geographical coordinate 24°51'22.5216"N, 67°9'41.8428"E) used in a pot experiment. It has a high relative humidity level around 70-80% and temperature range about 29 °C-38 °C.

Sample Collection: The soil was taken from the depth of 0-15 cm with a stainless steel trowel and brought to the laboratory for initial analysis. The soil was analyzed for EC, CEC, pH, SAR, organic matter, Soil texture, phosphorus, potassium and also the concentration of heavy metals (Pb, Zn, Cu and Cd). Four wild local reported grass species (Khabal, Jungle rice, Vetiver and Elephant grass) were acquired from an Industrial waste disposal point in Sharafi Goth, Karachi, Pakistan.

Initial Screening of Reemittiable Plant Species: Initially, the selected specimens were planted in contaminated soil for 20-25 days. The well, responsive species with vigorous growth were selected for pot experiment.

Experimental Setup: The methodological approach of this study was a version of a randomized complete block design. The pot experiment was conducted from October to December 2016. The average temperature during this experiment was approximately 19-25°C.Two types of soils were selected one having a normal range of soil characteristics (Healthy soil) and second (Contaminated soil). Plants were tested at three levels of contamination (100% healthy soil, 50% healthy + 50% contaminated soil, 100% contaminated soil) with three replicates, for their remediation potential. Wild local grass species were planted by 5-6 specimens in each pot allowed to grow filled with tap water whenever needed.

Sample Analysis: Each plant species was harvested after three month and separately washed with clean water. The observations were started with the measurement of whole plant length than separately measured root and shoot length. The freshly collected samples were oven dried at 60 $^{\circ}$C and ground for analysis. For the determination of Pb, Zn, Cd, and Cu in Soil and plant samples, 2gm each sample was taken in digestion tube. Nitric acid was added to digestion tube and placed it on digester for approximately 170 ^{o}C. Near the end of digestion one ml hydrogen peroxide (H_2O_2) added to it and filtered. Prepare its volume and further analyzed by Atomic absorption spectrophotometer (Hitachi-8100, Japan).

Data generated by this study was analyzed by STATISTICA-2013.

Results and Discussion

Table 1. shows The results of soil analysis used as indices for the evaluation of the remediation soil. The contaminated soil was saline-sodic, pH > 9, the concentration of heavy metals (Zn, Cu, Cd and Pb) exceed the safe level with K^+ more than 800 mg/kg that inhibit the plant growth.

The cultivation of native species (Khabal, Vetiver, Jungle rice and Elephant grass) significantly improved soil structure, reduced soil salinity, EC, SAR and neutralized pH. Among all four species, Khabal grass exhibited a higher degree of tolerance, neutralized the pH at all three levels of contamination. The lowest value of EC (1.36 dS/m), SAR around 2.7 and the safe level of P:K for vigorous plant growth were recorded in 100% contaminated soil shown in (Table 2). It showed that Khabal grass has the ability to tolerate saline-sodic soils and also has great potential to remediate soil contaminants. It has been also reported in previous researches that Khabal grass has the ability to reclaim saline-sodic soil and degrade recalcitrant organic compound and also petroleum sludge by the process of the rhizodegradation process (Shahandeh and Hossner, 2000; Abou-Shanab *et al.,* 2007). As Khabal grass contains fibrous and extended root system, that significantly enhances the microbial degradation activity in rhizosphere (Emenike *et al.,* 2018; Nguemté *et al.,* 2018).

In present study, higher biomass production is an important aspect because, the plant biomass can be used for different commercial purposes (Shukla *et al.,* 2011; Houben *et al.,* 2013), and in this case, maximum biomass in terms of average fresh weight and dry weight, 360 g and 257 g respectively, was observed in mix soils (50% healthy + 50% contaminated soil) in 90 days' experiment, was recorded for Elephant grass shown in (Table 3, Fig. 1). While the Khabal (43g) and Vetiver (34g) revealed the minimum average fresh and dry weight in (T1 & T2).

The highest average shoot and root length recorded in *Pennisetum purpureum* species 147 and 249 cm in 50 and 100% contaminated soil. Simultaneously, lowest average shoot and root length exhibited by *Vetiveria zizanioides i.e.* 32 and 25cm in (T1 & T2) (Fig. 2). These results showed that Elephant grass efficiently survived in moderately contaminated soils and produced high biomass as already reported that Elephant grass is a fast-growing species, with high biomass up to 30-40 dry t/ ha/ y. It has great potential for bleached pulp production, paper production and also used in composting process (Ayotamuno *et al.,* 2010; Nguemté *et al.,* 2018; Souza *et al.,* 2018).

The investigated grasses were also observed for their potential to accumulate toxic metals during the remediation process where different plant species showed different accumulation ranges for different elements as already reported by many researchers (Marchiol *et al.,* 2004; Rascio and Navari-Izzo, 2011; Tangahu *et al.,* 2011; Laghlimi *et al.,* 2015;). Highest bioaccumulation of Lead (0.8 mg/kg) was observed by vetiver grass in mix soils, however, the lowest Lead accumulation (0.04 mg/kg) was recorded for jungle rice as shown in Table 4. Vetiver grass well reported for its bioremediation potential which can tolerate >1500 mg/kg concentration of Lead and mainly used to treat septic tank effluent (Truong and Hart, 2001; Chen *et al.,* 2004). It is also known to remove the high concentration of Nitrogen (N) and Phosphorus (P) and used to control the high growth rate of blue-green algae (Truong, 1994; Chen *et al.,* 2004; Roongtanakiat *et al.,* 2007). Moreover, it has been observed that it has the ability to remove agrochemical pollutant and prevented the accumulation of toxin in the ecosystem (Danh *et al*., 2009).

The maximum accumulation (2.5 mg/kg) of Zinc was in mix soils, obsorbed by Elephant grass, whereas, the lowest accumulation was 0.07 mg/kg by Jungle rice grass Table 4. Elephant grasses wildly reported to treat petroleum hydrocarbon with the help of its deep root system by many researchers. Additionally, it produces the type of exudates which significantly facilitate a large number of microorganism that enhances the degradation of organic pollutant (Bauddh *et al.,* 2017; Souza *et al.,* 2018).

In the case of Copper (Cu) and Cadmium (Cd), the highest accumulation was observed by khabal grass. The uptake of Copper (Cu) was 2.5 mg/kg in mixed soils while uptake of Cadmium (Cd) was 0.31 mg/kg in the mixed soil. Khabal grass has the ability to accumulate high concentration of Copper (Cu) and Cadmium (Cd) by the process of rhizodegradation and remediate crude oil, benzofuran, fly ash and aged sludge type soil contamination (Bauddh *et al.,* 2017; Bharti *et al.,* 2017).

The objective of the present work was to analyze that the studied plant species either have a potential to degrade the industrial waste pollution or not, and results indicate that all species have the ability to reduce organic and inorganic pollutant. The jungle rice grass has been reported in previous variety of experimental conditions that it has the ability to phytoextract Zinc and Chromium (Subhashini and Swamy, 2015). In this case, however, results were contrary and the Elephant grass species was observed to be the high biomass species. The best hyperaccumulator species for the Lead was Vetiver grass, its uptake level was 0.8 mg/kg and high accumulation of Zinc (Zn) was observed in Elephant grass, with uptake level of 2.41 mg/kg in contaminated soil (Fig. 3). The high uptake level of Copper (2.44 mg/kg) and Cadmium 0.31 mg/kg was observed in the mixed soil of Khabal grass (Fig. 4). Statistical Analysis showed that the contaminated soil gave a much better reduction of pollutants like electrical conductivity, pH, sodium adsorption ratio and heavy metals. Biomass results reveal that the growth rate of all species was healthier and vigorous it exhibited that all species have the ability to tolerate in polluted conditions.

Table 1. The physiochemical properties of soil used in the pot experiment.

S. No.	Physiochemical properties of soil	Initial healthy soil Mean±SD	Initial contaminated soil Mean±SD
1	Electrical Conductivity (dS/m)	3.59 ± 0.20	3.8±0.67
2	pH (1:1)	8.86 ± 0.66	10.2±1.18
3	Organic Matter %	0.42 ± 0.04	0.3±0.12
4	Phosphorus (mg/kg)	51 ± 1.00	80.7±4.04
5	Potassium (mg/kg)	581.33 ± 23.46	806.7±16.07
6	Saturation %	31 ± 1	28.3±3.51
7	Texture	Loamy Soil	Sandy Loam
8	Salinity (dS/m)	1.97 ± 0.06	2.2±0.85
9	Lead (mg/kg)	2.15 ± 0.14	5.0±1.06
10	Zinc (mg/kg)	366.95 ± 6.18	539.6±13.86
11	Copper (mg/kg)	5.42 ± 0.03	6.7±1.27
12	Cadmium (mg/kg)	4.57± 0.18	6.5±1.09

Table 2. Affects on the physiochemical parameters of the soil after the growth of native grass species.

S. No.	Name of species	Treatments	pH (1:1)	EC (dS/m)	SAR	P (mg/kg)	K (mg/kg)	Saturation %
1	Khabal (*Cynodon dactylon*)	T1	7.0	1.46	3.21	37	153	37
		T2	7.0	1.36	2.70	32	153	32
		T3	7.0	2.01	3.10	29	375	36
2	Vetiver (*Vetiveria zizanioides*)	T1	7.5	1.60	3.60	35	153	38
		T2	7.5	1.60	4.30	31	153	32
		T3	7.5	1.73	2.50	32	290	36
3	Elephant (*Pennisetum purpureum*)	T1	8.1	2.73	5.90	40	95	37
		T2	8.5	3.12	7.40	37	95	32
		T3	8.2	1.87	5.20	29	153	31
4	Jungle rice (*Echinochloa colona)*	T1	8.0	1.78	3.20	32	250	37
		T2	8.5	2.17	4.00	40	187	32
		T3	8.2	1.89	4.20	37	250	31

T1 = Treatment 1(100 % healthy soil), T2 = Treatment 2 (50 % healthy + 50 % contaminated soil) & T3 = Treatment 3 (100 % contaminated soil).

Table 3. Average biomass of native species in three different treatments.

S. No.	Name of species	Treatment	Fresh wt. of plant (g) Mean ± SD	Dry wt. of plant (g) Mean ± SD	Shoot length (cm) Mean ± SD	Root length (cm) Mean ± SD
1	Khabal (*Cynodon dactylon*)	T1	49.14±4.67	40.74±4.75	91.16±9.19	118.97±18.19
		T2	56.63±5.27	49.62±5.16	63.56±5.94	152±15.83
		T3	124.67±21.7	112.72±11.7	35.86±3.06	216.89±20.98
2	Vetiver (*Vetiveria zizanioides*)	T1	46.63±4.93	39.28±3.72	32.98±3.50	103.17±10.44
		T2	43.44±4.34	34.46±3.82	35.61±3.65	25.58±2.65
		T3	55.62±5.73	51.11±5.06	40.75±4.81	130.30±11.95
3	Elephant (*Pennisetum purpureum*)	T1	340±30.14	248±23.81	120.04±10.6	120.51±10.63
		T2	215±22.91	191.91±18.5	147.15±14.6	111.33±10.50
		T3	360±30.55	257.62±23.1	102.01±11.5	249.67±15.70
4	Jungle rice (*Echinochloa colona*)	T1	75±8.07	68.79±6.36	35.97±3.76	88.59±8.20
		T2	100±14.54	82.30±8.46	29.17±2.99	59.99±5.22
		T3	140±22.30	90.24±9.05	53.35±5.72	69.68±6.50

T_1 = Treatment 1 (100% healthy soil), T_2 = Treatment 2 (50% healthy Soil + 50% contaminated soil) & T_3 = Treatment 3 (100% contaminated soil).

Table 4. The concentration of heavy metals in native grass species.

S. No.	Name of species	Treatment	Lead (Pb)	Zinc (Zn)	Copper (Cu)	Cadmium (Cd)
			BCF	BCF	BCF	BCF
1	Khabal (*Cynodon dactylon*)	T1	0.47	0.44	0.55	0.13
		T2	0.41	1.77	1.65	0.31
		T3	0.54	0.78	2.44	0.02
2	Vetiver (*Vetiveria zizanioides*)	T1	0.63	0.66	0.41	0.03
		T2	0.54	1.02	0.54	0.02
		T3	0.79	0.65	0.41	0.01
3	Elephant (*Pennisetum purpureum*)	T1	0.31	1.00	0.89	0.07
		T2	0.43	1.69	0.70	0.13
		T3	0.45	2.41	0.32	0.01
4	Jungle rice (*Echinochloa colona*)	T1	0.43	0.07	0.29	0.0
		T2	0.29	0.17	0.78	0.02
		T3	0.04	0.07	0.38	0.01

T1= Treatment 1 (100 % healthy soil), T 2= Treatment 2 (50 % healthy + 50 % contaminated soil), T3 = Treatment 3 (100 % contaminated soil) & BCF = Bioaccumulation factor (metal conc. in plant/pre-plantation metal conc. in soil).

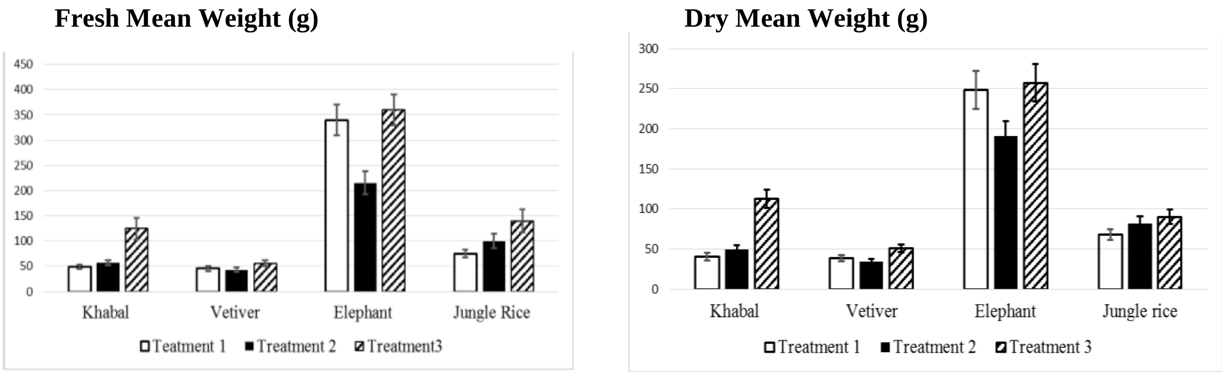

Fig. 1. Fresh and Dry mean weight of native species, Elephant grass exhibited high biomass.

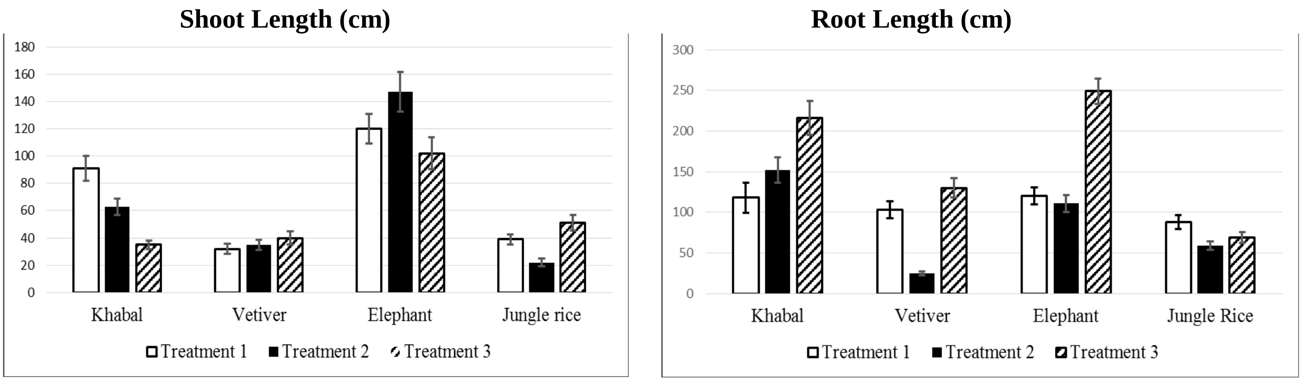

Fig. 2. Shoot and root length of native species, Elephant grass represented highest shoot and root length.

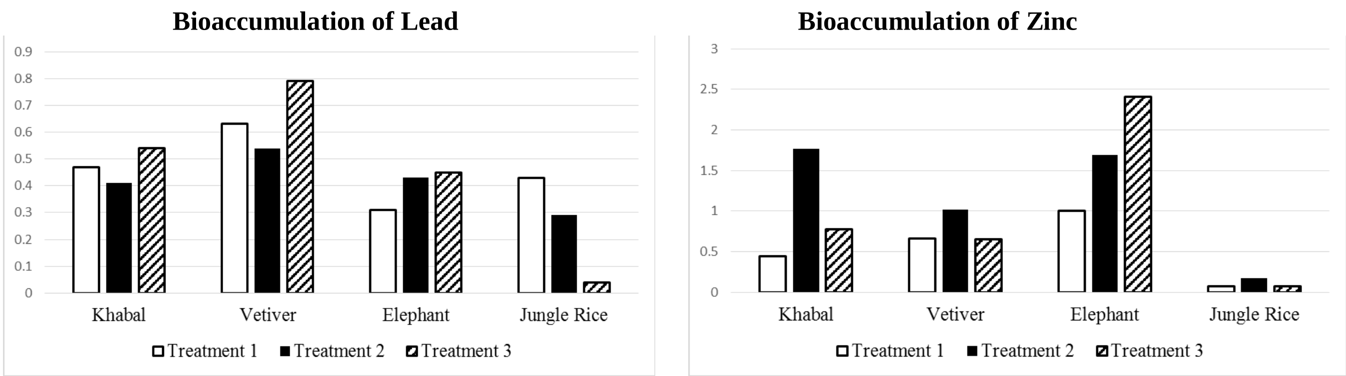

Fig. 3. High bioaccumulation rate of (Pb) observed in Vetiver grass and (Zn) observed in Elephant grass.

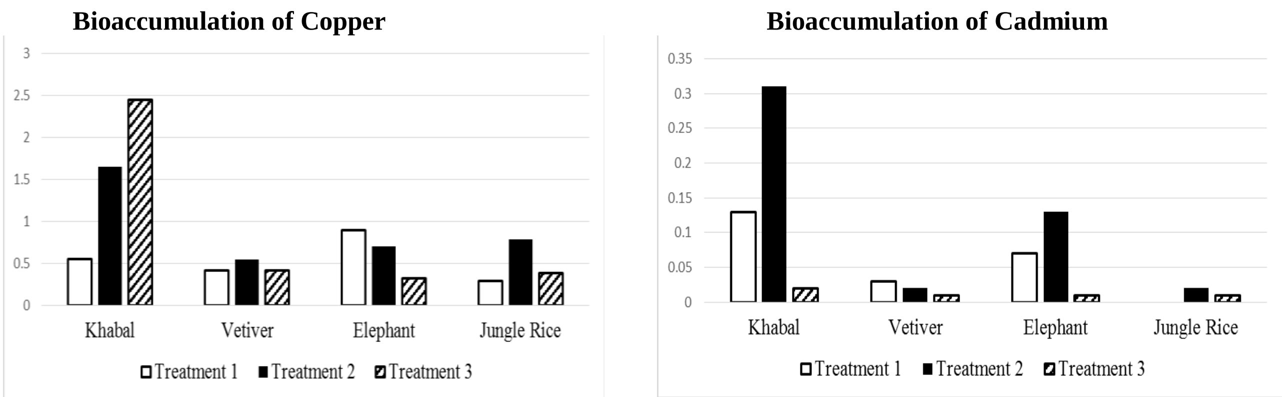

Fig. 4. Maximum bioaccumulation rate of (Cu) and (Cd) in Khabal grass.

Conclusion

From the overall performance of this study, it can be concluded that Khabal, Vetiver and Elephant grass has the potential to remediate soil pollutant. Furthermore, a remarkable reclamation of salinity and sodicity to be achieved by the use of khabal grass and elephant grass produce high biomass. These species can be recommended for the phytoremediation of Lead (Pb), Zinc(Zn), Cadmium (Cd) and Copper (Cu) for contaminated soils.

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