



Phytoextraction potential of different grasses for the uptake of cadmium and lead from industrial wastewater

Sana Ullah^{*1}, Zafar Iqbal¹, Sajid Mahmood¹, Kalsoom Akhtar² and Rehmat Ali¹

¹Soil and Environmental Sciences Division, Nuclear Institute for Agriculture (NIA), Tandojam Tando Jam 70050, Sindh, Pakistan

¹Soil and Environmental Sciences Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad 38000, Pakistan

²National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad 38000, Pakistan

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Abstract

Industrial wastewater contains a variety of contaminants like salts, organic carbon, and heavy metals. Among the heavy metals, cadmium (Cd) and lead (Pb) are considered highly toxic even at low concentration. These metals could enter the food chain through a process of phytoassimilation, hence, lethal for living beings. The present study aimed to investigate Cd and Pb phytoextraction in four grass species viz. Dhab (*Desmostachya bipinnata*), Sporobolus (*Sporobolus arabicus*), Kallar (*Leptochloa fusca*) and Para grass (*Brachiaria mutica*) from industrial wastewater. The grasses were grown hydroponically in plastic pots in industrial wastewater as growth medium under greenhouse conditions. The experiment trial was arranged following completely randomized design (CRD) with three replicates. Results showed that *B. mutica* had maximum shoot metal content (Pb = 21, Cd = 0.66 mg kg⁻¹ dry matter), shoot metal uptake (Pb = 201.8, Cd = 6.39 µg plant⁻¹), translocation factor (Pb = 0.73, Cd = 0.55), shoot dry matter (28.8 g pot⁻¹), and root dry matter (4.2 g pot⁻¹). Root Pb concentration was highest in *B. mutica* followed by *D. bipinnata* and *L. fusca*. Whereas *S. arabicus* showed depressed growth, minimum shoot metal accumulation and uptake potential. Thereby among the tested grass species *B. mutica* could be suitable option to remediate industrial wastewater contaminated with moderate levels of Pb and Cd.

Keywords: Metal uptake, para grass, kallar grass, dry biomass, metal concentration

Introduction

Heavy metals usually indicate environmentally bad metals which have higher atomic number, or atomic weight, density (3.5-7.0 g cm⁻³ or above), properties of metallic substance at room temperature, and are extremely toxic for living organisms even at lower concentration (Duffus, 2002; Mitra, 2019). These metals include lead (Pb), cadmium (Cd), chromium, nickel, arsenic and mercury which are well-known environmental contaminants due to their toxic nature, bioaccumulation and persistence in the environment (Latif *et al.*, 2019; Ali *et al.*, 2019). Heavy metal contamination distresses the environment and food security ultimately posing serious health concerns (Rai *et al.*, 2019; Mushtaq *et al.*, 2019). With rampant inflation in anthropogenic activities, the release of toxic metals into the environment is increasing day by day. According to an estimate, global annual release of Pb and Cd into the environment is 783,000 and 22,000 metric tons, respectively

(Singh *et al.*, 2003). Wastewater from different sources (domestic, municipal and industries) plays significant role in release of heavy metals like Pb and Cd. About 2 million tons of sewage sludge and effluents are polluting the world's water per day (Azizullah *et al.*, 2011). Issue is more serious in developing countries due to improper treatment of domestic, municipal and industrial waste. Such as in India 24% of wastewater (industry and domestic) is treated, whereas in Pakistan only 2% of total sewerage or wastewater is treated properly (IWMI, 2003; Minhas and Samra, 2004; Majeed *et al.*, 2018). Wastewater released from different industries contains a variety of toxic metals such as Pb, Cd, chromium and arsenic (Rehman *et al.*, 2018, 2019; Hussain *et al.* 2019; Afzal *et al.* 2019). Use of industrial effluents/wastewater for cultivation of crops has created the problem of heavy metal contamination in soil (Hussain *et al.*, 2019). Moreover, in developing countries, like Pakistan, shortage of good quality irrigation water also urges the farmers to use wastewater for irrigation purpose

*Email: msunny9887@gmail.com

(Qadir *et al.*, 2010). Use of contaminated wastewater to irrigate crops and vegetables increases the risk of entry of toxic metal into our food chain. For example, Ahmad *et al.* (2019) observed Pb and Cd contamination in wheat grain up to 0.2 and 1.6 mg kg⁻¹ respectively, due to irrigation with wastewater. Hussain *et al.* (2019) also observed Pb and Cd contamination in different vegetables (carrot, radish, and spinach) irrigated with wastewater.

In human beings, heavy metals cause severe health problems such as Pb poisoning especially in children and kidney disease due to Cd toxicity (WHO, 1997; Padmavathamma and Li, 2007; Rehman *et al.*, 2018). Intake of Pb and Cd above their permissible limits could leave carcinogenic impacts in human body (Salman *et al.*, 2019). Cadmium and Pb could easily cause acute and chronic risk even at their lower concentrations, causing renal damage, headache, hypertension and malformation in fetuses (Shi and Chatt, 2014; Zhou *et al.*, 2019). Svetlana *et al.* (2019) observed that higher levels of Pb and Cd caused chronic sinusitis in children. In plants, heavy metals retard shoot and root growth, reduce mineral uptake (Fe, Cu, Zn, Mn), and stimulate the production of reactive oxygen species (H₂O₂, O₂⁻) that damage plasma membrane (Zou *et al.*, 2019). Improper management to dispose-off wastewater can cause ground and/or drinking water contamination with toxic metals, thereby, it is imperative to strictly monitor the wastewater management (Hussain *et al.*, 2019; Rehman *et al.*, 2019).

Recently, much consideration has been paid to various techniques/ approaches in order to remove toxic metals

from effluents discharged in to water bodies such as membrane filtration, reverse osmosis, ion exchange, solvent extraction, absorption/adsorption, precipitation and electrochemical treatments (Miretzky *et al.*, 2006; Singh *et al.*, 2012; Liu *et al.*, 2018). But these techniques cover higher cost, technical expertise and are difficult to apply at large scale (Olguin and Sanchez-Galvan, 2012), thereby, phytoremediation is a well-suited technique. It is cost effective, easy to apply, and is environment friendly (Cheng, 2003; Sarwar *et al.*, 2017; Liu *et al.*, 2018). A hyperaccumulator to be used to extract water soluble heavy metals, should have fast growth, ample root system, higher biomass, resistant to higher concentration of metals, and retain higher metal concentration (Garbisu and Alkorta, 2001; Soda *et al.*, 2012; Rev *et al.*, 2017). In this regard, use of different grass species is gaining promising importance due to their tolerance to phytotoxicity, hinder natural succession of weeds, suitable for grazing, bulk biomass, rapid growth and compact root system. Well-developed root system in grasses triggers phytostabilization by retarding metal mobility and improves metal uptake and their accumulation in plant body hence make them more exploring phytoremediant agent as compared to shrubs, herbs, and trees (Ghosh *et al.*, 2017). Literature has confirmed the potential of different grass species (*Cynodon dactylon* L, *Chrysopogon zizanioides*, *Imperata cylindrical*) for the uptake of heavy metals like Pb and Cd from wastewater and contaminated soil (Pongthornpruek, 2017; Chen *et al.*, 2019; Kiiskila *et al.*, 2019; Zheng *et al.*, 2019).

Table 1: Some salient characteristics of grasses used in the experiment

Grass species	Origin	Salient feature	Salt tolerance potential [Root zone salinity (EC, dS m ⁻¹) causing 50% decrease in yield]	Reference
Dhab (<i>Desmostachya bipinnata</i>)	China, India, Pakistan and Africa	C4 perennial grass, used as fodder source in dry-saline areas	9.0	NIAB (2007); Gulzar <i>et al.</i> (2007); Ahmad <i>et al.</i> (2009); Asrar <i>et al.</i> (2017)
Kallar grass (<i>Leptochloa fusca</i>)	Australia, now well adapted to Pakistan and India	C4 perennial grass, used as animal fodder and reclamation agent for saline-sodic soils	22.0-14.6	Qureshi <i>et al.</i> (1982); Ashraf <i>et al.</i> (2012); Adabnejad <i>et al.</i> (2015)
Para grass (<i>Brachiaria mutica</i>)	African countries	C4 plant, known as buffalo grass, used as pasture, fodder	Salt tolerant	Ashraf <i>et al.</i> (2012); Mohanty and Patra, (2012)
<i>Sporobolus</i> (<i>Sporobolus arabicus</i>)		C4 perennial grass, used as desalinization tool for saline-sodic soils	21.7	Ashraf <i>et al.</i> (2012); Falla <i>et al.</i> (2017); Yobi <i>et al.</i> (2017)



Therefore, present experiment was conducted to investigate the growth response of four different salt tolerant grass species in wastewater and to assess the phytoextraction potential of four different salt tolerant grass species for Pb and Cd from wastewater in order to identify suitable grass species for the remediation of contaminated wastewater.

Materials and Methods

Plant material

Grass species viz. Kallar grass (*Leptochloa fusca*), Para grass (*Brachiaria mutica*), *Sporobolus* (*Sporobolus arabicus*) and Dhab (*Desmostachya bipinnata*) were brought from the Biosaline Research Station Pakka Anna, a substation of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The silent features of the grasses are presented in Table 1. Each grass was grown in plastic pots (three plants/cuttings per pot) containing gravel and three liters of wastewater which was aerated manually by placing pots outside on wooden sticks for two hours twice a day (morning and evening). Each grass was grown in three independent pots, representing three replicates. The pots were placed in the greenhouse of NIAB under natural conditions/ photoperiod, with mean temperatures of 23-26 °C (night-day) and relative humidity of 60-65%. Wastewater was replaced twice a week with its fresh counterpart till plants were harvested after three months. After harvesting root and shoot samples were dried at 70 °C in oven until constant weight and dry matter was recorded.

Table 2: Characteristics of industrial wastewater used in the experiment

Parameter	Value	Unit
pH	7.42	
SAR	254	
EC	3.57	dsm ⁻²
RSC	7	me L ⁻¹
TSS	3390	mg L ⁻¹
CO ₃ ⁻	ND*	mg L ⁻¹
HCO ₃ ⁻	17	mg L ⁻¹
Na ⁺	570	mg L ⁻¹
K ⁺	56	mg L ⁻¹
Total N	2.372	mg L ⁻¹
NH ₄ -N	0.07	mg L ⁻¹
NO ₃ -N	0.0006	mg L ⁻¹
Phosphorus	0.278	mg L ⁻¹
Organic carbon	42.3	mg L ⁻¹
Ca+Mg	10	me L ⁻¹

Cl ⁻	20	me L ⁻¹
Pb	0.92	mg L ⁻¹
Cd	0.03	mg L ⁻¹

ND*: Not detected

Water quality analysis

The wastewater used in this study was collected from industrial wastewater collecting system of village 'Chakaira 217 R.B.' Faisalabad, Punjab, Pakistan. The collected wastewater was stored in the plastic tanks once and analyzed for different quality indicators which are presented in Table 2. The pH was measured by pH meter (HI-8520, Hanna Instruments, UK), EC and total soluble salts (TSS) by conductivity meter (LF-538 WTW, Germany), carbonates (CO₃⁻), bicarbonates (HCO₃⁻), chlorides (Cl⁻), calcium (Ca⁺²) and magnesium (Mg⁺²) were determined by titration method as described by Richards (1954). Sodium (Na⁺) and potassium (K⁺) were determined by Flame Photometer (PFP7-Jenway, UK). Phosphorus was measured by Watanabe and Olsen (1965) method, organic carbon by Riehm and Ulrich (1954) method. Nitrogen (NH₄⁺-N, NO₃⁻-N, total N) was determined by following Kroon (1993) and Kjeldahl method with some modification. Wastewater was concentrated in water bath and the organic bound N was digested by adding the mixture of conc. H₂SO₄, K₂SO₄ and Se as catalyst.

Metal concentration, uptake and translocation factor (TF)

Root and shoot samples were ground and digested following the method described by Wolf (1982). After digestion, extracts of samples were used to determine Pb and Cd concentration by atomic absorption spectrometer (AA240FS, Varian, Australia). Shoot metal uptake was calculated by multiplying shoot dry matter with shoot metal concentration:

$$\text{Shoot metal uptake } (\mu\text{g/plant}) = \text{shoot dry matter (g)} \times \text{shoot metal concentration } (\mu\text{g/g})$$

Translocation factor (TF) was calculated by following formula (Soda *et al.*, 2012):

$$TF = \frac{\text{shoot metal concentration}}{\text{root metal concentration}}$$

Statistical analysis

Statistical differences among different grass species were assessed by one-way analysis of variance (ANOVA) at $p \leq 0.05$ and least significant difference (LSD) test was used to separate the significant means of treatments using Statistix 8.1.



Results

Wastewater characteristics

Different physicochemical parameters of wastewater

(Table 2) revealed that wastewater contained significant amount of essential plant nutrients (N, P, K, Ca, Mg), organic C while high levels of Na^+ (570 mg L^{-1}), SAR (254), RSC (7 mg L^{-1}), and TSS (3390 mg L^{-1}) were recorded. Heavy

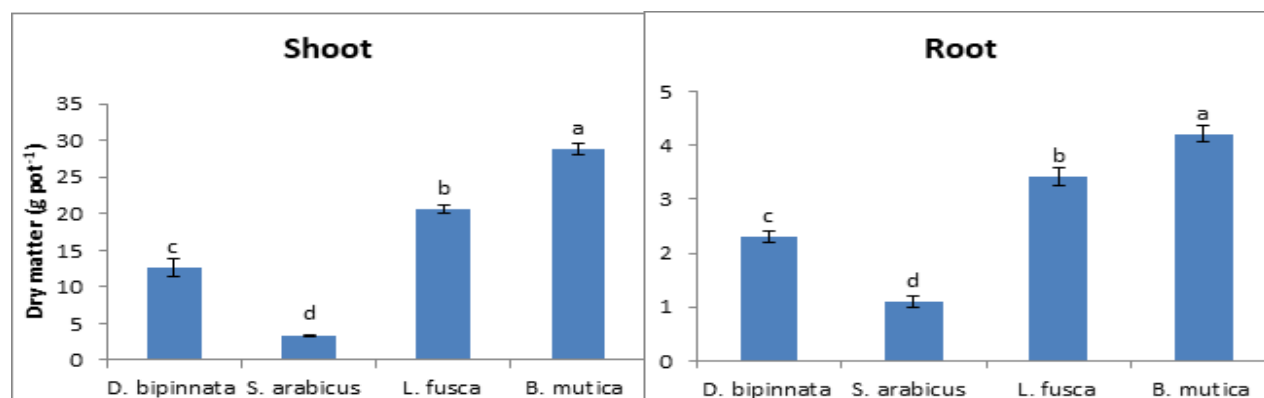


Figure 1: Effect of industrial wastewater on shoot and root dry matter yield of various grasses. Data represent the means of three replicates. The bars sharing similar letters do not differ significantly at $p \leq 0.05$.

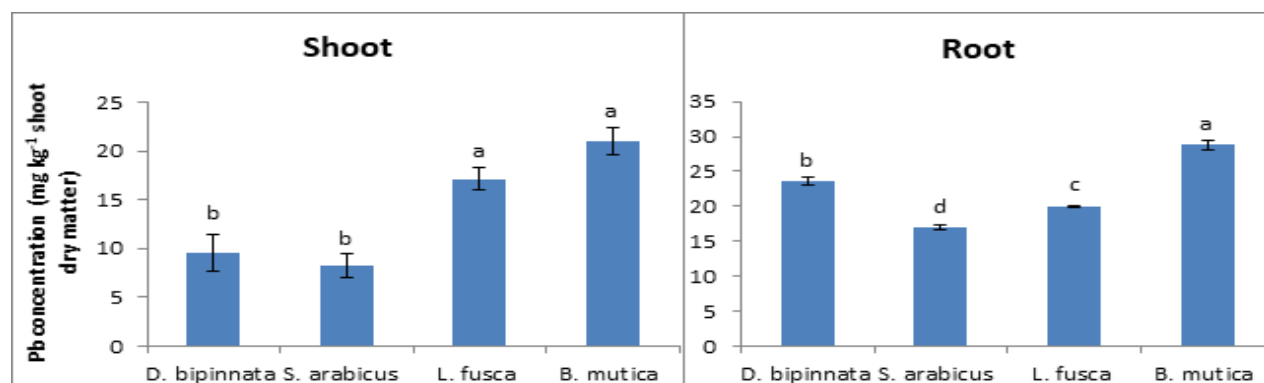


Figure 2: Effect of industrial wastewater on shoot and root Pb concentration of various grasses. Data represent the means of three replicates. The bars sharing similar letters do not differ significantly at $p \leq 0.05$.

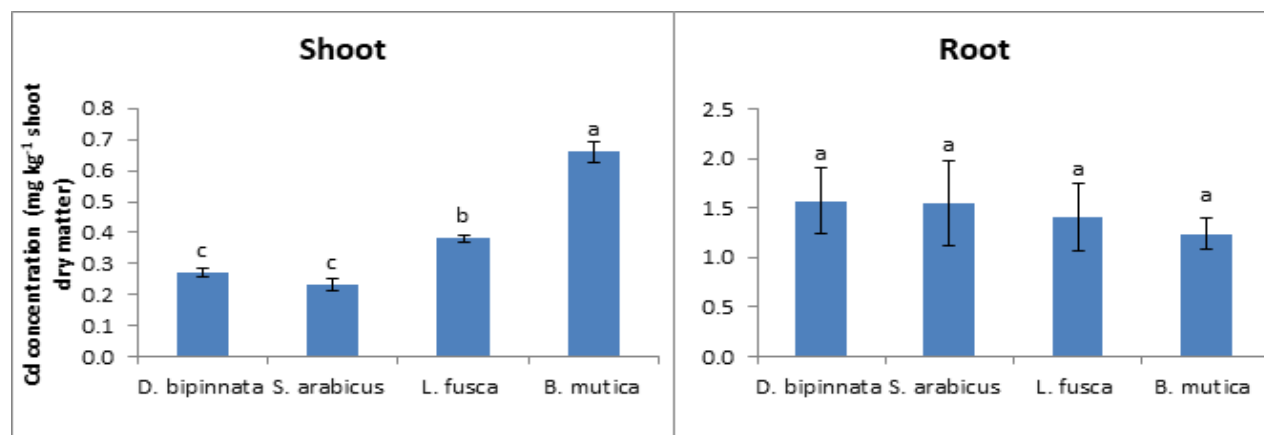


Figure 3: Effect of industrial wastewater on shoot and root Cd concentration of various grasses. Data represent the means of three replicates. The bars sharing similar letters do not differ significantly at $p \leq 0.05$.



metals Pb and Cd were found to be 0.92 and 0.03 mg L⁻¹, respectively.

Dry matter yield

Results regarding shoot dry matter yield are presented in Figure 1. *B. mutica* had maximum shoot dry matter yield (28.8 g pot⁻¹) as compared to other grass species, following *L. fusca* (20.6 g per pot) and *D. bipinnata* (12.6 g pot⁻¹), while the *S. arabicus* showed lowest dry matter yield of 3.4 g pot⁻¹. Similarly, in case of root dry matter yield (Figure 1), application of Chakaira-wastewater significantly affected the root dry matter of all grass species at $p \leq 0.05$. Root dry matter was observed in the sequence of *B. mutica* > *L. fusca* > *D. bipinnata* > *S. arabicus* ranging between 1.1 to 4.21 g pot⁻¹.

Shoot and Root metal concentration

Being statistically at par with each other, *B. mutica* and *L. fusca* had more Pb concentration in shoot, i.e. 21.05 and 17.14 mg kg⁻¹ dry weight, respectively, than *D. bipinnata* and *S. arabicus* with corresponding values of 9.55 and 8.19 mg kg⁻¹ dry weight, respectively (Figure 2). Data regarding root Pb concentration (Figure 2) revealed that highest root Pb concentration was recorded in *B. mutica* (28.82 mg kg⁻¹ dry weight) while the lowest was in *S. arabicus* (8.19 mg kg⁻¹ dry weight). Root Pb concentration in *D. bipinnata* and *L. fusca* was 23.61 and 20.06 mg kg⁻¹ dry weight, respectively. In case of shoot Cd concentration (Figure 3), high values were recorded for *B. mutica* (0.66 mg kg⁻¹ dry weight) followed by *L. fusca* (0.38 mg kg⁻¹ dry weight). The *S. arabicus* and *D. bipinnata* showed lower shoot Cd concentration, 0.23 and 0.27 mg kg⁻¹ dry weight

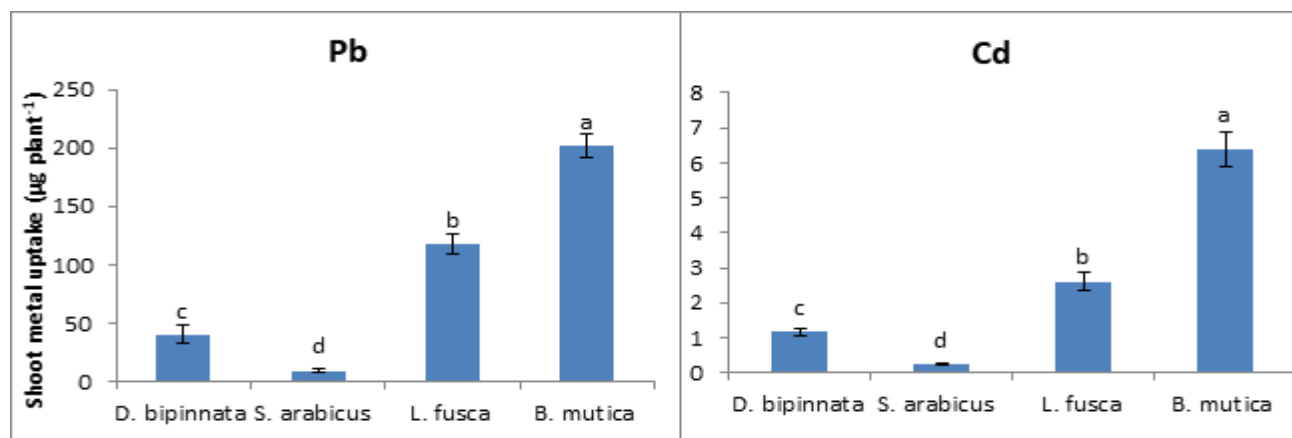


Figure 4: Effect of industrial wastewater on Pb and Cd shoot uptake of various grasses. Data represent the means of three replicates. The bars sharing similar letters do not differ significantly at $p \leq 0.05$.

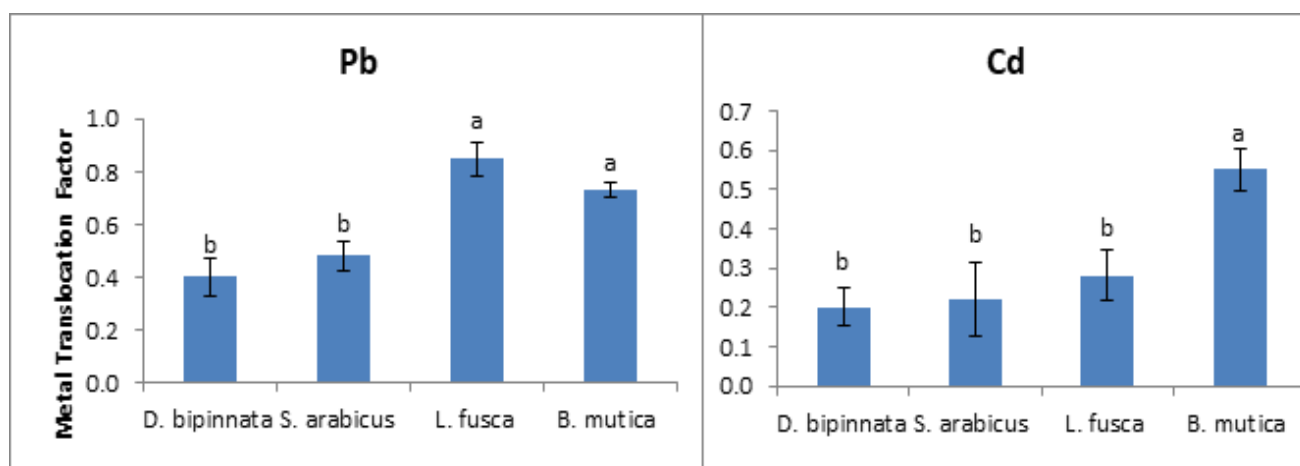


Figure 5: Effect of industrial wastewater on Pb and Cd translocation factor (TF) of various grasses. Data represent the means of three replicates. The bars sharing similar letters do not differ significantly at $p \leq 0.05$.

respectively, with non-significant difference among them. However, no significance difference was observed in root Cd concentration of different grass species (Figure 3) and it ranged from 1.24-1.57 mg kg⁻¹ dry weight.

Metal uptake and Translocation factor

Shoot metal uptake varied with different grass species and was in the range of 9.4-201.8 and 0.26-6.39 µg plant⁻¹ for Pb and Cd (Figure 4), respectively. Uptake for both the metals by different grass species was in the order *B. mutica* > *L. fusca* > *D. bipinnata* > *S. arabicus*. However, *B. mutica* indicated better efficiency with significant uptake of Pb and Cd, 201.8 and 6.39 µg plant⁻¹ respectively, in comparison to other grass species. The translocation factor (TF) for Pb and Cd did not exceed the unity (Figure 5). *B. mutica* and *L. fusca* gave higher TF-values for Pb 0.73 and 0.85, respectively, compared to other grasses. Moreover, TF did not differ significantly for *D. bipinnata*, *L. fusca* and *S. arabicus* except for *B. mutica* which showed highest TF-value 0.55 ($p \leq 0.05$).

Discussion

The pollution status is becoming worse especially in the developing countries due to modernization, such as Pakistan where industries share the major portion in water pollution (Sial *et al.*, 2006; Majeed *et al.*, 2018; Kadam *et al.*, 2018; Hussain *et al.*, 2018). These industries are generating over 435 million gallons of sewage and effluents daily (Ijaz *et al.*, 2016). A large quantity of this wastewater is discharged in the outer environment, and farmers near urban areas are likely to use this water for irrigation purpose due to shortage of good quality irrigation water. It contains toxic metals i.e. Cd (0.03 mg L⁻¹) and Pb (0.92 mg L⁻¹) (Table 2). Amount of Pb present in wastewater was above the permissible limit set by National Environmental Quality Standards (NEQS), Pakistan. Thereby, there is dire need for appropriate treatment of wastewater in order to prevent the entry of toxic metals into food chain.

Growth potential of grasses in wastewater showed variable influence on shoot and root dry biomass of grass species, especially *B. mutica* indicated good growth which might be due to the presence of organic matter and inorganic mineral ions like N, P, K, Ca, and Mg in wastewater which are necessary for plant growth and development (Adrover *et al.*, 2012; Rev *et al.*, 2017). The results of present study are consistent to the findings that use of urban wastewater as irrigation enhanced the photosynthesis, growth and dry matter yield of chickpea (Tak *et al.*, 2013). *S. arabicus* showed least growth potential compared to other grass species which might be

due to the suppressive effects of toxic elements (Cd, Pb) present in the wastewater (Nair *et al.*, 2008; Adrover *et al.*, 2012). These toxic metals may reduce plant growth by inhibiting photosynthesis, respiration and enzymatic activity, decreasing chlorophyll and nitrogen contents, increasing the production of reactive oxygen species, disequilibrium in nutrients, water and hormonal balance of plants (Li *et al.*, 2012; Lou *et al.*, 2017; Akhtar *et al.*, 2018). It was observed that grass species showed variable response regarding accumulation of Pb and Cd in roots and shoots in wastewater growth medium. However, the Pb and Cd concentration in roots of all grass species was more than shoot. This might be due to negative charges present on root surface capable to bind the metal cations and decrease root to shoot transport (Zhivotovsky *et al.*, 2011). These results are in line with the findings of Roongtanakiat *et al.* (2007) who found more concentration of heavy metals (Pb, Mn, Cu, Fe, Zn) in roots as compared to shoot of *Vetiveria zizanioides* growing in industrial wastewater. Moreover, Silva *et al.* (2016) also observed high Cd concentration in roots compared to above ground parts of forage grasses.

Results regarding shoot metal concentration and shoot metal uptake showed that *B. mutica* had maximum metal concentration (Pb 21 and Cd 0.66 mg kg⁻¹ dry matter) and uptake (Pb 201.8 and Cd 6.4 µg plant⁻¹) followed by *L. fusca*. Good response of *B. mutica* for uptake and accumulation of heavy metals (Pb, Cd) could be due to different genetically controlled mechanisms responsible for varying uptake and transportation potential of metal cations from soil/ solution to aerial parts of the plant such as composition and quantity of organic acids released through roots to mobilize/ chelate the metal cations (Hall, 2002; Sarwar *et al.*, 2010; Najafi *et al.*, 2015; Alves *et al.*, 2016). These results are in agreement with our recent study in which *B. mutica* showed good potential regarding the shoot metal concentration (Cd > 150 and Pb > 1000 mg kg⁻¹ dry matter) and shoot metal uptake (Pb 6000 and Cd 1200 µg pot⁻¹) under contaminated nutrient solution hydroponic culture (Ullah *et al.*, 2019). Mohanty and Patra (2012) observed significant Cr uptake (i.e. total accumulation rate 8.2 mg kg⁻¹ day⁻¹ and transportation index 6.16) with luxuriant growth and biomass, thereby, suggesting para grass an excellent plant for remediation of heavy metal contaminated wastewater and soil. The TF value refers to the efficiency of plant to transport the metal from root to above ground parts i.e. shoot, leaves. In our experiment, for both metals all grass species showed TF value less than one which shows the lower rate of metal transfer from root to shoot. Similar results were observed by Aran *et al.* (2017) who observed TF values less than



one for different heavy metals (Pb, Cr, Ni, Zn) in *L. laevigatum* growing under wastewater.

Conclusion

It can be concluded on the basis of current findings, among the test grass species that *B. mutica* showed better efficacy regarding metal transfer, accumulation, and uptake with luxuriant growth or biomass. Thereby, *B. mutica* could be a better option to filter out the toxic metals from wastewater.

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