EVALUATION OF HEAVY METAL ACCUMULATION IN SOIL AND BERSEEM (*TRIFOLIUM ALEXANDRINUM*) UNDER WASTEWATER IRRIGATION

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ABSTRACT: Since the quality of irrigation water is a significant factor in the sustainability of irrigated lands, a survey was conducted on the quality of the soil and fodder plant irrigated with Ruhi drain wastewater to establish the appropriateness of this water to irrigate the lands. Fodder crops like *Trifolium alexandrinum* which is irrigated by Rohi drain wastewater is fed upon by cattle and the meat of cattle ultimately fed upon by humans. Several physio-chemical characteristics of soil and *T. alexandrinum* were analyzed. In addition to this, macronutrient analysis as well as heavy metal analysis was also conducted. High value of pH and EC was observed in soil samples. Macronutrient analysis revealed higher nitrogen, phosphorus and potassium levels. The results revealed that soil and *Trifolium alexandrinum* that are being irrigated by Ruhi drain wastewater contained impermissible concentrations of Lead (Pb) and Cadmium (Cd). However, the concentration of Chromium (Cr) was found to be within permissible limit. The above results reveal that wastewater irrigation not only increases soil fertility by augmenting the nutrient content, but also brings with it the toxic metals which not only affects plant growth but poses considerable environmental as well as health problems. As such, there is strong need to treat the industrial effluents before they are used in agricultural activities to make fodder production sustainable and secure.

Keywords: Irrigation water**;** Soil pollution**;** Heavy metals**;** *Trifolium alexandrinum***;** Atomic absorption spectrophotometer**.**

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INTRODUCTION

Soil is an integral component of the ecosystem that interacts with all major Earth spheres, including the atmosphere, lithosphere, hydrosphere, and biosphere. It is a complex mixture of organic matter, liquids, gases, and microorganisms that maintain its balance and support life. Additionally, soil acts as a protective barrier, trapping toxic chemicals introduced either by anthropogenic activities or through natural sources (Ali *et al*., 2015). Metals are natural and ubiquitous in the environment. Some, such as calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn), are essential bio-elements for the normal functioning of living organisms. In contrast, others like lead (Pb), mercury (Hg), cadmium (Cd), and chromium (Cr) are toxic and harmful to both the environment and human health (Ali *et al*., 2015; Khan *et al*., 2018). While biogeochemical cycles help regulate their concentrations, heavy metals are non-biodegradable, persist in soils longer than in water or air, and accumulate in living organisms posing risks to ecosystems and human health (Khan *et al*., 2018).

Anthropogenic activities, including industrialization, mining, improper wastewater disposal, vehicular emission and construction and demolition activities have elevated heavy metal concentrations beyond acceptable levels. These pollutants disrupt the food chain by accumulating in soils and crops, thereby affecting soil fertility, plant health and crop productivity (Zafar-ul-Hye *et al*., 2020). The plants absorb heavy metals through roots and translocate them to aerial parts, entering the food chain (Nawab *et al*., 2015; Sahito *et al*., 2016). In developing countries like Pakistan, the reuse of untreated or partially treated industrial wastewater for irrigation has become common due to water scarcity. While it provides nutrients and resolves effluent disposal issues, this practice also contributes to heavy metal accumulation in soils and crops (Singh & Rathore, 2021; Mehmood *et al*., 2019). Long-term use of such water alters soil composition, affects microbial activity, and reduces agricultural productivity (Zhang *et al*., 2020).

Water's polar nature allows it to dissolve or carry contaminants, including heavy metals, from its surroundings. Naturally impure due to local geology and human activities, water quality deteriorates further due to overpopulation, deforestation, and industrial discharges (Salman *et al*., 2013). In Pakistan, the contamination of water bodies by heavy metals is an escalating issue (Ashraf *et al*., 2021). Heavy metals in wastewater may exist in dissolved or particulate form and can infiltrate sediments and higher trophic levels of ecosystems (Atique *et al*., 2020). The toxicity ranking of heavy metals for humans is as follows: Co < Al < Cr < Pb < Ni < Zn < Cu < Cd < Hg. Among these, lead, cadmium, and mercury have received significant attention due to their severe health impacts. Pb and Cd are potential carcinogens, while Hg affects the brain, lungs, kidneys, and fetal development (Chaoua *et al*., 2019; Kinuthia *et al*., 2020). The U.S. EPA sets regulatory limits of 0.001 ppm for Hg and 0.01 ppm for Pb in wastewater used for agriculture and 0.05 ppm and 0.1 ppm in agricultural soil, respectively (Kinuthia *et al*., 2020).

The reuse of wastewater in agriculture is especially prevalent in Punjab, where industrial hubs such as Lahore and Kasur contribute significantly to the economy. These cities host tanneries, textile mills and paint industries that discharge untreated or poorly treated effluents into nearby drains, such as the Rohi drain in Kasur (Umar, 2017; Khan & Zafar, 2015). The contaminated water from these drains is commonly used for irrigation, leading to environmental degradation and posing severe health issues in humans and plants (Mishra *et al*., 2018). These effluents not only contain heavy metals but also have high concentrations of salts, proteins, oils, and fats, contributing to high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Joiya *et al*., 2021). Groundwater, the primary source of drinking water in the region, is also being contaminated. Residents of these industrial zones suffer from various health problems including diarrhea, dysentery, peptic ulcers, nephritis, and liver diseases, particularly in children under five (Umar, 2017). The main objectives of this study were to assess the concentration of heavy metals in soil and *Trifolium alexandrinum* being irrigated with Rohi drain wastewater and analyze the range and trend of heavy metals and comparison with WHO standards in soil and *Trifolium alexandrinum* being irrigated with Rohi drain wastewater.

METHODOLOGY

Geographical position of study area: Lahore, the capital city of the Punjab province in Pakistan, is situated in the eastern part of the country along the bank of Ravi River. The sprawling city of Lahore is approximately 1.77 million hectares of which there has been a high degree of land use in urban infrastructure, agriculture and greenery. It is a large metropolitan area that is overcrowded and well-developed. The country has a semi-arid climate with the dominant features being hot summers, cool winters and moderate rainfall mainly during monsoon months between July and September. The meteorological data shows that there is greater annual precipitation on average than the desert regions in the south of Punjab, which warrants a relatively better habitat to develop agricultural activities and urban forests.

Collection of samples: The total of three soil samples and three plant samples (*T. alexandrinum)* were collected in November 2024 from the three different spots near the Ruhi drain. The soil samples were collected from top soil at the depth of 0–20 cm using soil auger. From each of the three sampling sites, the samples were gathered randomly. The soil and plants specie samples one from each sampling point were transferred in to polyethylene bags. These samples were labelled properly and transported to the laboratory. The detail of sampling points is presented in Table 1.

Table 1. Details of sampling points under study.

Sr no.	Sampling points	Description
1	SP 1	10 meters away from Ruhi drain
2	SP 2	Beside the Nishat Mills
3	SP 3	20 meters away from Ruhi drain

Soil sample preparation: The soil samples were subjected to air drying for seven days. After drying, the soil samples were sieved using 0.5 mm mesh for ensuring uniform size. Both the soil and plant samples were then preserved in zip lock bags.

Plant sample preparation: The plant samples used in this study were collected randomly from three different sampling locations around Ruhi drain, Lahore. The height of the shoot and root was determined in-situ using a meter rule. With the help of a cutter, the samples of root and shoot containing stem and leaves were collected carefully. Each sample was transferred to polyethylene bags and sealed cautiously. All the plant samples were labelled properly and transported to laboratory. In laboratory, the plant samples were washed with distill water and oven dried at 70°C. The dried plant samples were ground into powdered form with the help of a pestle and mortar. The samples were then preserved in zip lock bags for further analysis.

Determination of physio-chemical characteristics of soil: Weigh about 50 g of dried and sieved soil sample using weighing balance. The weighed soil sample was stirred with 200 ml of deionized water and allowed to stand at room temperature. A digital pH and EC meter (Milwaukee MW 802) was used for the determination of pH and EC of the soil samples. After determination, the combined electrode was removed from suspension and rinse with de-ionized water carefully in a separate beaker. Same procedure was repeated for rest of the samples (Vidyasagar and Vidyasagar, 2019).

Sample digestion: The digestion of soil and plant samples was done following the wet digestion method (Pierzynski, 2000). The 1 g of air-dried sample was taken in 50 ml beaker and 15 ml of aqua regia was added to it.

The mixture was allowed to stand at room temperature for 5 minutes. Then, the beaker containing mixture was placed on a hot plate at 180°C for 3 hours. The digested mixture was filtered via Whatman filter paper and then diluted with 50 ml deionized water. The filtrate was then transferred to a labelled glass bottles for further analysis.

Spectroscopic analysis of sample: The digested samples of soil and plants were transported to laboratory for the determination of heavy metal content. Atomic absorption spectrophotometer was employed for the detection of heavy metals present in soil and plant samples.

Determination of N, P and K content: Following procedures were used for the determination of nitrogen (N), phosphorus (P) and potassium (K) content of soil and plant samples.

Nitrogen analysis: Total nitrogen content in soil and plant samples were determined by following the procedure stated by Nelson and Sommers, (1980). 1.5 g of sample was taken in Kjeldahl flask and 50 ml of 0.1N solution of sulfuric acid (H_2SO_4) was added in it. Following digestion, the sample was mixed with sulfuric acid at 350-380°C. Potassium sulfate was added to increase the boiling point of H_2SO_4. During distillation, the 6 ml of 0.1N solution of sodium hydroxide was added into the digested sample. Ammonia liberated during distillation process reacted with excess of H_2SO_4 which was then titrated against sodium hydroxide and total N content was determined.

Phosphorus content (P): For the determination of phosphorus content present in soil and plant samples, the procedure outlined by Pierzynski (2000) was followed. 1000ppm solution of phosphate was prepared and 100ppm was taken from it. Sample complex was prepared by adding 2, 4, 6, 8 and 10 ml from 1000ppm solution in a separate flask and fill up to the mark with distilled water. 2 ml of each sample solution was taken into the flask and 10ml of complexing agent was added along with distilled water. Spectrophotometer was preheated for 20 minutes and it was set for absorbance at 820nm at which the absorbance of water was 0. Absorbance of each sample solution was noted and phosphorus concentration was determined.

Potassium content (K): The soil and plant samples were extracted using the ammonium acetate solution. The samples were shaken for 30 minutes and filtered afterwards. The filtrate of extracted samples was analyzed using atomic absorption spectrophotometer (AAS).

Chlorophyll content determination: The chlorophyll content of all the plant samples was determined according to the procedure stated by Rajput and Patil (2017). The samples were prepared using 80% acetone solution and analyzed using spectrophotometer at three different wavelengths (645 nm, 663 nm and 652 nm).

Sample digestion: The soil and plant samples were digested through wet digestion method in digestive furnace (Model: KDN-20C, China) at 180°C temperature for 180 minutes. The samples were then filtered and diluted using distilled water and preserved for further analysis.

Wastewater collection and characterization: The wastewater sample was collected using sterilized plastic bottles from different sampling points within the vicinity of Ruhi drain, Lahore. All the collected samples were merged together in order to make one composite sample. pH and EC of the sample were measured on site suing portable pH and EC meter. The samples were preserved using ice boxes to keep the quality intact. The sample was transported to laboratory for further analysis. The sample was digested using wet digestion method and heavy metal analysis was conducted using AAS.

RESULTS

Evaluation of physio-chemical parameters of soil

EC, pH and moisture content of soil: Physical characteristics of the collected soil samples was determined. SP1 showed the pH value of 4.2 while SP2 and SP3 showed a pH value of 5.1 and 4.5, respectively. Data in Figure 1 shows that maximum pH (5.1) was observed in soil sample SP2 and minimum pH (4.2) was observed in soil sample SP2. The EC of the soil samples ranged from 146.6 to 440 µS/cm. SP1 showed the EC value of 440 µS/cm while SP2 and SP3 showed a EC value of 146.6 µS/cm and 380 µS/cm, respectively. Data in Figure 1 shows that maximum value of EC (440 µS/cm) was observed in soil sample SP1 and minimum value of EC (146.67 µS/cm) was observed in soil sample SP2. The moisture content of the soil samples ranged from 16.03 to 20.93%. SP1 showed the moisture content of 16.03% while SP2 and SP3 showed moisture content of 20.93% and 17.62%, respectively. Data in Figure 1 shows that maximum moisture content (20.8%) was observed in soil sample SP2 while minimum moisture content (16.867%) was observed in soil sample SP1.

Evaluation of N, P and K in soil irrigated with Rohi drain wastewater: Macronutrient analysis of soil was also tested indicating that the levels of N content ranged from 1.24 to 3.23 mg kg^{-1}. SP1 showed 1.24 mg kg^{-1} while SP2 and SP3 showed 3.23 mg kg^{-1} and 1.73 mg kg^{-1}, respectively. Figure 2 shows that the maximum N content (3 mg kg^{-1}) was recorded SP2 while minimum N content (1.4 mg kg^{-1}) was recorded in SP1. The P content of the soil samples ranged from 0.33 to 1.32 mg kg^{-1}. SP1 showed the P content of 0.33 mg kg^{-1} while SP2 and SP3 showed P content of 1.32 mg $kg^{-1}$and 0.66 mg kg^{-1}, respectively. Figure 2 shows that the maximum concentration of P (1.2 mg kg^{-1}) was recorded in SP2 while minimum P content (0.4 mg kg^{-1}) was recorded in SP1. In case of K levels, SP1 showed the K content of 1.44 mg kg^{-1} while SP2 and SP3 showed K content of 1.91 mg kg^{-1} and 1.85 mg kg^{-1}, respectively. Figure 2 shows that the highest K content (1.953 mg kg^{-1}) was noted in SP2 in comparison to the control while minimum K content (1.452 mg $kg^{-1)}$ was noted in SP1.

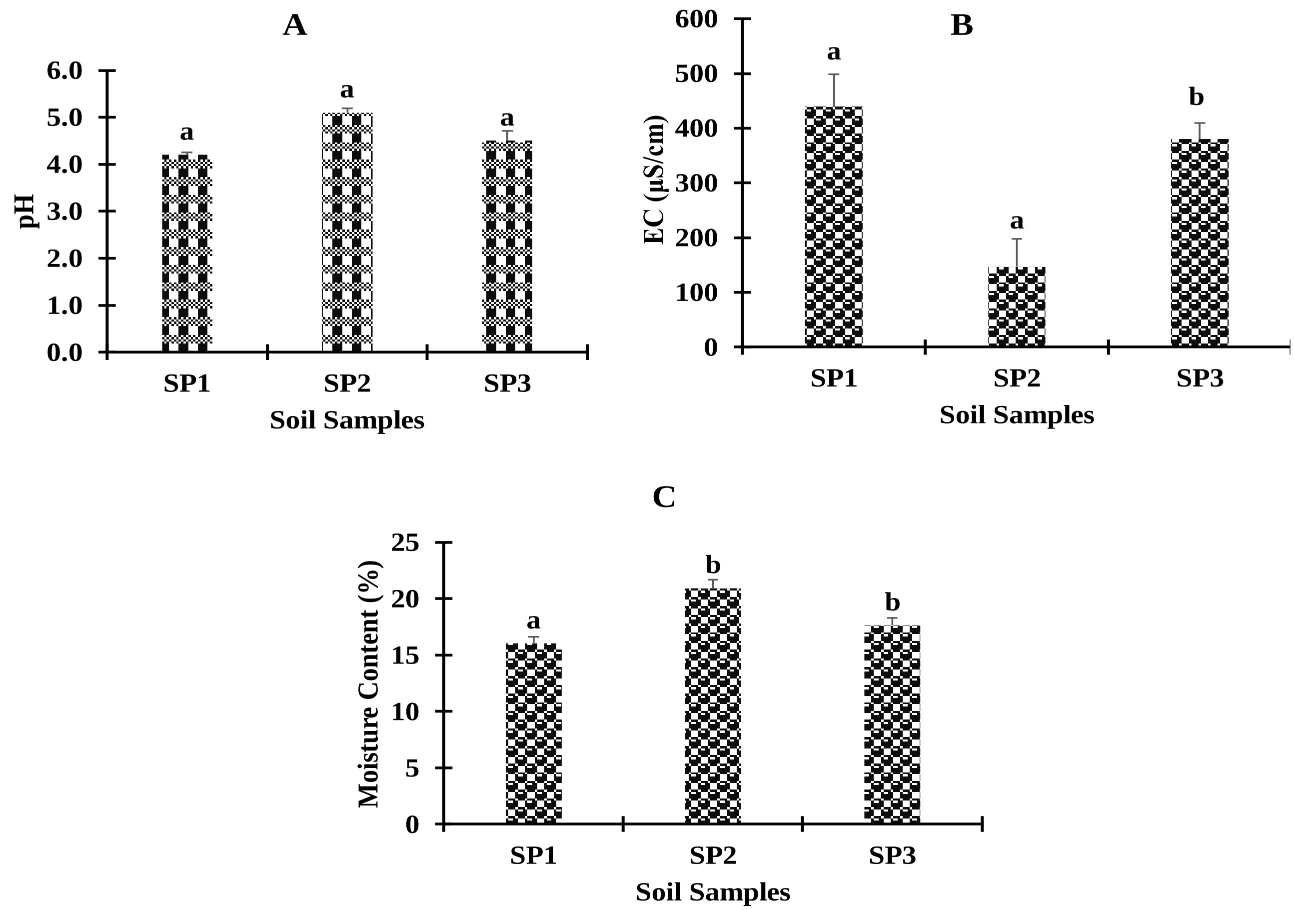

Figure 1. Evaluation of (A) EC, (B) pH and (C) Moisture content of soil samples

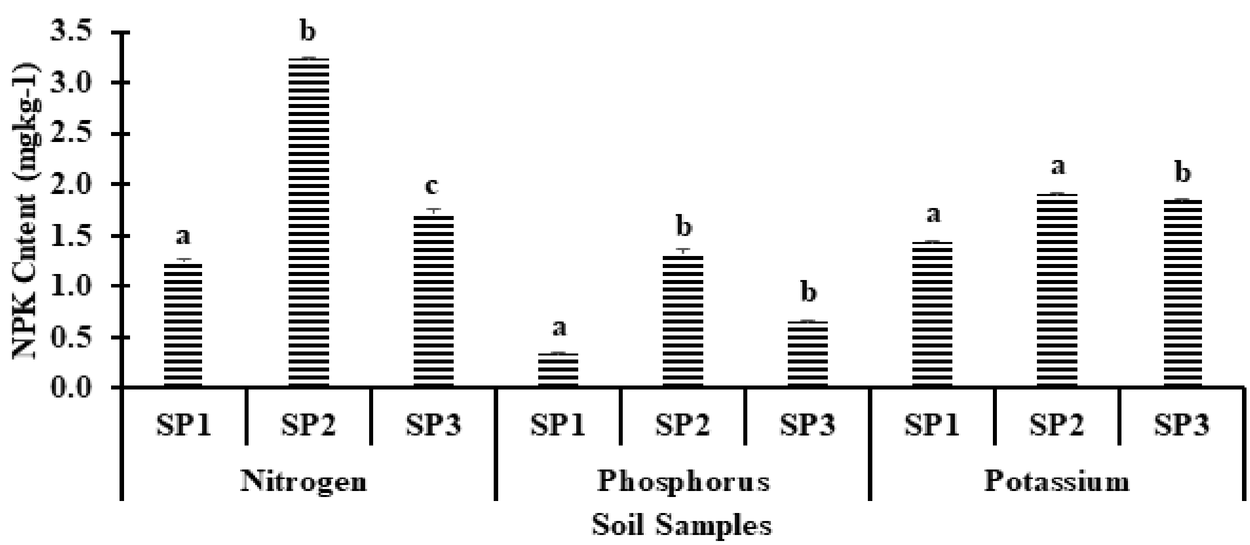

Figure 2. Evaluation of macronutrient content (NPK) of soil samples under study.

Evaluation of heavy metals (Cd, Cr, and Pb) in soil irrigated with Rohi drain wastewater: The cadmium (Cd) content of the soil samples ranged from 0.16 to 0.22 mg kg^{-1}. SP1 showed the Cd content of 0.22 mg kg^{-1} while SP2 and SP3 showed Cd content of 0.16 mg kg^{-1} and 0.20 mg kg^{-1}, respectively Figure 3 shows that the highest Cd content (0.241 mg kg^{-1}) was observed in soil sample of sampling point SP1 while lowest Cd content (0.084 mg kg^{-1}) was observed in soil sample SP2. The Chromium (Cr) content of the soil samples ranged from 0.066 to 0.193 mg kg^{-1}. SP1 showed the Cr content of 0.193 mg kg^{-1} while SP2 and SP3 showed Cr content of 0.044 mg kg^{-1} and 0.052 mg kg^{-1}, respectively. Figure 3 shows that the highest Cr content (0.193 mg kg^{-1}) was observed in soil sample SP1 and the lowest Cr concentration (0.044 mg kg^{-1}) was observed in soil sample SP2. The lead (Pb) content of the soil samples ranged from 0.16 to 0.27 mg kg^{-1}. SP1 showed the Pb content in soil 0.16 mg kg^{-1} while SP2 and SP3 showed Pb content of 0.18 mg kg^{-1} and 0.27 mg kg^{-1}, respectively. Figure 3 shows that the highest Pb content (0.27 mg kg^{-1}) was observed in soil sample SP1 and the minimum Pb content (0.16 mg kg^{-1}) was observed in soil sample SP2.

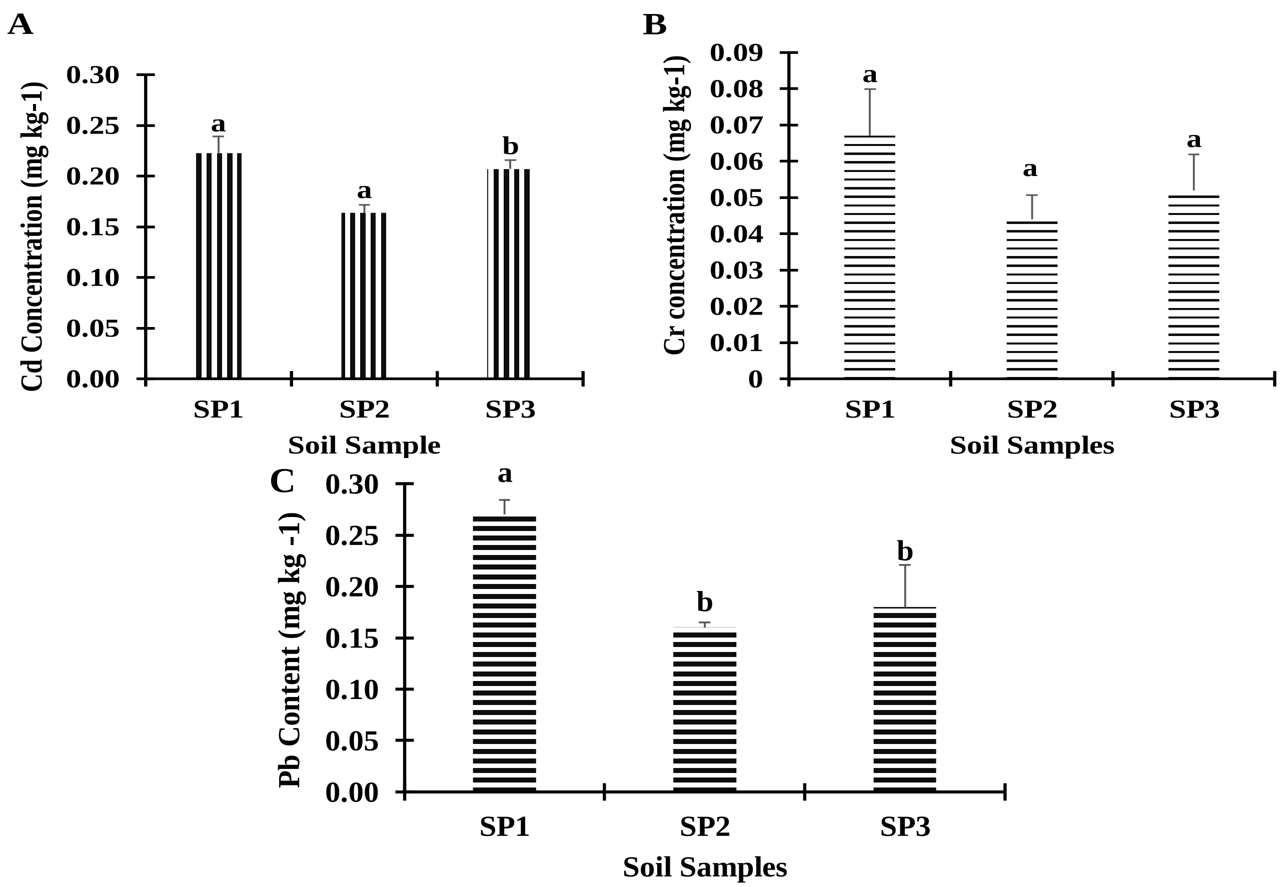

Figure 3. Evaluation of (A) Cd, (B) Cr and (C) Pb content in soil samples under study.

Evaluation of physio-chemical parameters of *Trifolium alexandrinum* irrigated with Rohi drain wastewater

Estimation of shoot length of *T. alexandrinum* irrigated with Rohi drain wastewater: The shoot length of all the plant samples was depicted in Figure 4. The shoot length of plant samples ranged from 23.3 cm to 26.1 cm. Sample SP1 showed the shoot length of 23.3 cm while SP2 and SP3 showed the shoot length of 26.1 cm and 25 cm, respectively. The maximum shoot length (26.167 cm) of *T. alexandrinum* was observed in sample SP2 while minimum shoot length (23.333 cm) was observed in sample SP1.

Estimation of root length of *Trifolium alexandrinum* irrigated with Rohi drain wastewater: The root length of all the plant samples was depicted in Figure 4. The root length of plant samples ranged from 9.1 cm to 11.8 cm. Sample SP1 showed the root length of 9.1 cm while SP2 and SP3 showed the root length of 11.8 cm and 9.6 cm, respectively. The maximum root length (11.8 cm) of *T. alexandrinum* was observed in sample SP3 while minimum shoot length (9.1 cm) was observed in sample SP1.

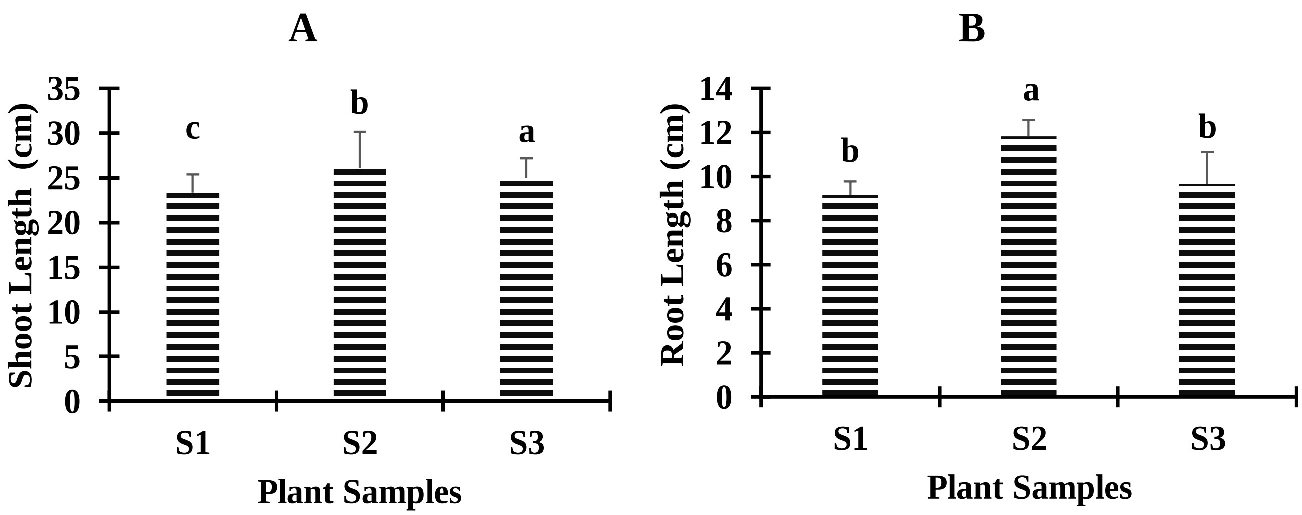

Figure 4. Evaluation of (A) shoot length and (B) root length of *Trifolium alexandrinum*

Estimation fresh and dry weight of shoot of *Trifolium alexandrinum* irrigated with Rohi drain wastewater: The fresh weight (FWS) and dry weight (DWS) of shoot samples was depicted in Figure 5. The shoot fresh weight of *T. alexandrinum* ranged from 38 g to 53g. Sample SP1 showed 38 g of the FWS while SP2 and SP3 showed the FWS of 53 g and 45 g, respectively. The highest FWS (53 g) of *T. alexandrinum* was observed in sample SP2 while lowest FWS (38 g) was observed in sample SP1. The shoot dry weight of *T. alexandrinum* ranged from 17.2 g to 27.3 g. Sample SP1 showed 17.2 g while SP2 and SP3 showed DWS of 27.3 g and 22.1 g, respectively. The maximum DWS (27.3 g) of *T. alexandrinum* was observed in sample SP1 while minimum DWS (17.2 g) was observed in sample SP3.

Estimation of fresh and dry weight of root of *Trifolium alexandrinum* irrigated with Rohi drain wastewater: The FWR and DWR of root samples was depicted in Figure 5. The FWR of *T. alexandrinum* ranged from 28 g to 29.6 g. Sample SP1 showed 28 g of the FWR while SP2 and SP3 showed the root fresh weight of 32.3 g and 29.6 g, respectively. The maximum FWR (32.3 g) of *T. alexandrinum* was observed in sample SP2 while minimum FWR (28 g) was observed in sample SP1. The DWR of *T. alexandrinum* ranged from 12.4 g to 16.3 g. Sample SP1 showed 12.4 g while SP2 and SP3 showed DWR of 16.3 g and 14.2 g, respectively. The highest DWR (16.3 g) of *T. alexandrinum* was observed in sample SP3 while lowest DWR (12.4 g) was observed in sample SP1.

Estimation of chlorophyll content of shoot of *Trifolium alexandrinum* irrigated with Rohi drain wastewater: The chlorophyll content of the plant samples ranged from 1.21 mg g^{-1} to 3.9 mg g^{-1}. The maximum chlorophyll (a) content (2.2 mg/g) was recorded in sample SP2 of *Trifolium alexandrinum* while minimum chlorophyll (a) content (1.5 mg/g) *alexandrinum* was recorded in samples SP1. The chlorophyll (b) content of 1.7 mg/g was recorded in sample SP2 of *Trifolium alexandrinum* which was found to be maximum while minimum chlorophyll (b) content (1.21 mg/g) was observed in sample SP1. The maximum value of total chlorophyll content (3.9 mg/g) was observed in sample SP2 while minimum total chlorophyll content (2.3 mg/g) was observed in sample SP1.

Estimation of N, P, and K in shoot of *Trifolium alexandrinum* irrigated with Rohi drain wastewater: The nitrogen (N) content in the shoots of *Trifolium alexandrinum* ranged from 1.28 to 3.2 mg kg^{-1}. SP1 showed the N content of 1.28 mg kg^{-1} while SP2 and SP3 showed N content of 3.2 mg kg^{-1} and 1.61 mg kg^{-1}, respectively. Figure 6 shows that the maximum concentration of N (3.2 mg kg^{-1}) in shoot of *T. alexandrinum* was recorded in sample SP2 and the minimum concentration of N (1.28 mg kg^{-1}) was recorded in sample SP1.The phosphorus (P) content in the shoots of *Trifolium alexandrinum* ranged from 0.93 to 1.75 mg kg^{-1}. SP1 showed the P content of 0.9 mg kg^{-1} while SP2 and SP3 showed P content of 1.75 mg kg^{-1} and 1.27 mg kg^{-1}, respectively. Figure 6 shows that the maximum concentration of P (1.75 mg kg^{-1}) in shoot of *T. alexandrinum* was recorded in sample SP2 and the minimum concentration of P (0.93 mg kg^{-1}) was recorded in sample SP1. The potassium (K) content in the shoots of *Trifolium alexandrinum* ranged from 0.94 to 1.67 mg kg^{-1}. SP1 showed the K content of 0.94 mg kg^{-1} while SP2 and SP3 showed K content of 1.67 mg kg^{-1} and 1.37 mg kg^{-1}, respectively. Figure 6 shows that the maximum concentration of K (1.67 mg kg^{-1}) in shoot of *T. alexandrinum* was recorded in sample SP2 and the minimum concentration of K (0.94 mg kg^{-1}) was recorded in sample SP1.

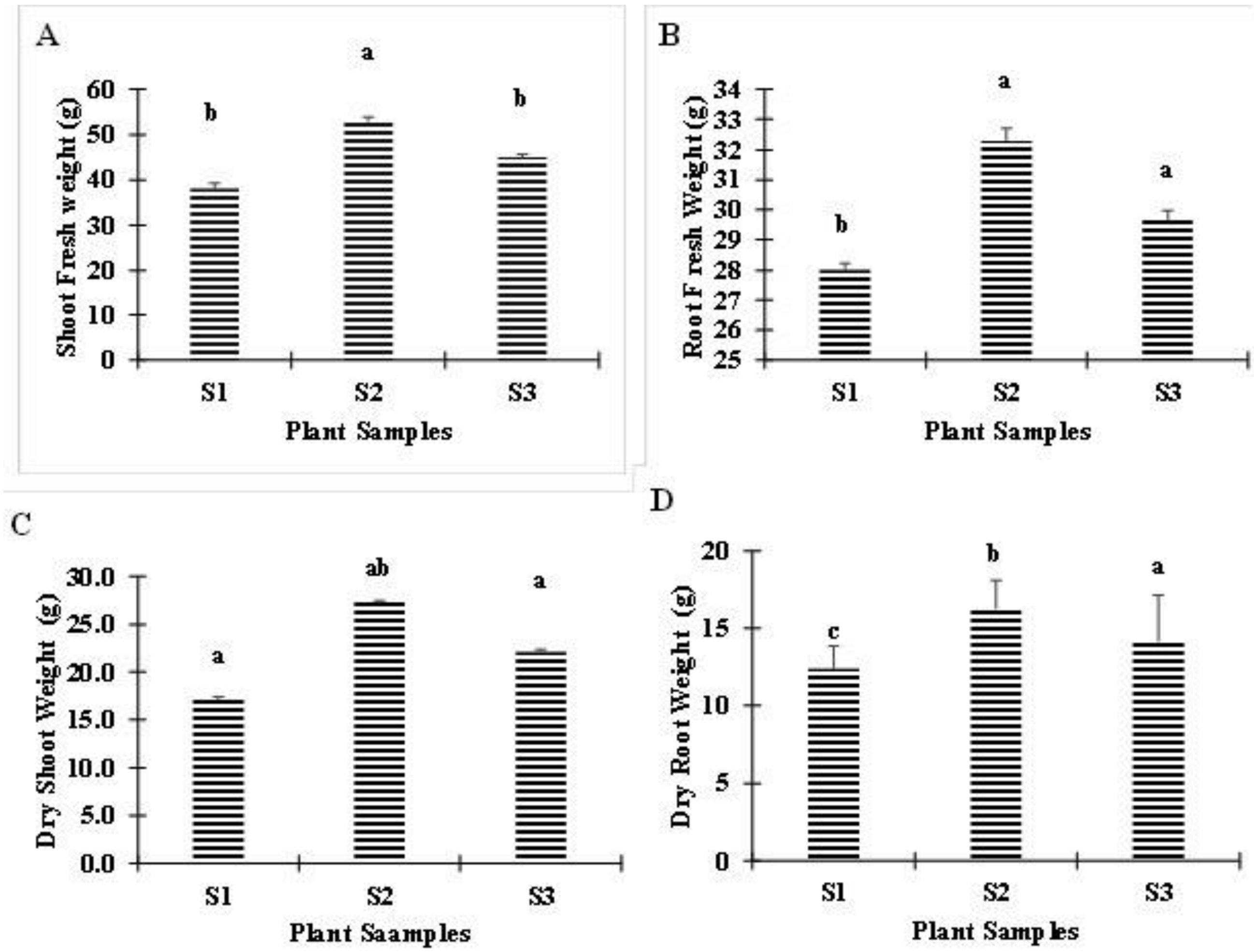

Figure 5. Fresh and dry weight of shoot and root of *Trifolium alexandrinum* irrigated with Rohi drain wastewater.

Table 2. Evaluation of chlorophyll content in *T. alexandrinum.*

Sr no.	Chlorophyll content	SP1	SP2	SP3	Unit
1.	Chlorophyll a	1.5	2.2	1.7	mg g^{-1}
2.	Chlorophyll b	1.21	1.7	1.26	mg g^{-1}
3.	Total Chlorophyll	2.36	3.9	2.7	mg g^{-1}

Estimation of N, P and K in root of *Trifolium alexandrinum* irrigated with Rohi drain wastewater: The nitrogen (N) content in the roots of *Trifolium alexandrinum* ranged from 1.87 to 3.19 mg kg^{-1}. SP1 showed the N of 1.87 mg kg^{-1} while SP2 and SP3 showed N content of 3.19 mg kg^{-1} and 3.09 mg kg^{-1}, respectively. Figure 6 shows that the maximum concentration of N (3.2 mg kg^{-1}) in root of *T. alexandrinum* was recorded in sample SP2 and the minimum concentration of N (1.87 mg kg^{-1}) was recorded in sample SP1. The phosphorus (P) content in the roots of *Trifolium alexandrinum* ranged from 2.09 to 3.17 mg kg^{-1}. SP1 showed the P content of 2.09 mg kg^{-1} while SP2 and SP3 showed P content of 3.17 mg kg^{-1} and 2.51 mg kg^{-1}, respectively. Figure 6 shows that the maximum concentration of P (3.17 mg kg^{-1}) in root of *T. alexandrinum* was recorded in sample SP2 and the minimum concentration of P (2.09 mg kg^{-1}) was recorded in sample SP1.

The potassium (K) content in the roots of *Trifolium alexandrinum* ranged from 1.75 to 2.71 mg kg^{-1}. SP1 showed the K content of 1.75 mg kg^{-1} while SP2 and SP3 showed K content of 2.71 mg kg^{-1} and 1.91 mg kg^{-1}, respectively. Figure 6 shows that the maximum concentration of K (2.71 mg kg^{-1}) in roots of *T. alexandrinum* was recorded in sample SP2 and the minimum concentration of K (1.75 mg kg^{-1}) was recorded in sample SP1.

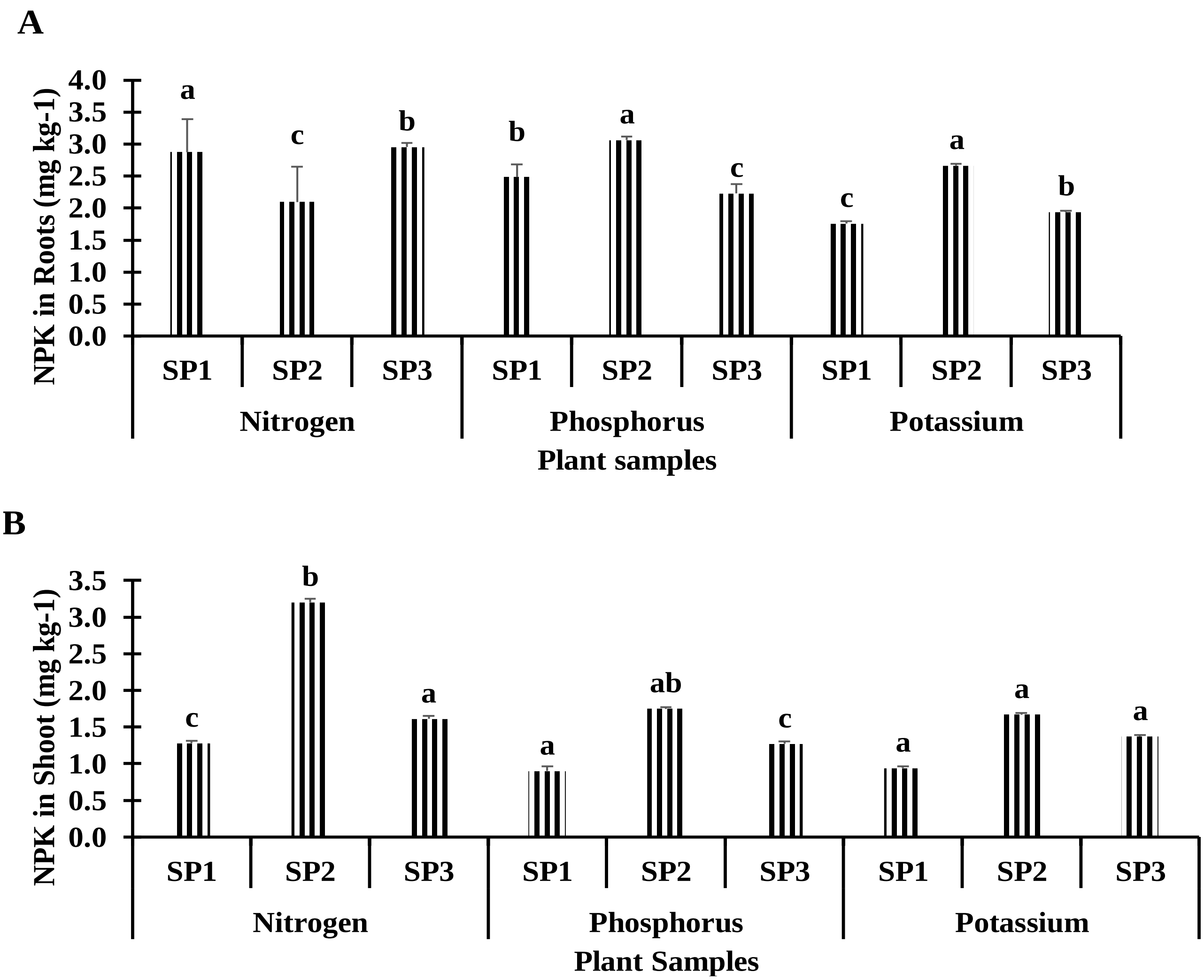

Figure 6. Evaluation of macronutrients (NPK) in (A) roots and (B) shoots of *T. alexandrinum*

Evaluation of Pb, Cd, and Cr accumulation in shoot of *Trifolium alexandrinum* irrigated with Rohi drain wastewater: In *Trifolium alexandrinum* shoot, the level of Cd heavy metal ranging from 0.14 to 0.17 mg kg^{-1}. SP1 showed 0.17 mg kg^{-1} while SP2 and SP3 showed Cd content of 0.14 mg kg^{-1} and 0.17 mg kg^{-1}, respectively. Figure 7 shows that the highest Cd content (0.17 mg kg^{-1}) in shoot of *T. alexandrinum* was noted in sample SP1 and lowest Cd accumulation (0.14 mg kg^{-1}) was noted in sample SP2.

Similarly, the Cr content ranging from 0.008 to 0.042 mg kg^{-1}. SP1 showed the Cr content of 0.04 mg kg^{-1} while SP2 and SP3 showed Cr content of 0.008 mg kg^{-1} and 0.02 mg kg^{-1}, respectively. Figure 7 shows that the highest level of Cr (0.04 mg kg^{-1}) in shoot of *Trifolium alexandrinum* was observed in sample SP1 and the lowest Cr level (0.008 mg kg^{-1}) was noted in shoot sample SP2. The Pb content of the plant samples ranging from 0.02 to 0.06 mg kg^{-1}. SP1 showed 0.067 mg kg^{-1} of Pb concentration in plants, while SP2 and SP3 showed Pb content of 0.02 mg kg^{-1} and 0.04 mg kg^{-1}, respectively. Figure 7 shows that highest Pb content (0.067 mg kg^{-1}) in shoot of *T. alexandrinum* was observed in sample SP1 and the lowest Pb content (0.025 mg kg^{-1}) was noted in sample SP2.

Evaluation of heavy metals (Pb, Cd, and Cr) in root of *Trifolium alexandrinum* irrigated with Rohi drain wastewater: The concentration of Cd in the roots of plant samples ranged from 0.13 to 0.25 mg kg^{-1}. SP1 showed the Cd content of 0.25 mg kg^{-1} while SP2 and SP3 showed Cd content of 0.13 mg kg^{-1} and 0.17 mg kg^{-1}, respectively. Figure 7 shows that the maximum concentration of Cd (0.25mg) kg^{-1}) in root of *T. alexandrinum* was observed in sample SP1 and the minimum concentration of Cd (0.13 mg kg^{-1}) was observed in sample SP2. The concentration of Cr in the roots of plant samples ranged from 0.14 to 0.67 mg kg^{-1}. SP1 showed the Cr content of 0.67 mg kg^{-1} while SP2 and SP3 showed Cr content of 0.14 mg kg^{-1} and 0.23 mg kg^{-1}, respectively. Figure 7 shows that the maximum concentration of Cr (0.67 mg kg^{-1}) in shoot of *Trifolium alexandrinum* was observed in sample SP1 and the minimum concentration of Cr (0.14 mg kg^{-1}) was observed in shoot sample SP2.

The level of Pb in plant samples ranged from 0.32 to 0.88 mg kg^{-1}. SP1 indicated 0.88 mg kg^{-1} of Pb, while SP2 and SP3 indicated Pb levels of 0.32 mg kg^{-1} and 0.51 mg kg^{-1}, respectively. Figure 7 shows that the highest Pb accumulation (0.88 mg kg^{-1}) in shoot of *T. alexandrinum* was observed in sample SP1 and the lowest level of Pb (0.32 mg kg^{-1}) was observed in sample SP2.

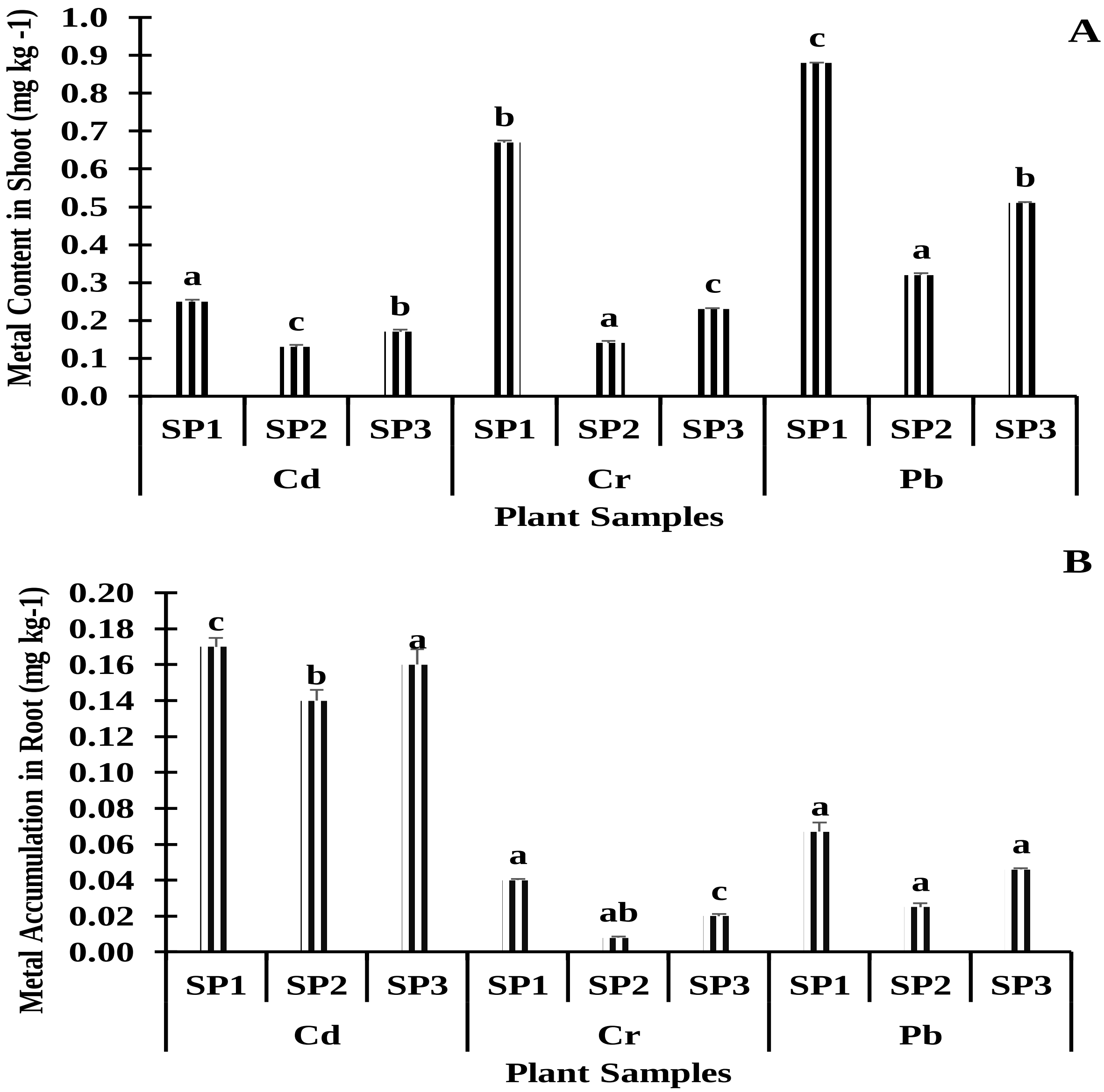

Figure 7. Evaluation of heavy metal content in (A) shoots and (B) roots of *T. alexandrium*.

Evaluation of water quality parameters: Physico-chemical characteristics of wastewater are presented in table 3. The EC and pH of wastewater was 1653 µS/cm and 8.2, respectively. The total dissolved solids (TDS) and total suspended solids (TSS) of wastewater was 1281 mg L^{-1} and 875 mg L^{-1}, respectively. The chemical oxygen demand (COD) and biological oxygen demand (BOD) of wastewater was 537 mg L^{-1} and 269 mg L^{-1}, respectively. The concentration of cadmium, chromium and lead was 0.004 mg L^{-1}, 0.51 mg L^{-1} and 0.15 mg L^{-1}, respectively.

Range of physio-chemical parameters of Rohi drain wastewater: Range of all studied physio-chemical parameters of samples of soil and plant (*Trifolium alexandrinum*) collected from agricultural area nearby Rohi drain is shown in Table 4.1. Data in Table 4 has shown that the pH, EC, N, P & K and Pb, Cd and Cr of soil samples irrigated with Rohi drain wastewater ranged from 7.867 to 8.033, 146.67 to 440, 1.4 to 3, 0.4 to 1.2, 1.452 to 1.953, 0.084 to 0.241, 0.066 to 0.193 and 0.038 to 0.211, respectively.

Data in Table 4 has shown that the height of shoot of (*Trifolium alexandrinum*), height of root of (*Trifolium alexandrinum*), fresh and dry weight of shoot of (*Trifolium alexandrinum*), FWR and DWR of (*Trifolium alexandrinum*), N, P, & K of shoot of (*Trifolium alexandrinum*), N, P, & K of root of (*Trifolium alexandrinum*), Cd, Cr & Pb of shoot of (*Trifolium alexandrinum*), and Cd, Cr, & Pb of root of (*Trifolium alexandrinum*), irrigated with Rohi drain wastewater ranged from 23.333 to 26.67, 9.167 to 11.833, 50 to 53, 28 to 29.667, 41.333 to 42.333, 17.333 to 22.667, 1.5 to 2.2, 1.2 to 1.7, 2.7 to 3.9, 1.33 to 3.2, 0.967 to 1.667, 0.947 to 1.633, 1.5 to 3.56, 2.033 to 3.033, 1.703 to 2.6, 0.177 to 2.247, 0.011 to 0.024, 0.046 to 0.081, 0.136 to 0.167, 0.008 to 0.041, and 0.019 to 0.042, respectively.

Table 3. Characterization of Ruhi drain wastewater.

Sr no.	Water quality parameters	Wastewater	Permissible limits (NEQs)	Unit
1.	pH	8.2	6.5-8.5	-
2.	EC	1653	3500	µS/cm
3.	TDS	1281	1000	mg L^{-1}
4.	TSS	875	200	mg L^{-1}
5.	COD	537	150	mg L^{-1}
6.	BOD	269	80	mg L^{-1}
7.	Cadmium	0.004	0.5	mg L^{-1}
8.	Chromium	0.51	0.5	mg L^{-1}
9.	Lead	0.15	0.1	mg L^{-1}

Table 4. Range of physio-chemical parameters of soil and plant (*Trifolium alexandrinum*) irrigated with Rohi drain wastewater.

Parameters of soil irrigated with Rohi drain wastewater		
Parameter	Minimum value	Maximum
ph	7.867	8.033
EC	146.67	440
Moisture content	16.867	20.8
N	1.4	3
P	0.4	1.2
K	1.452	1.953
Cd	0.084	0.241
Cr	0.066	0.193
Pb	0.038	0.211
Prameters of Trifolium alexandrinum irrigated with Rohi drain wastewater		
Parameter	Minimum value	Maximum
Height of shoot	23.333	26.67
Height of root	9.167	11.833
Fresh weight of shoot	50	53
Fresh weight of root	28	29.667
Dry weight of shoot	41.333	42.333
Dry weight of root	17.333	22.667
Chlorophyll (a) in shoot	1.5	2.2
Chorophyll (b) in shoot	1.2	1.7
Total chlorophyll in shoot	2.7	3.9
"N" in shoot	1.33	3.2
"P" in shoot	0.967	1.667
"K" in shoot	0.947	1.633
"N" in root	1.5	3.56
"P" in root	2.033	3.033
"K" in root	1.703	2.6
"Cd" in shoot	0.177	0.247
"Cr" in shoot	0.011	0.024
"Pb" in shoot	0.046	0.081
"Cd" in root	0.136	0.167
"Cr" in root	0.008	0.041
"Pb" in root	0.019	0.042

DISCUSSION

Heavy metals are toxic environmental pollutants. They can proliferate in soil and cause serious complications in food chain and ultimately cause severe deteriorating effects at different trophic levels of food chain. The fodder crops like *Trifolium alexandrinum* are being harvested in soil which is being irrigated via Rohi drain wastewater in Kasur, Lahore (Pakistan). In this study, the physio-chemical parameters of soil and plant samples of *Trifolium alexandrinum* that are being irrigated with Rohi drain wastewater were analyzed. The determination of physio-chemical parameters of soil and plant is necessary to determine whether the wastewater of Rohi drain is suitable for irrigation purposes or not. The physio-chemical properties of the soil irrigated with Rohi drain wastewater were analyzed and showed great deviations of the soil quality parameters which appeared to indicate the prolonged effect of wastes components on the soil characteristics. It was observed that higher pH was shown by sample SP2 while minimum Ph was shown by SP1. This might be due to the reason that presence of heavy metals causes the soil to become acidic. Similar findings were reported by Singh *et al*. (2020).

The capacity of solution to conduct the electricity is referred to as EC and it varies in number and type of ions in solution. In comparison to the normal soil, the EC value of the soil samples under study was found higher. Higher electrical conductivity values found in soils irrigated with wastewater imply salinity, which is due to the accumulation of soluble salts in repeated irrigation events (Javid *et al*., 2022). Any such salinity build-up may cause deleterious effects such as altering soil structure, decreasing water infiltration, as well as inhibiting the growth of plants. These results are consistent with those that indicate that long-standing irrigation with partially or untreated industrial wastewater may change soil physco-chemical properties, both negatively impacting (salinity, sodicity, and heavy metal accumulation) and positively (high organic matter and nutrients). Similar findings were reported by Sharma *et al*. (2016); Ahmad *et al*. (2019).

The moisture content and nutrient content (N, P and K) of soil samples being irrigated via Rohi drain wastewater was determined. The results showed that irrigation of wastewater significantly impinged on soil moisture content and nutrient content of soil samples. The moisture content and macronutrient values of soil samples were found lower than the standard issued by WHO. Industrial wastewaters usually contain more moisture than freshwater and thus the result was that soils irrigated with industrial wastewater had a considerable moisture content than those that were irrigated with freshwater. The occurrence of dissolved salts, suspended solid, and organic matter in wastewater could be the reason of lower macronutrient content, according to Javid *et al*. (2022). Another reason for lower macro-nutrient content might also be due to the presence of heavy metals present in wastewater being used for irrigation of soil sample used in this study. Our results are in alignment with Javid *et al*. (2022); Sharma *et al*. (2016).

The influence of Ruhi drain wastewater on the growth attributes of *Trifolium alexandrinum* collected from the vicinity of Ruhi drain was deternmined. Among the collected samples, the minimum growth features including root length, shoot length, root and shoot fresh and dry biomass were shown by sample SP1. This reduction in growth attributes of *Trifolium alexandrinum* might be due to the presence of heavy metals. Accumulation of Pb heavy metal in plant bodies causes growth retardation. Similarly, Cd and Cr also cause chlorosis and poor growth of plants. Similar findings were reported by Mahmood & Malik (2014) (Khan *et al*., 2018). The untreated industrial wastewater that provided the source of irrigation also had dramatic effect to the growth properties and the nutrient content of *Trifolium alexandrinum*. The length of roots and shoots, as well as fresh and dry biomass showed decrease, which means that salinity, sodicity, and presence of dangerous metals in wastewater had a negative impact on plant physiology and mitosis (Sharma *et al*., 2016). Biomass decrease goes in line with the stressed-induced deprivation of photosynthesis and the degradation of water and nutrient absorption due to the interception of heavy metals in the root metabolic processes (Ahmad *et al*., 2019).

In the case of macronutrient analysis, the change in the N, P, and K values of the roots and the shoots was analyzed. Although wastewater supplied extra nutrients, the toxicity of heavy metals, excess salts perhaps led to a limitation of nutrient translocation between roots and shoots, thus giving rise to nutrient imbalances. Cases of such antagonistic counter-reactions have been cited occurring between Pb, Cd, and Cr relative to vital nutrient ions that have decreased mobility and further absorption in plant tissues (Mahmood & Malik, 2014). The analysis of metal accumulation proved that there are significantly higher concentrations of Cd, Cr and Pb in the roots and shoots with lower levels being localized in roots. The trend demonstrates the defensive strategy of the plant in which the translocation of the metals is limited to the aerial portions (which is also evident in other forage crops that are grown in contaminated soils) (Khan *et al*., 2018). Nonetheless, the heavy metal content in the shoots was higher than levels that are permitted to be used as animal feed causing possible harm to the livestock via the food chain. In summation, raw industrial wastewater not only hampered growth properties of T. alexandrinum but also triggered the perilous amount of metal accumulation representing the potential of effluent treatment before agricultural usage. Our results are in line with the results reported by Khan *et al*. (2018).

Conclusion: Wastewater irrigation has increased significantly due to water scarcity. There is a need to develop strategies to combat with water scarcity and pollution issues in order to avoid its detrimental effect on soil and plants irrigated with untreated wastewater. The study of soil and *Trifolium alexandrinum* irrigated with Ruhi drain wastewater showed that there were significant changes to soil physicochemical characteristics and strong enrichment of the plant with heavy metals. Wastewater irrigation reduced the soil moisture content, pH and macronutrient level (N, P, K) of soil samples and plants irrigated through Ruhi drain wastewater where maximum reduction was observed in sample SP1. The following growth parameters were negatively impacted; root and shoot length, shoot and root biomass, Pb, Cd and Cr accumulated in roots, and shoots showed concentration higher than the FAO/WHO standards allowable in fodder. These results point out the duality of wastewater irrigation, which entails buttressing on the benefits as well as the risks posed to environment and the food chain, in particular; this shows the great urgency of effluent treatment before it is reused in agricultural practices.

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