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Impact of industrial effluent on selected grass species

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Abstract
Industrial wastewater contains a variety of substances such as organic carbon, salts and heavy metals, which along with arsenic (As), cadmium (Cd), lead (Pb) and nickel (Ni) are known to be highly toxic even in very low concentrations. This study was carried out to test the impact of industrial effluent from the Industrial Estate Islamabad, the capital city of Pakistan, on four local grass species of the Pothohar region, i.e., *Panicum maximum* (Mombasa grass), *Cenchrus ciliaris* (Dhaman grass), *Cynodon dactylon* (Khabbal grass) and *Chloris gayana* (Rhodes grass). These grasses were grown in tap water (T0), industrial effluent-tap water (ratio 50:50; T1), and in industrial effluent (T2). The concentrations of As, Cd, Pb, and Ni accumulated by these grasses were appraised with inductively coupled plasma optical emission spectrometry (ICP-OES). All four grasses differed significantly in terms of their accumulation of different types of metal elements in the shoot and root organs. The analysis of industrial effluent, and root and shoot biomass samples of all four grasses did not have high amounts of heavy metals with reference to the levels of the metals documented by WHO. It is generally recommended that the effluents from the Industrial Estate Islamabad can be used with some caution for growing forage grasses including the four species tested in this study.

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Introduction

Water is a fundamental input for agriculture and a key factor of food security. Agriculture is a major consumer of water and accounts for 70% withdrawal from the global pool (Dionisio Pérez-Blanco et al., 2020). Intensive crop cultivation and changing rainfall patterns have created water shortage and negatively impacted agricultural production in many regions of the world (Xiao et al., 2021). In Pakistan, there is a shortage of water for agriculture too. The issue is exacerbated by Pakistan's reliance on cotton, sugar cane and rice crops because of the crop's huge water usage. Further, water pollution is intensifying the problem. The anthropogenic activities are polluting the natural water reservoirs by adding poisonous chemicals as a waste of industry. Over 80% of wastewater in the world flows back into the environment as untreated and unused (NRDC, 2023). Moreover, industrialization and urbanization are continuously deteriorating the water quality because of industrial effluents and sewage water contamination (Rahmana et al., 2021).

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Polluted water can lead to a variety of problems, affecting both the environment and human health. This water when used for irrigation can lead to soil contamination, affecting the quality of crops and posing risks to human health through the food chain (Munzel et al., 2023). It may adversely affect crop growth, leading to reduced agricultural productivity, contributing to malnutrition and food insecurity (Lin et al., 2022). Industrial effluents are being used for crop production nowadays. Industrial effluents refer to liquid waste streams generated as a byproduct of industrial processes. These effluents often contain various pollutants, chemicals and contaminants that can have adverse effects on the environment, if not properly treated before being discharged into water bodies or other disposal systems. The consumption of fruits, vegetables or animal products contaminated with heavy metals can lead to adverse health conditions, including neurological disorders, kidney damage and developmental issues especially in children (Vasilachi et al., 2023).

Industrial processes in various sectors such as manufacturing, mining, chemical production and food processing can produce effluents with a wide range of substances, including heavy metals, organic compounds, oils, suspended solids and other harmful materials (Ayilara and Babalola, 2023). Heavy metals are among the contaminants found in higher concentrations in the industrial wastewater. These heavy metals may include lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), copper (Cu), nickel (Ni), etc. The continuous release of these metals, degrade the environment and negatively impact the life on earth. Excessive metal concentrations in cultivated soils alter the quality of food and have an adverse effect on human health. This raises the danger of kidney and liver failure, infertility and reproductive abnormalities; causes cancer, nervous system breakdown, mental sickness and other toxicity issues (Khan et al. 2011; Oyugi et al., 2021). Some heavy metals, such as arsenic, cadmium and certain forms of chromium, are classified as human carcinogens by international health agencies (Balali-Mood et al., 2021). Prolonged exposure to these metals through contaminated crops can increase the risk of development of cancer.

Industrial effluents are now being widely used to grow agricultural crops including fruits and vegetables. Many metal elements are important for plant growth in small amount, but their excessive levels in soil can cause toxicity to crops growing therein (Shah et al., 2010). The impact of industrial effluents on vegetation is complex and varies depending on the specific pollutants involved, their concentration and the resilience of plant species. When plants consume excessive amounts of heavy metals, their physiological processes are altered, triggering changes to the growth patterns of their roots and shoots (Malkowski et al., 2019). Plants may absorb and accumulate pollutants from contaminated water, leading to bioaccumulation in plant tissues. This can pose a risk to herbivores and other organisms higher up the food chain.

Further, plants have their own resistance systems against pollutants and for purifying heavy metals (Asati et al., 2016). Some plants can absorb heavy metals but do not accumulate them in their biomass. Instead, these plants may store the metals in vacuoles or bind them in a non-toxic form leading to phyto-stabilization (Yan et al., 2020). Grasses are considered as robust plant species which can uptake and accumulate considerable amounts of heavy metals and other inorganic elements (Agnieszka et al., 2020). Based on these findings, we hypothesized that the industrial effluent from the Industrial Estate Islamabad is enriched with a variety of heavy metals and commonly occurring grass species in most regions of Pakistan might uptake and accumulate high amounts of heavy metals contained in the industrial effluent. Thus, the principal objective of the present investigation was to appraise the levels of different heavy metals in the industrial effluent and the extent of their accumulation in four commonly occurring grasses, *Panicum maximum* (Mombasa grass), *Cenchrus ciliaris* (Dhaman grass), *Cynodon dactylon* (Khabbal grass), and *Chloris gayana* (Rhodes grass).

Material and Methods

Study area

The present study was conducted at the National Agricultural Research Centre (NARC), Islamabad. Industrial effluent was collected from the Industrial Estate Islamabad (IEI) from a drain in I-9 Sector. This drain contained effluent from various industries including steel melting furnaces, re-rolling mills, metal working and engineering, galvanizing, flour mills, marble cutting and polishing units, and oil and ghee industries. There are about ten pharmaceutical industries in the IEI with the occasional washing of formulation vessels, which produce wastewater in minor quantities.

Study design

The study design used in this research was a randomized complete block design (RCBD). This study focused on three water treatments for growing four grass species under confined conditions as shown in **Table 1**. Each treatment had three replications for each species, i.e., 12 replications in each treatment, hence a total of 36 samples.

Table 1. Description of treatments applied in this study

Treatments	Composition (%)	
	Tap water	Industrial effluent
T0	100	0
T1	50	50
T2	0	100

The species tested in this study were *Panicum maximum* (Mombasa grass), *Cenchrus ciliaris* (Dhaman grass), *Cynodon dactylon* (Khabbal grass) and *Chloris gayana* (Rhodes grass). For transplanting, a 1 m^2 quadrat was used in the field of each particular grass species at random places to cut out tufts of each species in required number. Three-month old tufts of grasses were transplanted from the field to the pots for the experiment. The transplanted tufts were treated with tap water for two weeks to achieve equilibrium. After two weeks, the treatments were applied to pots till harvesting. Grasses were harvested after eight weeks, air-dried and crushed for further analysis. Before harvesting, vegetative growth data was collected using standard protocols.

Water quality analysis

The water quality indicators tested in this study were: pH, electrical conductivity (EC), total dissolved substances (TDS) and heavy metal concentration. The pH, EC and TDS were measured by conventional digital meters (Buck et al., 2002; Corwin and Yemoto, 2020).

Vegetative growth analysis

Growth indicators measured in this study were leaf count, tiller count and plant height. The vegetative growth data was recorded after eight weeks of the initiation of the effluent treatments.

Biomass analysis for heavy metals

Chemical analysis of grass biomass for heavy metal detection was performed using inductively coupled plasma optical emission spectroscopy (ICP-OES) equipment. The heavy metals analyzed in this study were arsenic (As), chromium (Cr), nickel (Ni) and lead (Pb).

The oven-dried samples were macerated into a fine powder for digestion. A 0.5 g powder was mixed with 0.5 mL di-acid and 50 mL distilled water. The material was placed in a chemical digester for 20-40 minutes. After cooling, an aliquot (15 mL) of distilled water was added to the remaining sample and then heavy metal analysis was carried out.

Results and Discussion

Water quality analysis of the industrial effluent in comparison with that of tap water

Table 2 shows results for water quality analysis. Industrial effluent had lower pH (7.78) than that of tap water (8.87), but both being alkaline in nature. The pH values detected in this study are generally in agreement with a similar study conducted in the Industrial Estate Peshawar, Pakistan (Tariq et al., 2006). Electrical conductivity of the industrial and tap waters was 1.27 and 0.73 dS m^{-1}, respectively, whereas Tariq et al. (2006) reported EC value of 1.06 dS m^{-1} of the industrial effluent collected from Peshawar. The amount of TDS in the industrial effluent used in the current study was 889 mg L^{-1} being higher than that of tap water (510.31 mg L^{-1}). The TDS ranges found in this study are in agreement with those of the study performed using industrial effluent from Hayatabad Industrial Estate, Peshawar by Khan et al. (2023) in which TDS ranged between 560 and 942 mg L^{-1}.

Table 2. Water quality analysis of industrial effluent and tap water

Indicators	Industrial effluent	Tap water
pH	7.78	8.87
EC (dS m^{-1})	1.27	0.73
TDS (mg L^{-1})	889	510.3

Vegetative growth analysis

The leaf count of four grasses after eight weeks of transplanting under three water treatments is shown in **Figure 1**. The highest leaf count was observed under T0 (100% tap water) in all grass species except Mombasa grass; this grass showed comparatively higher leaf count under T2 (100% industrial effluent) than that in the other treatments. The lowest leaf count was observed in Dhaman grass under T2 while the highest leaf count was of Rhodes grass under T0. Dhaman, Khabbal and Rhodes showed the highest leaf count under T0, whereas Mombasa grass showed the highest leaf number under T2.

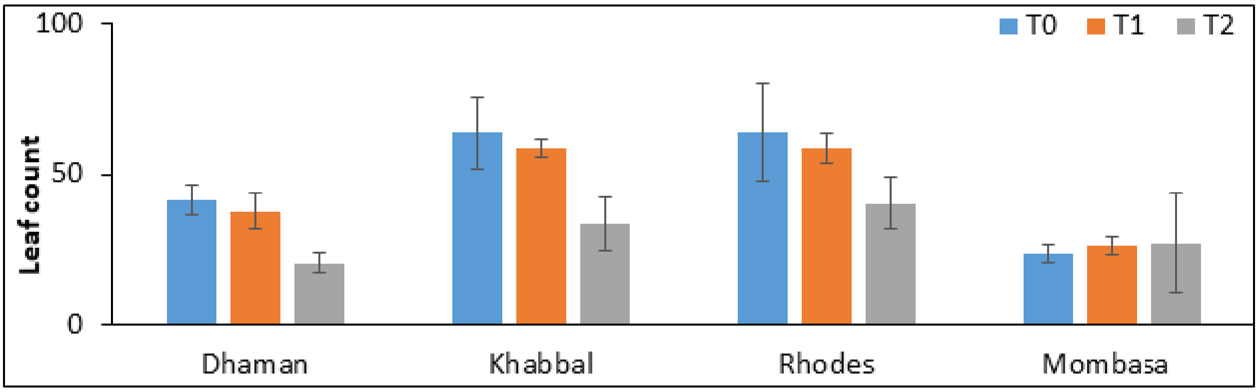

Figure 1. Leaf count of four grass species under three industrial effluent treatments

Figure 2 illustrates tiller count of grass species under three treatments. Highest tiller count was observed in Rhodes grass under T0 and T1 followed by Khabbal under T0. Mombasa grass showed relatively lower tiller count under all the treatments, and hence its poor performance. In case of industrial effluent treatments (T1 and T2), Rhodes grass showed the highest tiller count followed by Khabbal, Dhaman and Mombasa grass, respectively. Overall, industrial effluent application to the grass species reduced the tiller count. This study was found in agreement with the findings of Bhatti et al. (2017), who investigated the impact of industrial effluents on tree species growth. The results indicated the negative impact of industrial effluent on growth of all four grasses in terms of tiller production.

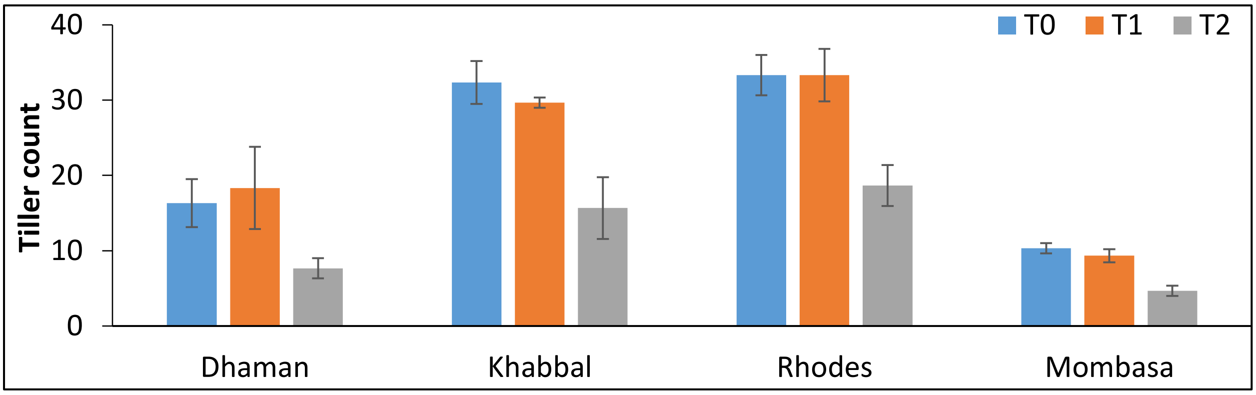

Figure 2. Comparison of tiller count of four grass species under three industrial effluent treatments

Plant height of the four grasses achieved after eight weeks under three treatments is shown in **Figure 3**. All grasses attained good height under T0 except Dhaman grass; this grass showed maximum height (101 cm) under T1 followed by T2 and T0, respectively. Under T2, Mombasa grass showed maximum height followed by Dhaman, Rodes and Khabbal, respectively.

Alghobar and Suresha (2016) investigated the impact of waste water on the growth of grass and agricultural crops. They reported that the investigated grass species showed better performance in terms of height gain with waste water. In the current study, some species showed higher height growth with industrial effluent treatments, but some indicated better growth with tap water treatment.

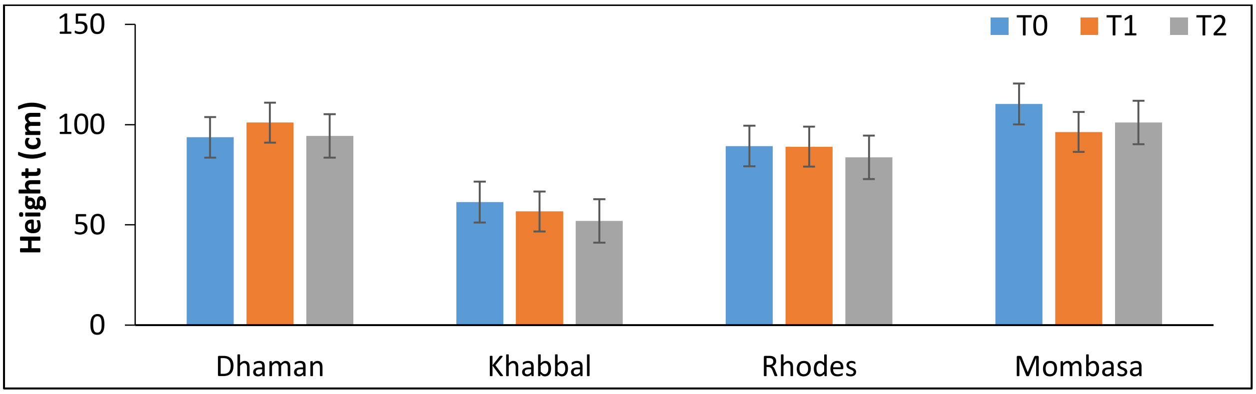

Figure 3. Comparison of plant height of four grass species under three industrial effluent treatments

Soil analysis for heavy metals

Analysis for heavy metals' presence in the original soil is shown in **Table 3**. Nickel was found to be in the highest amount in the soil sample (0.28 mg kg^{-1}), followed by Cr 0.19 mg kg^{-1}, Pb 0.08 mg kg^{-1} As 0.04 mg kg^{-1}, respectively. Other heavy metals like Co and Cd were found in trace amounts.

Table 4 depicts the heavy metal concentration in the industrial effluent samples. The industrial effluent contained As, Pb, Cr and Ni, arsenic being comparatively in lesser amount in the analyzed sample. All the heavy metals were found within permissible limits when compared to Pak-NEQS set limits (National Environmental Quality Standards (NESQ), 1997).

Table 3. Heavy Metal concentration in original soil sample

Heavy metal	concentration (mg kg^{-1})
Pb	0.08
Cr	0.19
Ni	0.28
As	0.04

Table 4. Heavy metal concentration in the industrial effluent sample

Heavy metal	concentration (mg L^{-1})	Pak-NEQS limits (mg L^{-1})
Pb	0.5	0.5
Cr	0.4	1.0
Ni	0.4	1.0
As	0.08	1.0

Shoot analysis for heavy metals

Shoot biomass of the four grasses grown under different treatments was analyzed for heavy metal concentration. **Figure 4** illustrates the concentration of Pb in shoots of Dhaman, Khabbal, Rhodes and Mombasa grasses. The highest accumulation was observed in Dhaman, Khabbal and Mombasa under T2, i.e., 0.06 mg L^{-1}. In the Rhodes grass comparatively low accumulation was observed under all treatments. Under the control treatment T0, less accumulation of Pb was found in all grass shoot samples. The results of the study were in line with the findings of Ullah et al. (2020) in which Pb concentration in the shoots of grasses remained unaffected.

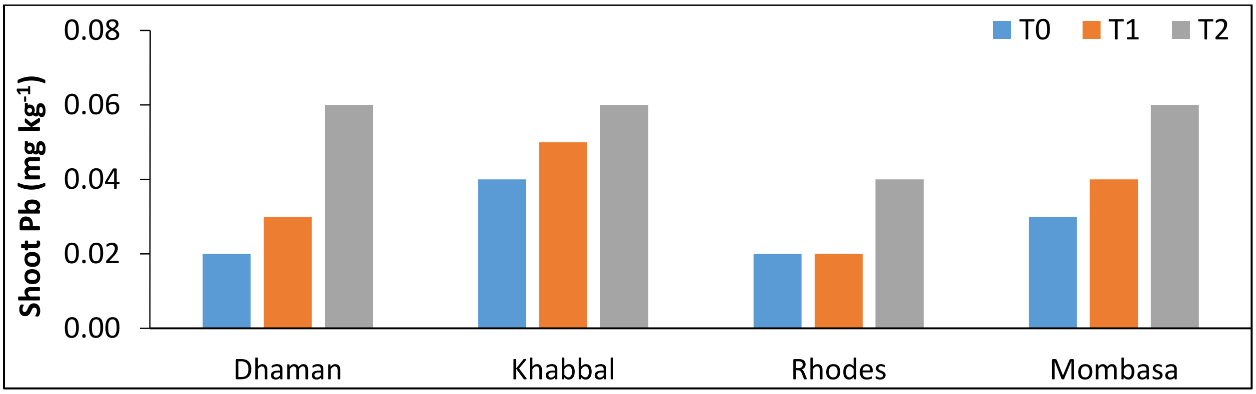

Figure 4. Concentration of Pb in the shoots of four grasses under industrial effluent treatments

The concentration of Cr in grass shoot samples under T0, T1 and T2 is shown in **Figure 5**. Under T2 (100% industrial effluent), comparatively higher accumulation of Cr was observed in all grass shoot samples than that in the other treatments. The highest accumulation (0.11 mg kg^{-1}) was observed in Khabbal grass under T2 and the lowest (0.04 mg kg^{-1}) was found in Dhaman and Mombasa under T0.

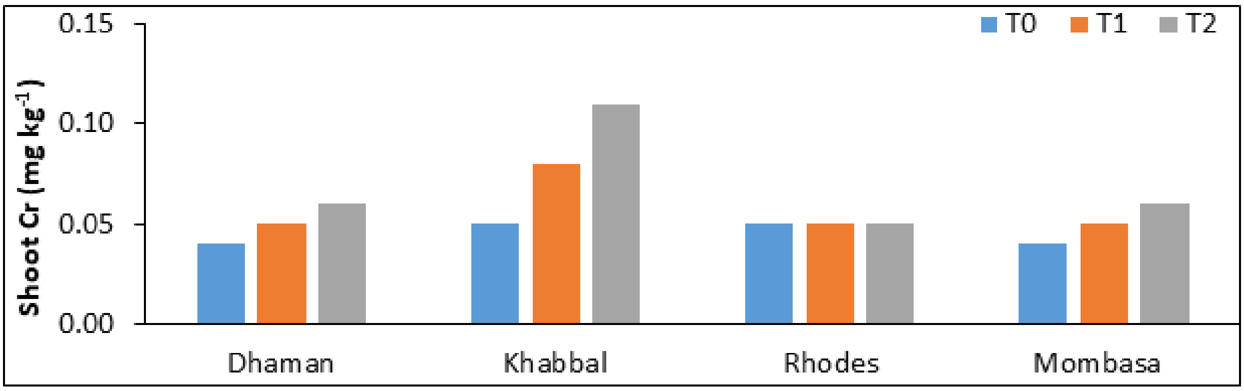

Figure 5. Concentration of Cr in the shoots of four grasses under industrial effluent treatments

Figure 6 demonstrates the concentration of Ni in the shoot samples of all four grasses under three different industrial effluent treatments. Khabbal grass showed the highest accumulation under 100% industrial effluent (T2), being 0.1 mg L^{-1}. The control treatment results indicated lower accumulation of Ni than that in the other treatments. The least accumulation of Ni was observed in Dhaman shoots under T0 (0.02 mg kg^{-1}). Under T1, Khabbal shoots showed higher Ni accumulation than that in the other grass species.

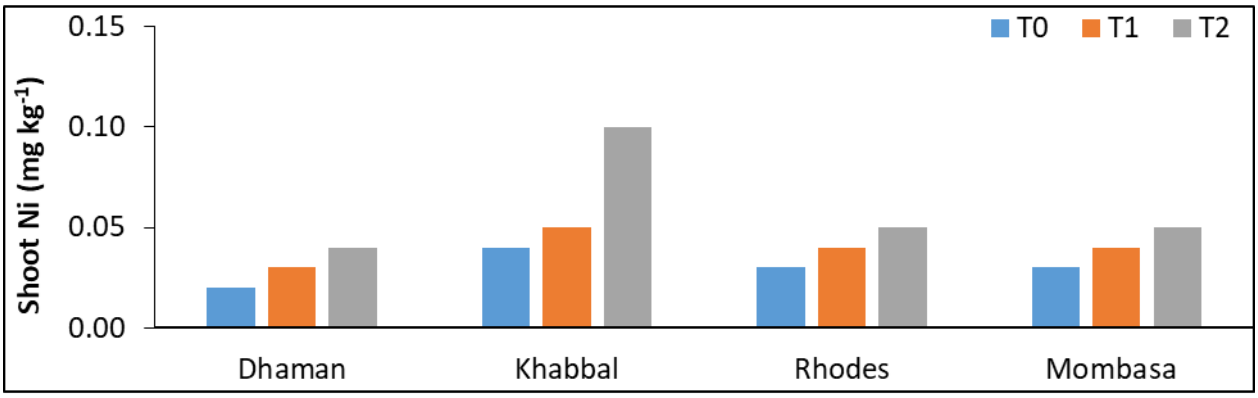

Figure 6. Concentration of Ni in the shoots of four grasses under industrial effluent treatments

The highest accumulation (0.09 mg kg^{-1}) was observed in Mombasa grass under T2 followed by T1 (**Figure 7**). No accumulation of As was observed in Dhaman and Khabbal under all the treatments except for Khabbal under T2 having accumulation of 0.02 mg kg^{-1}. Under T0, only Mombasa grass showed a little accumulation of 0.01 mg kg^{-1}.

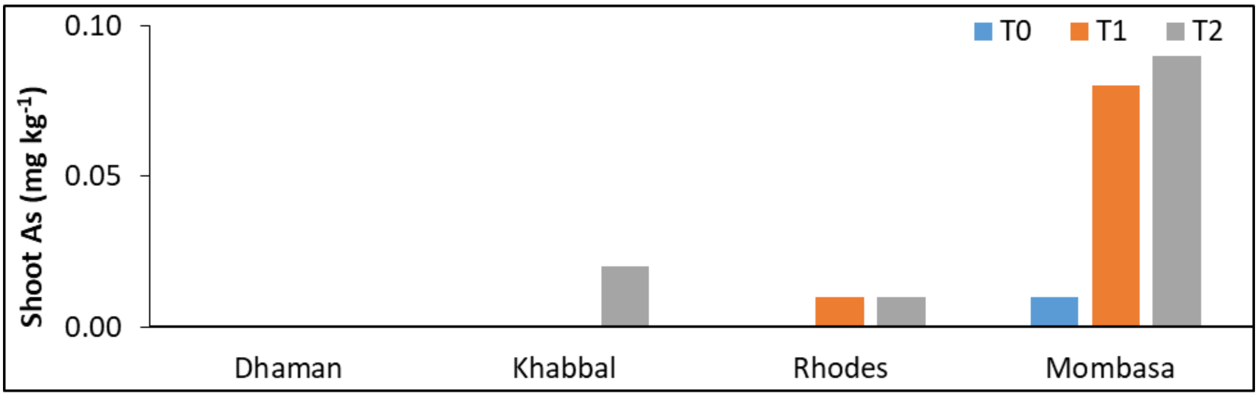

Figure 7. Concentration of As in the shoots of four grasses under industrial effluent treatments

Analysis of roots for heavy metals

The roots of grass samples were also analyzed for the appraisal of heavy metals. In the roots of Khabbal grass, the highest accumulation of 0.13 mg kg^{-1} was observed under T2 (**Figure 8**). The lowest accumulation was found in Mombasa roots under T1. Furthermore, in the T2 (100% industrial effluent) treatment higher Pb accumulation was recorded in the roots compared with those in the other treatments except for Mombasa grass, that had higher accumulation under T0.

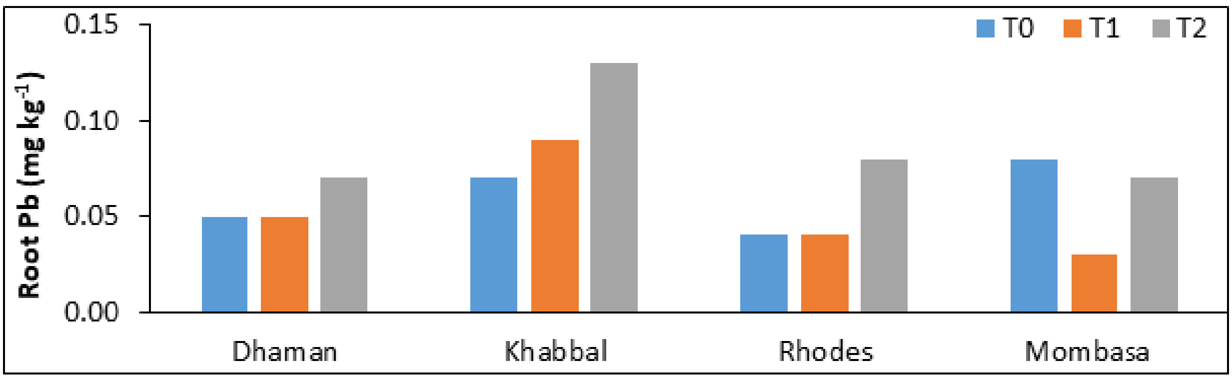

Figure 8. Concentration of root Pb in four grasses under industrial effluent treatments

Highest accumulation (0.69 mg kg^{-1}) of Cr was observed in Dhaman roots under T2 and the lowest (0.09 mg kg^{-1}) in Mombasa root samples under T0 (**Figure 9**). All the root samples showed higher accumulation of Cr under 100% industrial effluent treatment of all other root samples from different treatments. Dhaman root samples also showed a significant Cr accumulation (0.51 mg kg^{-1}) under T1.

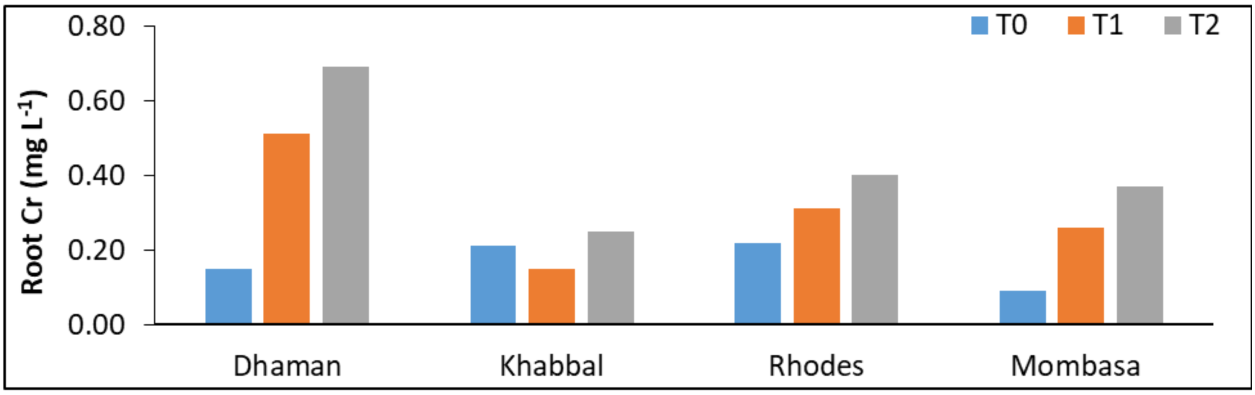

Figure 9. Concentration of root Cr in four grasses under industrial effluent treatments

Nickel accumulation in the roots of all four grasses was found to be the highest under T2 (**Figure 10**). The highest Ni accumulation was observed in Mombasa roots treated with 100% industrial effluent (T2). Under T0, Mombasa roots showed the least accumulation of Ni of that of all grass species.

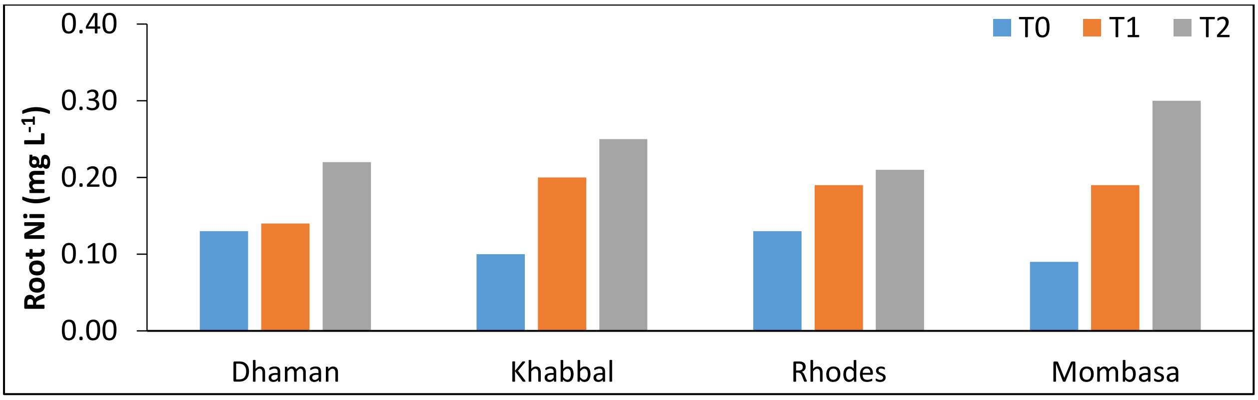

Figure 10. Concentration of root Ni in four grasses under industrial effluent treatments.

Overall, greater accumulation was observed in all the roots all four grasses under T2 (**Figure 11**). The highest accumulation (0.04 mg kg^{-1}) was found in Mombasa grass under T2. No accumulation of As was observed in the roots of Khabbal and Rhodes under T1. Under T0, only trace amount of As was observed in the roots of Khabbal and Rhodes.

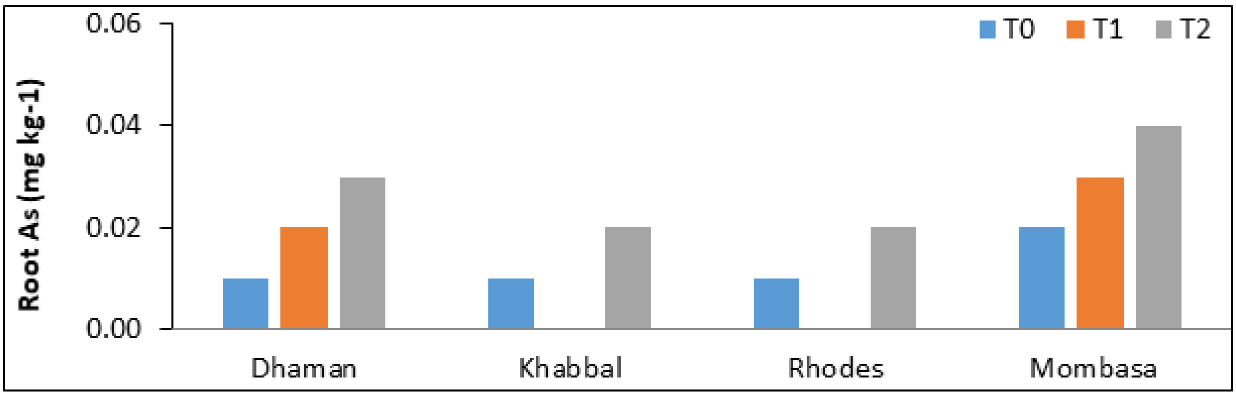

Figure 11. Concentration of root As in four grasses under industrial effluent treatments

While comparing the Pb accumulation in shoots and roots in the current study, the roots of all grasses accumulated higher amount of Pb than that in the shoots. These results contradict to those of Ullah et al. (2020) who observed higher accumulation of Pb in the shoots than that in the roots of different grass species. However, Bassegio et al. (2020) observed a many-fold accumulation of Pb in the roots of *Brassica juncea* plants compared with that of the shoots when the plants were exposed to varying concentrations of Pb in the growth medium. In view of Kumar et al. (1995) most plants accumulate a substantial amount of metals in the roots limiting their uptake to the shoots. However, the reverse was observed in the current study in case of Cr accumulation in shoots and roots. Generally, like that observed in the case of Pb, Cr is also supposed to be accumulated more in roots than that in shoots (Mangabeira et al. 2011), because higher accumulation of Cr in the roots may limit the uptake and accumulation of this element to above-ground plant parts and hence protects the photosynthesizing tissues from Cr toxicity.

The pattern of accumulation of Ni in all four grasses was similar to that of Pb, i.e., most of the Ni absorbed by all four grasses was retained in the roots thereby limiting its uptake and accumulation in the shoots. However, while critically observing the accumulation pattern of As, the four grasses differed significantly in terms of the concentrations of this element in shoots and roots. All grasses except Mombasa retained a significant amount of As in their roots, whereas Mombasa did otherwise being high accumulator of As in the shoots. Thus, Mombosa grass can be categorized as arsenic accumulator like several other plant species (Campos et al., 2016).

Conclusion

The results of the present study revealed that the industrial effluent from the Industrial Estate Islamabad contains heavy metals but are within the safe limits as declared by WHO. These effluents have higher electrical conductance and TDS values than those of tap water. However, it is recommended that industrial effluent from the Industrial Estate of Islamabad could be used to grow grass species for livestock grazing with some caution as Mombasa has been found to accumulate As more in the shoots than that in the roots.

Author(s), Editor(s) and Publisher's declarations

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Conflict of interest

The authors declare no conflict of interest.

Source of funding

None declared.

Contribution of authors

Planning and conduction of experiment: ZFI, MIA, SK, MAG. Research supervision: MIA. Data acquisition, analysis, and interpretation: ZFI, SK. Write-up of initial draft: ZFI, MIA, SK, MAG, MKR, HBA, OES. Review of final draft: ZFI, MIA, SK, MAG, OES.

Ethical approval

This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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