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Nutrient dynamics and ion partitioning in *Cynodon dactylon* under combined salinity and metal stress in hypersaline environments

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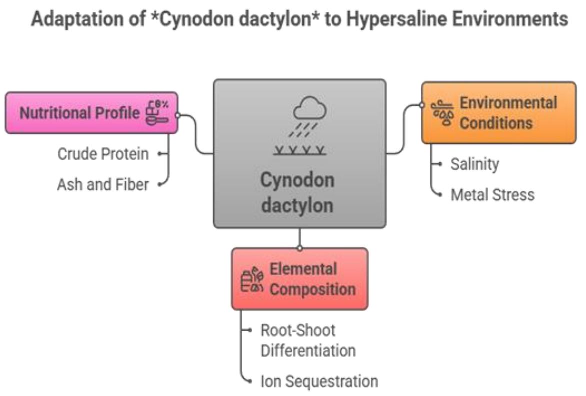
Graphical abstract



Abstract

Combined salinity and metal stresses are exerted in hypersaline environments and have a significant effect on the dynamics of nutrients and physiological performance of plants. The research has studied the elemental composition, nutritional conditions and adaptive mechanisms of *Cynodon dactylon* in hypersaline areas of the Salt Range, Pakistan. Surface and groundwater samples, soil samples, and plant samples at 5 representative sites were collected using standard analytical methods and analyzed using ANOVA, Tukey HSD and canonical correspondence analysis (CCA). The chemistry of soil and water differed significantly across sites, with electrical conductivity values ranging between 4.1 and 18.6 dS m^{-1}, which led to different patterns of uptake of various elements in plants. Plant tissues displayed evident root-shoot differentiation with roots having a higher concentration of Mn and Ca (up to 412 and 9,840 µg g -1 dry weight), and shoots having a higher concentration of Fe and Cu (up to 387 and 368 µg g -1 dry weight). Such tissue-specific partitioning implies that ion sequestration will be regulated as a major form of adaptation in hypersaline conditions. The nutritional profile revealed an average amount of crude protein (6.8-9.4%), higher levels of ash and fibre, which were evidence of augmented mineral loading and structural adaptation to stress. Our findings indicate that Bermuda grass has acclimatized to the prevailing conditions and can thrive in hypersaline environments, making it a potential fodder grass for grazing animals.

Keywords: Bermuda grass, Crude fiber, Crude protein, Lake, Metals, Salt Range, Stress

1. Introduction

Grasses are the most diverse group of flowering plants, with over 10,000 species divided into 610 genera (Akbari *et al.,* 2024). Grasslands are more widely distributed than other blooming plants around the planet. Different grasses make up the majority of the vegetation in semiarid areas. Humans can eat some grass types, such as cereals, and animals can eat them as fodder. Grass plants respond to environmental challenges in a variety of ways, altering their development, growth, photosynthesis, yield, and water relationships. However, the degree of response to these changes varies across plant species and between phases of development (Rehman *et al.,* 2024). It is well

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known that plants undergo several growth, physiological, and biochemical changes in response to salt stress. Overall, plant growth might be hindered by salt stress. Salinity causes a decline in plant growth, including shoot and root length, leaf and root water content, leaf dry weight, and transpiration (Nefissi *et al.,* 2021; Abd El-Moneim *et al.,* 2021; Tang *et al.,* 2022).

Bermuda grass, or *Cynodon dactylon*, is a perennial grass that grows throughout the world but is native to warm temperate and tropical climates. It is indigenous to southern Europe, Asia, Australia, and East Africa. The weed Cynodon has a number of possible therapeutic uses (Tashsin *et al.,* 2021). *C. dactylon* is distributed in tropical and warm temperate regions, makes excellent hay considered fodder grass, and can tolerate moderate to high salinities (Irshad *et al.,* 2024). According to Zhus *et al.* (2023), Bermuda grass species are categorized as semitolerant to drought stress based on their salinity tolerance, which varies between 6 and 10 dS m^{-1}. The morphophysiological characteristics of plants, particularly grasses, determine their ability to withstand drought and salinity (Zuffo *et al.,* 2022). Grass is well known for its highly adaptable nature, allowing it to thrive in a wide range of environments. It is commonly found in sandy or saline soils of open sites such as roadsides, fields, canals, orchards, dumps, and areas contaminated with heavy metals such as lead, zinc, and copper (Nazish *et al.,* 2024).

Khabeki Lake (32°37 N, 72°12E) is a shallow brackish water lake of approximately 283 ha in the Soon Valley. This lake is located at an elevation of 740 m above mean sea level. Local rainfall and various intermittent streams rising in nearby hills feed this lake. This stagnant water lake is fed mainly by rainfall during the winter monsoon and summer monsoon, as well as runoff. Uchalli Lake (32° 33 N, 72° 01'E) covers an area of 943 ha with depths of 0.2-6.0 m at an altitude of 764 m above sea level. This area is a brackish to saline water wetland surrounded by agricultural areas. Uchalli Lake is also fed by rainfall and runoff and is flooded throughout the year (Bhatti *et al.,* 2018). The Khewra salt mine (32°5600 N, 73°44E) (**Table 1**), located at the base of the Salt Range, is the second largest salt mine in the world and contains numerous valuable salts, such as sodium chloride (NaCl), potassium chloride (KCl), and calcium sulfate dihydrate ($CaSO_4$. $2H_2O$).

Table 1. Meteorological records and locations of the selected study sites in the Salt Range, Pakistan.

Sr. no	Site Name	Temperature (°C)		Annual Rainfall (mm)	Latitude	Longitude	Elevation
		Max.	Min.				
1	Khabeki lake	25.8	11.54	669	32°37'18.84"N	72°12'50.76"E	740 m
2	Uchalli Lake	20.5	10.2	670	32° 33' 36.00" N	72° 01' 12.00" E	764 m
3	Pir Da Khara	28.65	14.5	905	32°38'44.05"N	72°44 '29.36"E	295 m
4	Khewra Mine	33.15	21.29	544	32°56 '52.76"N	73°40 '30.27"E	359 m

The recorded conditions in the Khewra salt range were annual rainfall: 900 mm; the maximum temperature in June was 45.7 °C; the minimum temperature in January was 1.8 °C; and the soil type was sandy loam (Nazish *et al.,* 2024). Pir Da Khara is a famous shrine whose natural spring is located in the Salt Range. This spring starts from Pind Kathoa and extends up to 20 km, passing through the hills, and its water is saline. The climatic conditions in the Pir Da Khara recorded during the study were as follows: annual rainfall: 905 mm; maximum temperature in June: 28.65 °C; minimum temperature in January: 14.5 °C; and loam soil type. This pioneer study of grasses in Pir Da Khara recorded the response of selected grasses to water in the spring. In previous studies, the response of Bermuda grass to different kinds of environmental conditions was explored.

The project investigates the widespread *Cynodon dactylon* growing alongside natural lakes and springs adapt to prevailing environmental conditions and the relationship between *C. dactylon* traits and the surrounding environment. To date, no study has been conducted that is strictly related to documenting the response of *C. dactylon* growing alongside lakes and springs in the Salt Range to answer these questions. The salt range of Pakistan has unique environmental and soil conditions. Most previous investigations have relied on controlled pot experiments, which often fail to capture the complexity of field-scale interactions between soil chemistry, groundwater composition, and plant nutrient acquisition. Thus, in our study, we measured the elemental content and proximate composition of Bermuda grass along with water and soil analyses of the study areas. The objectives of this research were to i) quantify elemental and proximate composition variability in *C. dactylon* across hypersaline sites, ii) determine the potential role of *C. dactylon* as fodder in saline regions or its role in phytoremediation, and iii) evaluate the relationships among different traits of *C. dactylon*, water and the soil of lakes and springs.

2. Materials and methods

The native grass species *Cynodon dactylon* was collected from two different types of habitats, viz. natural lakes and springs in 2021-2022. Natural lakes, including Uchalli Lake and Khabeki Lake, and springs, i.e., Pir Da Khara and Khewra, from the Salt Range, Pakistan, were selected for this study. The four study sites were visited to collect water and soil samples during June-July. The collection of plant samples was performed at the vegetative stage directly from the fields to determine the element content and proximate composition. Five plants were collected from each site. Meteorological data, including the mean average temperature and mean average rainfall over a year (2021-2022), along with latitude, longitude and elevation, were recorded for the four study sites.

2.1. Soil physiochemical analysis

Soil is the medium that supplies plants with available nutrients through roots. Physical and chemical analyses of the soil were carried out to determine the efficiency of supplying macronutrients to the soil as described by Estefan *et al*. (2013). The soil organic matter content was calculated via the accepted technique developed by Walkley and Black (1934). By using Olsen's (1954) sodium bicarbonate technique, the phosphorus content of the soil was measured. An Atomic Absorption Spectrophotometer (AAS, Perkin Elmer Analyst 400) was used to evaluate the presence of heavy metals in the soil after it had been digested using the Tri-Acid digestion procedure as described in Estefan *et al*. (2013). All the samples were analysed at the High Tech Laboratory of the Pharmacy Department, University of Sargodha.

2.2. Water analysis

Water samples were collected from the four study sites (lakes and springs). The collected water was analysed for pH, EC, Ca+Mg, Na, sodium absorption ratio (SAR), carbonate, bicarbonate, chloride, total dissolved solids (TDS), and total suspended solid (TSS) contents, following the methods described by Estefan *et al*. (2013). The water samples were also analysed for heavy metals. The samples were digested for heavy metal determination according to APHA (2005). The concentrations of heavy metals in the filtrate of the digested water were estimated by using an atomic absorption spectrophotometer (AAS, Perkin Elmer analyst 400).

2.3. Elemental analysis

Dried plant material (roots, stems and leaves) was ground in a dry mill. One gram of this dried material was digested with sulfuric acid and hydrogen peroxide following the Wolf method (1982). Macronutrients and trace metals (Fe, Mn, Cu, Zn, Cd, Pb, Ni, Co, and Cr) were measured after acid digestion using atomic absorption spectrophotometry (AAS). Flame photometry was used to measure sodium (Na^+) and potassium (K^+) concentrations independently.

A flame photometer (Perkin Elmer A Analyst 700) was used to measure the levels of Na, K, Ca and Mg in the grasses. The standard curves were created using a graded series of standards with Na, K, Ca, and Mg concentrations ranging from 10, 20, to 100 µg/g. The flame photometer Na, K, Ca, and Mg readings were compared with the standard curve, and the calculated values are expressed as µg/g dry weight.

2.4. The potassium to sodium (K^+/Na^+) ratio

The potassium to sodium (K^+/Na^+) ratio was used to evaluate ion homeostasis in hypersaline circumstances. Despite high Na^+ concentrations at all sites, *C. dactylon* maintained a steady K^+/Na^+ ratio, demonstrating selective ion uptake and exclusion of excess sodium at the root-soil interface.

2.5. Phosphorus (P) contents

The phosphate contents in the roots were determined by using the method of Yoshida (1977).

2.6. Determination of heavy metal content

For the determination of heavy metals, 500 mg of plant material was digested by the diacidic digestion method described by Estefan *et al*. (2013). For this purpose, the plant material was first digested with concentrated HNO_3, followed by a diacid mixture of HNO_3 and $HClO_4$. After digestion, the contents were filtered and diluted by increasing their volume to 50 mL. The concentrations of heavy metals were determined via an atomic absorption spectrophotometer.

2.7. Proximate composition

The collected grass samples were analysed for proximate composition analysis of the dry matter, moisture, ash, crude fibre, crude fat, crude protein, nitrogen-free extract, and net energy contents by using the methods of the AOAC (2006).

2.8. Statistical analysis

The data obtained from the water and soil analyses were subjected to statistical analysis using SPSS-10. The values calculated in this way were used to determine the significance of the soil physicochemical attributes at each site. Tukey's HSD values were calculated to determine the significance of various morphological, physiological, and biochemical attributes and the proximate compositions of different grass species collected from four different habitat types (natural springs and lakes). Canoco for Windows version 4.5 was used, and conical corresponding analysis (CCA) was performed on the data obtained from the study of morphophysiological and biochemical variables, elemental content and proximate composition.

3. Results and discussion

3.1. Water and soil analysis

Water and soil analysis revealed that the study sites were saline to hypersaline, with the Khewra Mine being the most saline and Khabeki Lake the least saline. The alkaline nature of the study sites is demonstrated by the pH range of the examined springs and lakes, which was between 7.1 and 8.31. The EC of the water at the four study sites was recorded in the following decreasing sequence: Khewra Mine>Pir Da Khara>Uchalli Lake>Khabeki Lake. The EC of the water samples from the four sites exceeded the limit set by the FAO for irrigation water (**Table 2**). In addition to higher levels of EC, the sodium content, chloride content, total dissolved solids, total soluble solids and sodium absorption ratio were also found to be higher than the limits set by the FAO for irrigation water (Abadi *et al.,* 2025). Uchalli Lake has the highest TDS value among the four study sites. Similar results were reported by Bhatti *et al.* (2018).

In addition to the salinity and alkalinity of the study sites, metal toxicity was also noted in the water samples from these sites. Among the heavy metals, copper (Cu) and manganese (Mn) had the highest levels across the four study sites. In addition to copper and manganese (Mn), iron (Fe), lead (Pb) and chromium (Cr) were also present at high concentrations at the study sites. Chromium was the single metal that exceeded the set limits of the FAO at all the study sites. Cadmium (Cd), cobalt (Co), copper and nickel (Ni) were the four metals with concentrations higher than the FAO limits at the three study sites except for Khabeki Lake (**Table 2**). Manganese metal exceeded the FAO limits at three study sites, with the exception of Uchalli Lake, whereas iron, lead and zinc were within the limits set by the FAO for irrigation water. The presence of these metals at the study sites (Uchalli and Khabeki Lakes) has also been reported (Bhatti *et al.* (2018).

Analysis of the soil from four study sites was carried out, as plants obtain their required nutrients from the soil. The pH of the soil varies from 7.2 in Pir Da Khara to a maximum of 8.1 in Uchalli Lake. A high pH indicates the alkalinity of the soil at the study sites. Soil pH significantly affects the availability of nutrients for plant absorption (Brown *et al.,* 2009; Rousk *et al.*, 2009; Xiao *et al.*, 2016). The EC of the soil was highest at the same site where the highest EC of the water was reported, which clearly indicates that the water at these sites is the cause of the salinity of the soil. The organic matter, phosphorus, potassium and saturation percentages of the soil did not vary significantly across the sites.

Among the heavy metals recorded in the soil, the amount of iron was greater at all the study sites, followed by manganese, lead and chromium. Copper and zinc have their highest values at the Khewra Mine, and their source is the drainage of water from the mine, which is deposited in the soil. Boron had comparable values at the four study sites. The characteristics of the soil are crucial to the development of the species (Dotaniya *et al.*, 2016). The electrical conductivity (EC) and organic matter content in cultivated soils tend to increase over time (Zhong *et al.,* 2025).

Table 2. Physio-chemical properties of the water collected from the four study sites.

Sr. No.	Name	pH	EC µS/cm	Ca+Mg mEq/L	Sodium mEq/L	Bicarbonate mEq/L	Chloride mEq/L	SAR	TSS µS/cm	TDS µS/cm
1	Khabeki Lake	8.15	780	20.0	94.2	3.21	126.8	12.5	18.56	196.82
2	Uchali Lake	8.31	1100	22.2	95.4	3.2	139.9	12.7	18.91	198.98
3	Pir Da Khara	7.1	14400	112.4	121.8	3.2	198.5	36.5	18.46	192.82
4	Khewra Mine	7.3	20780	113.6	124.3	4.0	196.8	39.3	18.81	194.98
Sr. No.	**Name**	**Cd mg L⁻¹**	**Cu mg L⁻¹**	**Co mg L⁻¹**	**Cr mg L⁻¹**	**Fe mg L⁻¹**	**Mn mg L⁻¹**	**Ni mg L⁻¹**	**Pb mg L⁻¹**	**Zn µg/g**
1	Khabeki Lake	0.10	0.13	0.05	1.2	0.30	0.21	0.20	2.4	0.20
2	Uchali Lake	0.70	1.72	0..06	1.9	0.40	0.13	0.31	1.4	0.50
3	Pir Da Khara	0.41	3.8	0.44	1.36	2.2	3.8	0.56	1.96	0.66
4	Khewra Mine	0.80	3.7	0.16	1.38	2.14	3.7	0.48	1.30	0.65

Table 3. Physio-chemical properties of the soil collected from the four study sites.

Sr. No.	Name	pH	EC µS/cm	O.M %	P mg/Kg	K mg/Kg	Saturation %	Texture	Cu µg/g	Cd µg/g
1	Khabeki Lake	8.0	9.4	0.77	3.4	386	36	Loam	0.54	0.08
2	Uchali Lake	8.1	12.03	0.91	2.9	352	36	Loam	0.76	0.1
3	Pir Da Khara	7.2	35.8	0.56	4.8	318	26	Loam	1.4	0.07
4	Khewra Mine	7.6	45.9	0.42	3.7	258	28	Loam	7.4	0.1
Sr. No.	**Name**	**Co µg/g**	**Cr µg/g**	**B µg/g**	**Fe µg/g**	**Mn µg/g**	**Ni µg/g**	**Pb µg/g**	**Zn µg/g**	
1	Khabeki Lake	0.06	1.38	0.44	4.9	1.2	0.20	1.3	0.62	
2	Uchali Lake	0.44	1.36	0.43	6.0	1.4	0.31	1.96	0.72	
3	Pir Da Khara	0.16	1.9	0.48	7.0	2.4	0.48	1.4	1.0	
4	Khewra Mine	0.15	1.2	0.68	6.5	2.8	0.56	2.4	6.2	

The findings of the present investigation are consistent with this observation, as areas surrounding lakes and springs receive saline water from these water channels. The EC of the water samples from the four sites exceeded the limit set by the FAO for irrigation water. This relatively high level of EC at the Khewra Mine and Pir Da Khara site was due to the addition of water drainage from the salt mine, while surface runoff from the areas surrounding the two lakes may be a possible reason for the relatively high EC (**Table 3**). In addition to higher levels of EC, the sodium content, chloride content, total dissolved solids, total soluble solids and sodium absorption ratio were also found to be higher than the limits set by the FAO for irrigation water (Abadi *et al.,* 2025). Soil acidity or alkalinity is affected by a number of variables, including the rate of decomposition of organic matter, the availability of nitrogen, the rate at which minerals weather and the environmental and management conditions.

Table 4. Descriptive statistics for the different water and soil parameters from four study site at Salt Ranage.

Parameters	Water					Parameters	Soil				
	Min	Max	Mean	SD	CV%		Min	Max	Mean	SD	CV%
pH	7.0	8.38	7.71	0.56	7.3	pH	7.0	8.20	7.725	0.40	5.36
EC uS/cm	778.0	22782	9265	9009.48	97.2	EC uS/cm	9.30	46.4	25.725	16.15	68.3
Ca+Mg mEq/L	19.0	113.80	67.05	48.0	71.6	O.M %	0.41	0.92	.665	0.196	29.3
Na mEq/L	94.0	124.40	108.92	14.79	13.6	P mg/Kg	0.47	3.80	2.61	1.32	45.8
HCO_3- mEq/L	3.0	4.10	3.40	0.378	11.1	K mg/Kg	256	387	329	49.42	14.9
Cl mEq/L	125.90	198.60	165.51	33.93	20.5	SAT %	24.0	38.0	31.566	5.15	16.1
SAR	12.40	39.40	25.25	13.24	52.4	Cu µg/g	0.63	7.50	2.51	2.97	330.8
TSS µS/cm	18.38	19.0	18.68	0.209	1.1	Cd µg/g	0.01	0.20	0.0829	0.063	84.6
TDS µS/cm	192.80	199.38	195.90	2.39	1.2	Co µg/g	0.05	12.0	1.18	3.41	2306.3
Cd mg L^{-1}	0.10	0.90	0.504	0.286	56.9	Cr µg/g	1.10	1.92	1.41	0.228	16.9
Cu mg L^{-1}	0.12	3.82	2.33	1.589	68.0	B µg/g	0.14	0.70	0.5067	0.107	26.3
Co mg L^{-1}	0.04	0.45	0.179	0.164	91.9	Fe µg/g	0.70	6.50	4.527	2.38	47.0
Cr mg L^{-1}	1.10	1.95	1.46	0.293	20.0	Mn µg/g	1.15	3.10	1.933	0.744	40.5
Fe mg L^{-1}	0.20	2.30	1.26	0.955	75.8	Ni µg/g	0.12	0.56	0.390	0.148	37.4
Mn mg L^{-1}	0.11	3.85	1.95	1.872	95.5	Pb µg/g	1.20	2.60	1.784	0.466	26.2
Ni mg L^{-1}	0.10	0.98	0.388	0.153	39.5	Zn µg/g	0.61	6.40	2.138	2.454	322.8
Pb mg L^{-1}	1.20	2.50	1.76	0.471	26.3						
Zn mg L^{-1}	0.10	0.68	0.50	0.204	40.6						

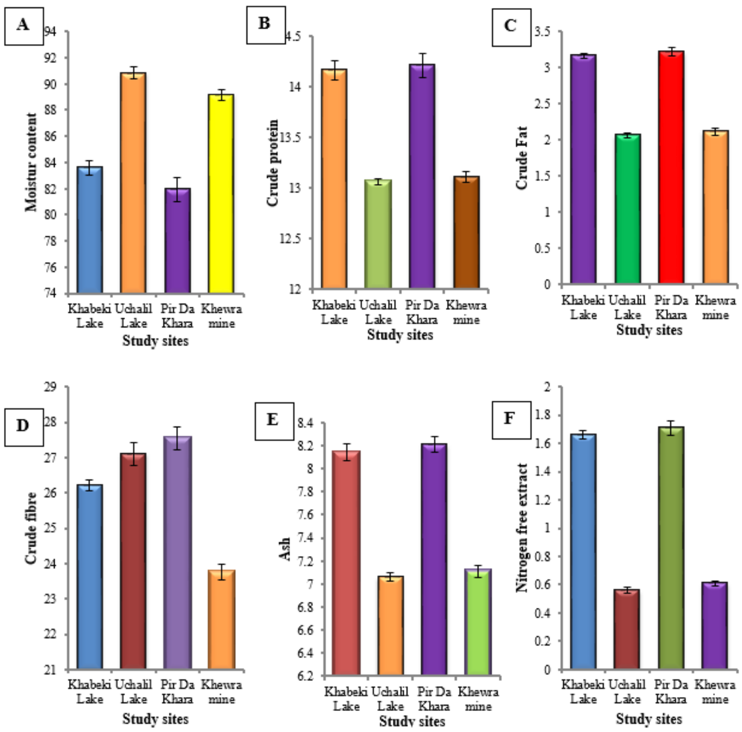

Figure 1. (1a-1f) Proximate analyses of *Cynodon dactylon* from different study sites of the Salt Range.

From descriptive statistics, it is evident that heavy metals dominate the water samples with average concentrations of Cu of 2.33 mg L^{-1}, Mn of 1.95 mg L^{-1}, Pb of 1.76 mg L^{-1}, Cr of 1.46 mg L^{-1}, Fe of 1.25 mg L^{-1}, Cd of 0.50 mg L^{-1}, Ni of

0.388 mg L^{-1}, Co of 0.179 mg L^{-1}, and in soil with Fe of 4.527 mg/kg, Cu of 2.51 mg/kg, Zn of 2.138 mg/kg, Mn of 1.933 mg/kg, Pb of 1.784 mg/kg, Cr of 1.41 mg/kg, Co of 1.18 mg/kg, B of 0.506 mg/kg, Ni of 0.390 mg/kg, and Cd of 0.083 mg/kg (**Table 4**). The higher concentration in the sampled water may be attributed to the release of effluent directly from water springs and lakes in contact with the mineralization of deposits.

3.2. Proximate analysis

The data regarding the proximate composition of *Cynodon dactylon* are presented in **Table 7**. The moisture content provides a precise measurement of significant feed material that excludes moisture, bases, and volatile acids (Gaikwad *et al.,* 2017). In the present study, the moisture content of Bermuda grass ranged from 81.58% to 90.53% and was highest at Uchalli Lake (**Figure 1A**). The present findings are in line with those of Gursoy *et al*. (2021) and Harun *et al*. (2022), who studied some grasses (*Cenchrus ciliaris*, *Cynodon dactylon*, *Panicum antidotale, Sorghum helepense*, and *Chrysopogon zizanioides*) from northern areas of Pakistan.

Table 5. Mean values of elemental contents in roots of *Cynodon dactylon* from four study sites at Salt Range.

Site	Nitrogen	Phosphorus	Potassium	Sodium	Calcium	Magnesium	Zinc
Khabeki Lake	0.8923±0.002	0.257±0.001	0.676±0.003	20.5±0.10	59.52±0.008	15.093±0.003	16.15±0.05
Uchhali Lake	0.81±0.01	0.273±0.001	0.635±0.003	29±0.10	23.05±0.008	15.163±0.021	8.3±0.01
Pir Da Khara	0.8823±0.002	0.167±0.001	0.826±0.003	24.5±0.10	69.52±0.004	15.053±0.003	15.15±0.05
Khewra Mine	0.8±0.01	0.183±0.001	0.785±0.003	25±0.10	33.05±0.003	15.123±0.021	7.3±0.01
Mean	0.8462±0.04	0.22±0.05	0.7305±0.081	24.75±3.14	46.28±18.91	15.108±0.04	11.725±4.13
Site	Iron	Manganese	Copper	Cobalt	Cadmium	Lead	Nickel
Khabeki Lake	43.237±0.03	9.5±0.01	8.58±0.01	2.0337±0.003	0.5623±0.002	15.963±0.02	1.82±0.01
Uchhali Lake	44.1±0.10	93.137±0.03	15.83±0.03	3.4±0.01	0.9137±0.003	23.137±0.02	1.55±0.01
Pir Da Khara	38.237±0.03	14.5±0.01	6.58±0.01	1.0337±0.003	0.4623±0.002	10.963±0.02	2.82±0.01
Khewra Mine	39.1±0.10	98.137±0.03	13.83±0.03	2.4±0.01	0.8137±0.003	18.137±0.02	2.55±0.01
Mean	41.168±2.65	53.818±43.76	11.205±3.93	2.2168±0.88	0.688±0.19	17.05±4.57	2.185±0.54

Table 6. Mean values of elemental contents in shoots of *Cynodon dactylon* from four study sites at Salt Range.

Site ↓	Nitrogen	Phosphorus	Potassium	Sodium	Calcium	Magnesium	Zinc
Khabeki Lake	0.213±0.001	20.05±0.05	1.153±0.003	19±0.10	0.594±0.0026	48.52±0.008	223.88±0.01
Uchhali Lake	0.3177±0.0005	20.493±0.05	0.879±0.003	22.4±0.01	0.663±0.021	12.05±0.008	198.74±0.02
Pir Da Khara	0.113±0.001	18.05±0.05	1.103±0.003	21±0.10	0.554±0.003	58.82±0.008	173.88±0.01
Khewra Mine	0.2177±0.0005	18.493±0.05	0.829±0.003	24.4±0.01	0.623±0.021	22.05±0.008	148.74±0.02
Mean	0.2153±0.075	19.272±1.07	0.991±0.14	21.7±2.06	0.608±0.039	35.36±19.0	186.31±29.22

Table 7. Mean values of proximate composition of *Cynodon dactylon* from four study sites at Salt Range.

Site ↓	Moisture Content %	Crude Protein%	Crude Fat%	Crude Fibre%	Ash%	Net Energy K Cal/100g	Nitrogen Free Extract
Khabeki Lake	83.58±0.01	14.16±0.01	3.16±0.01	26.2±0.10	8.16±0.01	108.58±0.01	1.66±0.01
Uchhali Lake	90.83±0.03	13.06±0.01	2.06±0.01	27.1±0.10	7.06±0.01	115.83±0.03	0.56±0.01
Pir Da Khara	81.58±0.01	14.21±0.01	3.21±0.01	27.533±0.05	8.21±0.01	106.58±0.01	1.71±0.01
Khewra Mine	88.83±0.03	13.11±0.01	2.11±0.01	23.567±0.05	7.11±0.01	113.83±0.03	0.61±0.01
Mean	86.205±3.93	13.635±0.57	2.635±0.57	26.1±1.61	7.635±0.57	111.2±3.93	1.135±0.58

The crude protein content ranged from 13.06% to 14.21% across the four study sites. Higher levels of salt stress are associated with lower crude protein concentrations, perhaps because of the inability to properly use nitrogen molecules, reduced protein synthesis, and increased activities of protein hydrolysing enzymes. The crude protein was highest at Pir Da Khara (**Figure 1B**). These findings are consistent with those reported by other studies, including Kumari *et al*. (2018) for *Sorghum* and Kumar *et al*. (2018) for *Dichanthium annulatum*. However, in the present study, a higher percentage of crude protein resulted in better adaptations of the selected grass species, i.e., *Cynodon dactylon,* to cope with the saline conditions at the study sites.

The amount of crude fat of *Cynodon dactylon* ranged from 2.06% to 3.21% across the four study sites (**Table 5**). In the present study, the lower values of crude fat in *C. dactylon* were due to differences in the soil fertility level or sampling strategies. The crude fat content was highest at Pir Da Khara (**Figure 1C**). The crude fibre content was found to range from 23.56% to 27.53% in *C. dactylon* across the four study sites. The crude fibre content was highest at Pir Da Khara (**Figure 1D**). According to Tedeschi *et al.* (2021), fibre is frequently seen as detrimental to nutritional quality. This phenomenon is closely related to the digestibility of the feed; as the fibre content of the feed decreases, digestion becomes simpler, and the energy value of the feed increases (Cooney *et al.,* 2023). The results of the present study are similar to those of previous studies, which reported relatively high fibre values (Cooney *et al.,* 2023; Rafay *et al.,* 2013).

The ash content in the Bermuda grass ranged from 7.06% to 8.21% across the four study sites. The ash content is a rough indicator of the total mineral content in various fodders or feeds (Castillo *et al.,* 2025). The ash content was highest at Pir Da Khara (**Figure 1E**). The presence of saline conditions in the study areas may be a key factor for this significant variation in ash content in the studied grass. The present findings are consistent with those of Ahmed *et al*. (2017) and Abbas *et al.* (2023), who reported that plants exposed to high salinity levels accumulate much more Na and Cl, increasing the ash content, and variation in soil and other habitat features affects the ash content of plants.

In the present study, the concentration of nitrogen-free extract was high in *Cynodon dactylon.* This shows the response of this grass under stress conditions, which helps to survive under harsh conditions in the environment of the study areas. The nitrogen-free extract was present at the highest level at Pir Da Khara (**Figure 1F**). The findings of the present study are in line with those of Rafay *et al.* (2013), who reported that Cholistan grasses have a greater NFE (nitrogen-free extract) percentage than most other forages. The net energy was in the range of 106.58 kcal/100 g to 115.83 kcal/100 g in Bermuda grass across the four study sites. The nitrogen-free extract was recorded in the range of 0.56-171 across the four study sites. The net energy was highest at Pir Da Khara

3.3. Root and shoot elemental analysis

The data regarding the elemental contents of *Cynodon dactylon* in the roots and shoots are presented in **Tables 5 and 6**, respectively. The mean potassium (K) values ranged from 0.635 µg/g to 0.826 µg/g in the roots and 0.829 µg/g to 1.51 µg/g in the shoots at the four study sites. The value of K was highest at Pir Da Khara (**Figure 2A**). Potassium helps in the regulation of water in plants (Brown *et al.,* 2022). It is the most abundant cation in the cytoplasm and increases the osmotic potential of plant cells (Rocha *et al.,* 2021).

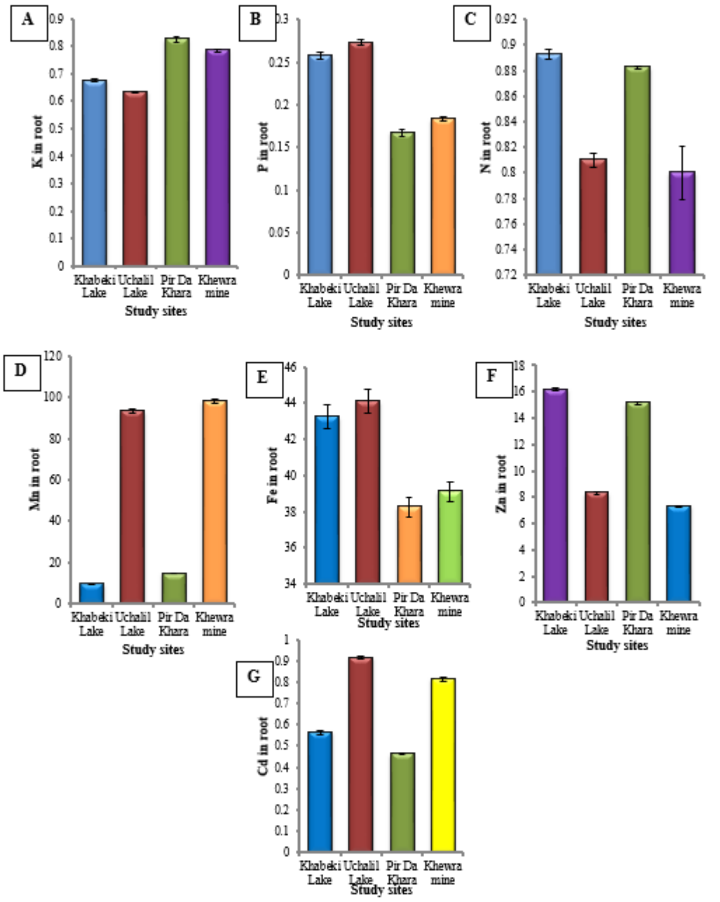

Figure 2. (2A-2G): Elemental (K, P, N, Mn, Fe, Zn and Cd) analysis of root of *Cynodon dactylon* from different study sites at the Salt Range.

The potassium content of grasses at the four study sites was recorded, which revealed that these grasses have the ability to cope with saline conditions. Under conditions of water stress, banana and olive trees both have low levels of K^+, as reported by Mahouachi (2007). Mineral nutrients such as potassium can be moved from the soil to the root and shoot systems via water. Phosphorus (P) was found in selected grasses at relatively high soil pH values, which is a response to adjusting to the prevailing conditions of the environment. The value of K was highest at Uchalli Lake (**Figure 2B**) (**Table 6**). Nucleic acids can be used by plants to absorb soluble organic phosphates. Inorganic phosphorus is easily incorporated into organic compounds in roots and shoots (Sanchez, 2007).

The mean nitrogen (N) value ranged from 0.8 to 0.829 µg/g in the roots and 0.113 to 0.3177 µg/g in the shoots across the four study sites. Nitrogen is taken up by plants in the form of ammonium and nitrates, which are absorbed best at acidic and neutral pH values, respectively (Enesi *et al.,* 2023). The low nitrogen content may be caused by the alkaline pH of the soil at the study sites. The mean P values ranged from 0.167 µg/g to 0.273 µg/g in the roots and 18.05 µg/g to 20.05 µg/g in the shoots. Phosphorus is less available in soils because it is bonded to calcium and magnesium at relatively high pH values (De-Bashan *et al.,* 2022; Barik *et al.,* 2018). The value of nitrogen (N) was highest in Khabeki Lake (**Figure 2C**).

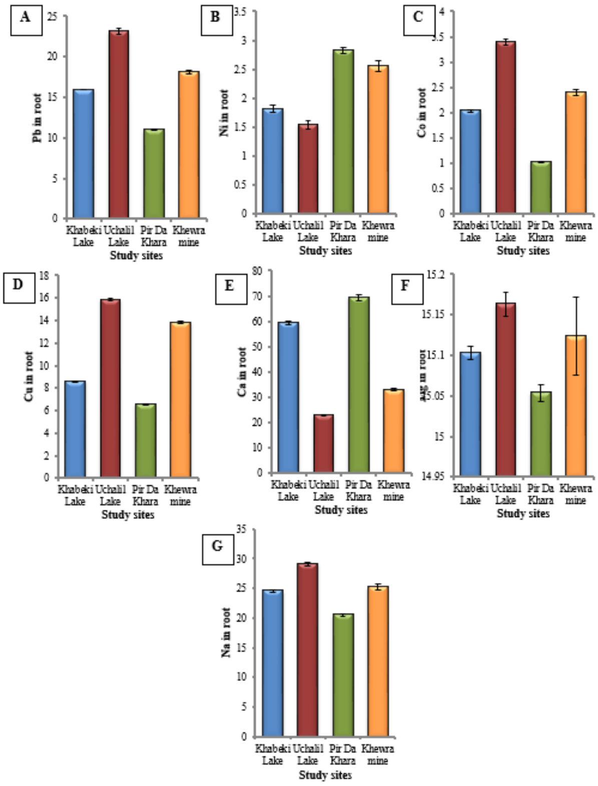

Figure 3. (3A-3G): Metal (Pb, Ni, Co, Cu, Ca, Mg, and Na) analyses of root *Cynodon dactylon* from different study sites at the Salt Range.

Table 8. Analysis of variance for proximate composition from four study sites at Salt Range.

SOV	df	Moistur content	Crude protein	Crude Fat	Crude fibre	Ash	Net Energy	Nitrogen free extract
Treatment	3	55.3403**	1.2125**	1.2125**	8.49889**	1.19521**	56.5625**	1.2125**
Error	8	1.1168	0.0211	0.00615	0.20667	0.01048	1.9534	0.00305
Total	11							
Khabeki Lake		83.58b	14.16a	3.16a	26.20b	8.14a	108.58b	1.66a
Uchalil Lake		90.83a	13.06b	2.06b	27.10a	7.06b	115.83a	0.56b
Pir Da Khara		81.91b	14.21a	3.21a	27.53a	8.21a	106.58b	1.71a
Khewra mine		89.16a	13.11b	2.11b	23.77c	7.11b	113.83a	0.61b
Mean		86.37	13.64	2.64	26.15	7.63	111.21	1.14
CV		1.22	1.07	2.98	1.74	1.34	1.26	4.87

**Significant, ** Highly significant (P<0.01) Means followed the same letter are not significantly different at P=0.05*

The mean values of manganese (Mn) in the roots of Bermuda grass ranged from 9.5 µg/g to 98.137 µg/g, whereas those in the shoots ranged from 43.05 µg/g to 89.52 µg/g. The value of Mn was highest in Uchalli Lake (**Figure 2D**). Manganese is a trace metal that is abundant in various environmental matrices, such as soils, sediments and biological materials (Sheded *et al.,* 2006). Similar reports are available in the literature, in which the authors documented concentrations of zinc and manganese (Younas *et al.,* 2024; Haidu *et al.,* 2017).

The amount of Mn in the grasses was found to be relatively high, which may be caused by the pH of the soil in the study area. Mn is found in rocks, soil and water, and although it is rarer than Fe, it is frequently found in water containing Fe (Jennings *et al.,* 2016). The value of Fe was highest in Uchalli Lake (**Figure 2E**). Aquatic environments often have relatively high metal concentrations (Cheng *et al.,* 2014). The major cause of Fe in water and soil and its bioaccumulation in the food chain is the tendency of the metal to be transferred by sediments (Chen *et al.,* 2022). The possible cause of the high Fe concentration in Bermuda grass is that the addition of Fe to water and soil is both natural and anthropogenic.

The mean zinc (Zn) values ranged from 8.3 µg/g to 16.15 µg/g in the roots and from 144.74 µg/g to 233.88 µg/g in the shoots of *Cynodon dactylon.* The value of Zn was highest at Khabeki Lake (**Figure 2F**). Plants take up Zn^{2+} from the soil through their roots, and it is an essential component of the biochemical pathways that are responsible for the production of carbonic hydrase, auxin and cytochrome (Ejaz *et al.,* 2022; Liu *et al.,* 2020). The extent of erosion and chemical composition of limestone are the main factors affecting Zn concentrations in soil (Brown, 2016). In this study, the Zn concentration in grasses significantly varied, which may be due to relatively high levels of phosphorus and Mn in the soil analysed. The iron concentrations ranged from 38.23 gm/g to 44.1 and from 330.6 µg/g to 387.01 µg/g in the roots and shoots, respectively.

The value of Cd was highest in Uchalli Lake (**Figure 2G**). The Cd concentration ranged from 0.462 µg/g to 0.913 µg/g in roots and from 1.99 µg/g to 2.55 µg/g in shoots of *Cynodon dactylon*. Household items included detergents and shampoo. Both lakes included in the present study receive runoff water from surrounding areas, which contain Cd from various sources that might be responsible for the accumulation of Cd in water and soil and ultimately be taken-up by grasses, whereas the drainage water from the mines may contain it and add it to the water of spring Pb in the roots.

3.4. Study sites at Salt Range

Pb concentrations in the roots ranged from 10.96 µg/g to 23.13 µg/g, whereas in the shoots, 18.05 µg/g to 20.49 µg/g was detected. Plants have a limited ability to take in lead (Pb) from the soil, yet they might be able to do so to some degree at high quantities (Genchi *et al.,* 2020). In the present study, Pir Da Khara and Khewra Mine springs receive drainage from the salt mine, whereas two lakes receive surface runoff from the surrounding residential areas, which may build up Pb in these resources in the soil as well, and plants take up Pb from the soil (**Figure 3A**).

The mean values of Ni ranged from 1.55 µg/g to 2.82 µg/g in the roots and from 8.45 µg/g to 10.45 µg/g in the shoots of *Cynodon dactylon* across the four study sites. The value of Ni was highest at Pir Da Khara (**Figure 3B**). Nickel (Ni) is a naturally existing element in soil and water (Shahbaz *et al.,* 2019). Ni is found in dust particles from the weathering of rocks, forest fires and volcanic eruptions (Eisler, 1998) and contributes to the soil by dissolving in rainwater and becoming part of groundwater. The high pH of the soil recorded in the present study may reduce Ni accessibility to plants, and high organic matter can reduce Ni absorption from the soil because of the strong binding of Ni particles to organic matter.

The mean values for Co ranged from 1.033 µg/g to 3.4 µg/g in the roots and from 4.28 µg/g to 6.17 µg/g in the shoots across the four study sites. The value of Co in roots was highest at Uchalli Lake (**Figure 3C**). The positive outcomes of Co include increased drought resilience in seeds and a delay in leaf senescence caused by the restriction of ethylene production. Cobalt interferes with the homeostasis of iron in many organisms, including plants, and fights with iron for access to transporters (Van-Praag *et al.,* 2000; Bai *et al.,* 2025). The concentration of Cu in the roots ranged from 6.52 µg/g to 15.83 µg/g, whereas in the shoots, it ranged from 25.18 µg/g to 368.44 µg/g. The value of Cu in roots was highest at Uchalli Lake (**Figure 3D**). Copper is a common soft heavy metal found in nature and is an essential trace element for all forms of life. Copper is an integral part of the biochemistry of all organisms since it is found in numerous metalloenzymes and respiratory pigments (Jodral-Segado *et al.,* 2006; Bai *et al.,* 2022; Gatasheh *et al.,* 2025).

The value of calcium (Ca) was highest at Pir Da Khara (**Figure 3E**). Soil calcium uptake is pH- and soil concentration dependent. When there is more calcium in the soil, the plant absorbs more calcium (Farris *et al.,* 2024). Calcium uptake is reduced under stress in contrast to the P and K contents. The magnesium (Mg) content in the roots ranged from 15.053 µg/g to 15.163 µg/g, whereas that in the shoots ranged from 12.05 µg/g to 58.82 µg/g. The value of Mg was highest in Uchalli Lake (**Figure 3F**). Chlorophyll molecules require magnesium as a core element, and magnesium plays a role in both energy conservation and conversion (Weng *et al.,* 2022). In the present study, the availability of magnesium in *C. dactylon* across the four study sites was due to its ability to survive, as the presence of magnesium affects chlorophyll production, which is the primary requirement for photosynthesis.

The sodium (Na) concentration varied from 24.5 µg/g to 29.0 µg/g and from 19.0 µg/g to 24.4 µg/g in the roots and shoots, respectively. The value of Na was highest at Uchalli Lake (**Figure 3G**). The presence of sodium in the roots and shoots of grasses is possibly caused by the NaCl dissolved in the water of the study sites, from which it is absorbed through the soil. The mean values of calcium in the roots ranged from 23.05 µg/g to 69.52 µg/g and 0.554 µg/g to 0.663 µg/g in the shoots of *C. dactylon* across the four study sites.

3.5. Proximate composition

The crude fatty acid content, ash content and crude protein content of *Cynodon dactylon* were positively associated with Khabeki Lake, whereas the crude fibre content at the Pir Da Khara site and the net energy at the Khewra Mine were positively associated. The moisture content and nitrogen-free extract were associated with Uchalli Lake and Khabeki Lake, respectively. The TSS, TDS and pH of the water, along with three heavy metals, i.e., Cd, Zn and Cr, were associated with Uchalli Lake, whereas HCO^{3-}, EC and Cu, single heavy metals, were associated with the Khewra Mine. All these water properties are negatively associated with the Pir Da Khara site (**Table 8**).

The higher crude fibre and ash levels detected in *C. dactylon* are most likely due to structural strengthening and increased mineral loading as adaptive responses to salt and metal stress. While increasing fibre content may lower digestibility and energy availability for ruminants, a higher ash level implies significant mineral buildup, which can be advantageous or detrimental depending on metal composition. This trade-off emphasizes the importance of evaluating forage quality based not only on protein content but also on digestibility and metal safety.

3.6. Elemental content

Many elements found in *Cynodon dactylon* were strongly associated with the Uchalli Lake site. These elements include phosphorus, magnesium, copper, cadmium, iron, cobalt, and sulfur. Lead and boron are found in roots, whereas calcium, phosphorus, lead, cobalt, cadmium and nickel are present. Mg, Mn and S in shoots and Ca in roots were associated with Pir Da Khara (**Table 9**). B and N were found in shoots, whereas K, Ni and N in roots were associated with the Khewra Mine. Copper in shoots, Zn in roots and Cu in shoots were associated with the Khabeki Lake site. The pH, TSS and TDS of the water were positively associated with Uchalli Lake, whereas EC, HCO^{3-} and a few elements were found in the water associated with the Khewra Mine (**Table 10**).

Table 9. Analysis of variance for Element content in roots from four study sites at Salt Range.

SOV	K in root	P in root	N in root	Mn in root	Fe in root	Zn in root	Cd in root	Pb in root	Ni in root	Co in root	Cu in root	Ca in root	Mg in root	Na in root
Treatment (df=3)	0.024**	0.0084**	0.0069**	7020.09**	25.745**	62.622**	0.1334**	76.457**	1.0729**	2.8669**	56.5625**	1430.1**	0.0062^{NS}	37.207**
Error (df=8)	0.00014	0.00004	0.00036	1.62	1.105	0.0188	0.00018	0.1705	0.015	0.0052	0.0174	1.72	0.00201	0.3233
Total (df=11)														
Khabeki Lake	0.68c	0.26b	0.89a	9.50d	43.24a	16.15a	0.56c	15.96c	1.82c	2.03c	8.58c	59.52b	15.10a	24.53b
Uchalil Lake	0.64d	0.27a	0.81b	93.14b	44.10a	8.30c	0.91a	23.14a	1.55d	3.40a	15.83a	23.05d	15.16a	29.10a
Pir Da Khara	0.83a	0.17d	0.88a	14.50c	38.24b	15.15b	0.46d	10.96d	2.82a	1.03d	6.58d	69.52a	15.05a	20.50c
Khewra mine	0.79b	0.18c	0.80b	98.14a	39.10b	7.30d	0.81b	18.14b	2.55b	2.40b	13.83b	33.05c	15.12a	25.20b
Mean	0.73	0.22	0.85	53.82	41.17	11.73	0.69	17.05	2.19	2.22	11.21	46.29	15.11	24.83
CV	1.62	2.69	2.25	2.37	2.55	1.17	1.97	2.42	5.61	3.25	1.18	1.72	0.30	2.29

**Significant, ** Highly significant (P<0.01), Means followed the same letter are not significantly different at P=0.05*

Table 10. Analysis of variance for element content in shoots from four study sites at Salt Range.

SOV	K in shoot	P in shoot	N in shoot	Mn in shoot	Fe in shoot	Zn in shoot	Cd in shoot	Pb in shoot	Ni in shoot	Co in shoot	Cu in shoot	Ca in shoot	Mg in shoot	Na in shoot
Treatment (df=3)	0.0776**	0.021**	1.0203**	1429**	2541.2**	3131.8**	1.206**	4.1965**	4.2463**	1.792**	93567**	0.0064**	1430.06**	1.2833**
Error (df=8)	0.00015	0.00013	0.0027	2.01	9.23	4.45	0.00346	0.10342	0.00598	0.0078	47.7	0.00053	0.51	0.06592
Total (df=11)														
Khabeki Lake	1.15a	0.21b	1.95c	79.48b	330.60d	223.88a	2.10b	20.05a	10.95a	5.28b	328.44b	0.59bc	48.52b	19.20b
Uchalil Lake	0.88c	0.32a	1.81d	43.05d	337.02c	198.74b	2.55a	20.49a	10.45b	6.17a	65.18c	0.66a	12.05d	19.27b
Pir Da Khara	1.10b	0.11c	2.95a	89.52a	380.60b	173.88c	1.10d	18.05b	8.95c	4.28c	368.44a	0.55c	58.52a	20.10a
Khewra mine	0.83d	0.22b	2.81b	53.05c	387.02a	148.74d	1.55c	18.49b	8.45d	5.17b	25.18d	0.62ab	22.05c	20.55a
Mean	0.99	0.22	2.38	66.28	358.81	186.31	1.82	19.27	9.70	5.23	196.81	0.61	35.29	19.78
CV	1.24	5.27	2.18	2.14	0.85	1.13	3.23	1.67	0.80	1.69	3.51	3.80	2.02	1.30

*Significant ** , Highly significant (P<0.01), Means followed the same letter are not significantly different at P=0.05*

3.7. CCA plots for the elemental content and proximate composition of Cynodon dactylon

CCA triplots of morphophysiological and biochemical parameters and proximate compositions of selected grass species across the selected sites are presented separately, which reveal significant variation in the response of plants to the water of natural lakes and springs. The environmental variables (EVs) are shown in red, while the heavy metals are taken as supplementary variables (SVs) to provide a better understanding of the results and are shown in blue. Moreover, abbreviations comprising two alphabets have been used instead of the complete names of the plants to make the graphs more discernible (**Figure 4A** and **4B**).

Canonical correspondence analysis (CCA) indicated significant correlations between environmental variables and plant elemental composition. The first CCA axis showed a salinity-metal gradient that distinguished lake sites from salt mine sites, whereas the second axis reflected variance in nutritional and proximate content. These patterns show that *C. dactylon's* nutrient uptake and adaptive responses are substantially influenced by site-specific water and soil chemistry.

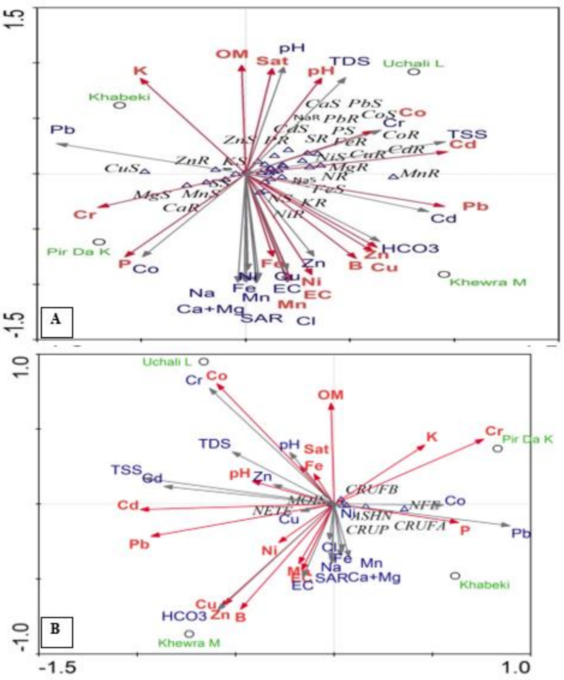

Figure 4. (4A and 4B): CCA Plots for elemental content and (A) CCA Plots for proximate composition of *Cynodon dactylon* (B).

3.8. Adaptive mechanisms of Cynodon

The observed tissue-specific distribution of elements in *C. dactylon* is the result of a number of physiological and biochemical changes that work together to improve tolerance to salt and metal stress. The preferential buildup of manganese (Mn) and calcium (Ca) in root tissues indicates an exclusion-based strategy in which potentially harmful ions are held within the root system to limit their transfer to aerial, photosynthetically active tissues (Roman, 2021). Such root sequestration is frequently linked with vacuolar compartmentalization, in which excess metals are immobilized in root vacuoles, lowering cytosolic toxicity and limiting oxidative damage.

In contrast, the substantially greater concentrations of iron (Fe) and copper (Cu) in shoot tissues suggest regulated xylem loading and selective translocation of critical micronutrients necessary for photosynthesis, electron transport, and enzymatic activity. This unequal root-shoot partitioning demonstrates the plant's capacity to balance nutritional and detoxifying needs (Kaur *et al.,* 2023).

Maintaining a healthy K^+/Na^+ ratio is crucial for salt tolerance, as potassium is required for enzyme activation, stomatal control, and osmotic balance. *C. dactylon's* capacity to limit Na^+ buildup while maintaining K^+ uptake indicates the presence of specialized membrane transporters and ion channels that maintain cellular homeostasis in high-stress situations. This process most likely contributes significantly to the species' resistance in hypersaline settings (Noor *et al.,* 2023). The CCA triplots further confirmed that ion accumulation and nutritional traits of *C. dactylon* are tightly coupled with environmental stress gradients, reinforcing the role of soil–water chemistry as a primary driver of adaptive strategies in hypersaline ecosystems.

3.9. Implications for fodder use

Although *C. dactylon* had adequate crude protein levels, the presence of potentially hazardous metals such as lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) raises questions about its acceptability as animal feed. Despite adjusting elemental units to biologically plausible concentrations (µg g^{-1}), certain metal levels still exceeded FAO/WHO standards for animal feed.

Chronic exposure to such elements through fodder eating may endanger grazing livestock, including bioaccumulation and possible transmission into the food chain (Swain *et al.,* 2024). As a result, while *C. dactylon* has excellent ecological adaptability and nutritional potential, its use as fodder in hypersaline environments should be approached with caution and supported by formal risk assessment tools such as target hazard quotient (THQ) calculations and controlled feeding trials.

4. Conclusion

The elemental profile of Bermuda grass revealed that it contains nutrients and heavy metals, which indicates that it is highly adaptable to prevailing conditions. The nutritional results confirmed that these grasses in the Salt Range of Punjab, Pakistan, also contain relatively high crude protein, crude fat, crude fibre and carbohydrate levels, which indicates that they can be used as fodder for animals. *Cynodon dactylon* demonstrates strong ecological adaptability to hypersaline environments through effective ion regulation and tissue-specific nutrient partitioning. However, its potential use as fodder should be considered cautiously and validated through comprehensive metal risk assessments and toxicological studies.

5. Suggestions and Recommendations

The present investigation is limited to elemental and proximate composition analyses and does not quantify metal bioavailability, animal exposure pathways, or toxicological thresholds. Consequently, while the species demonstrates promising ecological resilience and nutritional attributes, its suitability as fodder under hypersaline conditions should be considered conditional. Future studies should incorporate comprehensive risk assessments, including metal bioaccumulation factors, target hazard quotient (THQ) calculations, and controlled feeding trials, to determine the safety and practicality of its use in livestock systems.

Ethics approval and consent to participate

The plants were collected from different Lakes at Khushab, Pakistan (public land) and governing body Higher Education Commission, Pakistan and University of Education, Khushab Campus, Pakistan gave its approval for the collection of samples as per Ethics and Guideline Committee of the University of Education, Khushab Campus (Approval No. 019-S13/2018 UE).

Consent for publication

Not applicable

Availability of data and materials

Data are provided within the manuscript or supplementary information files.

Competing Interest

All authors declare that there are no competing interests.

Clinical trial number

Not applicable

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Authors' contributions

Muhammad Farooq wrote the original manuscript, collected and analyzed the samples. Abdul Ghani supervised the study; Muhammad Nadeem and Muhammad Arif worked on methodology; Ahmed Muneeb, Bushra Huma and Toqeer Abbas reviewed the article; Iqra Parvez, Roha Ramash and Muhammad Asim Sultan reviewed and edited the article. All authors contributed to the manuscript.

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