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RESEARCH PAPER

Plants as bioindicators of heavy metal pollution

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ABSTRACT:

This research was designed to assess metal deposition (Zn, Cr, As, Cd, Hg and Pb) and changes in photosynthetic pigments, total nitrogen and protein in six plant species (*Brachiaria eruciformis, polypogon monspeliensis, Phragmites australis, Cynodon ductylon, Prosopis farcta* and *Typha domingensis*) near Turaq region which was designated as polluted site and comparing it to control site. The results indicated a significantly increased concentration of studied metals in the contaminated area. *Phragmites australis* shows maximum accumulation of Cr, As, and Hg. The accumulation of Zn, Pb and Cd were highest in *Prosopis farcta, Cynodon ductylon* and *polypogon monspeliensis,* respectively. Pigments and proteins were found in lower amounts in plants grown on polluted soil. Metals were negatively correlated with pigments, total nitrogen and proteins in all of the studied plants. The data demonstrated the different plants were variant in metal absorption capacities in both regions.

KEY WORDS: Wild plants; Heavy metals; Photosynthetic pigments; Total nitrogen; Protein.
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1. INTRODUCTION:

Heavy metal pollution is turning into global issue of greatest concern. Harmful elements can exist in the ecosystem for a prolonged duration and might ultimately build up to levels that put public health in danger and cause environmental deterioration (Xu et al., 2019).

There are two sorts of HMs: essential and non-essential. Chromium, zinc, copper, manganese, nickel, iron, and are essential micronutrients, but excessive amounts can be harmful. Heavy metals such as lead, cadmium and mercury are extremely hazardous to life (Sandeep et al., 2019). According to Environmental Protection Agency (EPA), the majority of environmental pollution involves heavy metals like As, Cr, Cu, Hg, Pb, Ni and Cd (Hu et al., 2020).

Heavy metals can be accumulated in the environment easily, once the ratio of them surpasses the allowable limit, bio-magnification occurs throughout the food chain, affecting every organism on the planet. Therefore, their elimination from the environment becomes a priority (Liu et al., 2020).

When wastewater is used to irrigate the soil, it causes heavy metals to build up in the agricultural soil, crops and vegetables can assimilate extra amount of metals, affecting the quality and safety of the food we eat. Many vegetables gather large amounts of these metals from contaminated soil, and causes severe health complications after consuming them (Khan et al., 2018). Another source of heavy metal pollution are emissions from vehicles which influences on the environment and roadside vegetations negatively. Roadside plants are strong enough to withstand huge levels of heavy metals and may possibly help in mitigating pollution problems (Altaf et al., 2021).

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Plants are important for regulating the homeostasis of the basic minerals required by plants and they evolved a variety of methods to tolerate high metal stress. Moreover, not all plants are uniformly active against the toxic effects of excessive levels of heavy metals. They have changed their morpho physiology, anatomy and molecular connections to deal with changes in their environment (Jogawat et al., 2021). Whenever plants are exposed to large amounts of heavy metals, cellular and root secretions start to prevent them from penetrating the cell, thus providing the first protection barrier for the plant (Ghori et al., 2019).

Heavy metals absorption by plants depends on its concentration, soil pH and plant type and their physiological efficiency, certain species can metabolize different types of heavy metals. This means that plants have different abilities to take in and store heavy metals (Abdelhafez and Li, 2015).

Exposure to heavy metals causes protein denaturation, enzyme inactivation, decreasing chlorophylls and photosynthesis as well as changes in the organelles structure and disturbance in metabolic reaction (Hossain et al., 2012) (Kalaivanan and Ganeshamurthy, 2016).

Furthermore, it induces the formation of reactive oxygen species including hydrogen peroxide ($H_2 O_2$), superoxide radical ($O \cdot - 2$) and hydroxyl radical (OH). This reactive species triggers lipid peroxidation, mostly of cell membranes, which makes them leaky, as well as destruction of macromolecules and breaking of DNA strands which is collectively termed oxidative stress (Ahmad et al., 2012) (Cui et al., 2022).

Photosynthetic pigments such as chlorophylls and carotenoids, are fundamental for plants because they convert light energy into chemical energy (Yang et al., 2020). The chlorophyll reaction represents one of the most impressive plant responses to stress. Additionally, it was thought that carotenoids acted as antioxidants by capturing free radicals, transferring electrons to dual-bond structures and inhibiting cellular damage and chloroplast membrane degradation (Uarrota et al., 2018).

The recent study focused on the evaluation of potential relationships among heavy metals in plants grown at roadside and wastewater discharge sites and comparing them to the control area. The investigation also considered how the stress of the metals in that location might impact plant proteins and photosynthetic pigments. The ability of this plants in accumulation of hazardous metals and using them in further remediation processes and in ecosystem restoration.

2. MATERIAL AND METHOD

2.1. Site description

In the current study, two distinct sites were chosen. The first one was polluted and positioned south-west of Erbil along a wastewater channel near Turaq region at an elevation of 355 meters above sea level between longitude 43º55'27'' E and latitude 36º10'07''N. The other site was un polluted rural area within Koya district located between longitude 44º49'53''E and latitude 35º58' 06''N about 85 km away from polluted site. (Fig.1).

Figure 1: Map of studied sites (Polluted site – Turaq region) and (Control site – Koya region)

2.2. Sample collection

Leaves of six wild plant species were collected in both areas. The plants belonged to three families: four species from the Poaceae family, which were: *Brachiaria eruciformis* (Sm.) Griseb. *Polypogon monspeliensis* (L.) Desf. *Phragmites australis* (Cav.) Trin. *Cynodon dactylon* (L.) Pers. *Prosopis farcta* (Banks. & Sol.) Mac. from the family (Mimosaceae) and *Typha domingensis* Pers. From Typhaceae family. All samples were identified by Prof. Dr. Abdullah Shakur Sardar from Education College - Salahaddin University Herbarium (ESUH). The samples were stored in labelled plastic bags before being transported to the laboratory for additional analysis.

2.3. Plant digestion and heavy metal analysis

To assess the heavy metal concentration in plant leaves, leaf samples were dried in an oven for 72 hours at 70 ºC. An electrical grinder was used to grind dried leaves. The powdered samples were digested using 0.5g from the ground samples by adding 10 ml $H_2 O_2$ and 10 ml $H_2 SO_4$, they were heated for 1 hour at 420 ºC, after cooling, Whatman No.42 filter paper was used to filtrate the solution. Then distilled water was added to reach the final volume of 100 ml (Rashid et al., 2016). The measurement of heavy metals (As, Cr, Hg, Pb, Cd and Zn) was conducted by Inductively coupled plasma optical emission spectroscopy (ICP- OES) method manufactured by PerkinElmer (USA).

2.4. Photosynthetic pigments and carotenoids

To determine chlorophyll a, chlorophyll b and carotenoids, the spectrophotometric method as described by Lichtenthaler (1987) was used at plant physiology lab. of Education college. 0.5g of fresh leaves were immersed in 10ml of absolute ethanol for 24 hours in dark conditions. This procedure was performed three times to extract the chlorophyll completely. The final volume was 30 ml. After making sure that chlorophyll is completely extracted, chlorophyll a, chlorophyll b and carotenoids were measured spectrophotometrically at three wavelengths: 665nm, 649nm, and 470nm as follows:

Chlorophyll a (mg/g) = $(13.36 \times A664.2) - (5.19 \times A648.6) \times 1000/VW$

Chlorophyll b (mg/g) = $(27.43 \times A648.6) - (8.12 \times A664.2) \times 1000/VW$

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Carotenoids (mg/g) = $\{(1000\times A470) - (2.13 \times \text{Ch a}) - (97.64 \times \text{Ch b})\}/209\times 1000/VW$

Where, V: Volume of the extract (L)
W: Weight sample (g)

2.5 Protein contents of leaves

The Kjeldahl method was applied to measure total nitrogen, as explained by Ryan et al. (2001).Total protein was obtained by multiplying the total nitrogen by the factor 6.25 (Al-Dalali and Al-Hakim, 1987).

Reduction percentage of photosynthetic pigments, total nitrogen and protein were calculated using the following equation used by Salih and Aziz (2019).

$$R\% = 100 - \frac{\text{The concentration in the polluted site}}{\text{The concentration in the control site}} \times 100$$

2.6 Statistical Analysis

The data are presented as mean ± standard error. Unpaired T test were applied to identify statistically significant differences between the two groups. Correlations between different metal concentrations and metals with pigments, total nitrogen, and protein variables were evaluated by a bivariate correlation test with Pearson's correlation coefficient using the GraphPad Prism 8. A p-value below 0.05 was considered statistically significant.

3. RESULTS

3.1. Heavy metals content

The heavy metal content (Zn, Cr, As, Cd, Hg, and Pb) in the leaves of six plant species from two distinct locations (pollute and control) is provided in table 1. The results revealed that the polluted site had significantly recorded higher accumulation of heavy metals in leaves of plants that were studied than the control area.

In this study, zinc content means values (mg/kg) ranged from 93.80 to 227.40 and 47.80 to 124.20 in the polluted and control sites, separately. *Prosopis farcta leaves had the highest zinc content in the contaminated area, while Typha domingensis had the lowest amount in the control site.*

At the contaminated site, *phragmites australis* leaves accumulated 86 mg/kg of chromium whereas *Polypogon monspeliensis* leaves accumulated 11 mg/kg. The average value for the control area was between 2.80 and 8.60 mg/kg.

P. australis deposited the greatest amount of arsenic, with a mean value of 4.60 –10.00 mg/kg for both sites (control and polluted), while *P. farcta* accumulated the least, with a mean value of 0.80–5.60 mg/kg.

The level of Cd in the polluted region varied from 0.40 ± 0.02 to 0.60 ± 0.04 and was significantly ($p < 0.05$) higher in all studied plants from the control region, which was 0.20 ± 0.05 and 0.28 ± 0.03. Among the studied plants, P. *monspeliensis* accumulated the maximum amount of Cd at the polluted site.

The concentration of Hg in plants grown in polluted region is considerably greater than the control area. Amongst *Phragmites australis* exhibits the peak value 2.93 ± 0.32 mg/kg in their leaves. By contrast, *P. monspeliensis* accumulates the lowest amount, 1.60 ± 0.20 mg/kg in the polluted site.

Accumulations of Pb at the unpolluted site ranged between 8.02 ± 0.87 and 10.27 ± 0.93 (Table 1). *Cynodon ductylon* had the highest average level of lead at the polluted site (16.20 mg/kg), followed by *Typha domingensis* (16.00 mg/kg). The lead concentration in all plant samples of control region in our study was within the range of the standard value of 5–10 mg/kg according to the WHO acceptable limits while in polluted site exceeds that range (WHO, 2005).

Table 1: Concentrations of heavy metals (mg/kg) in the plant leaves of polluted and control sites.

Heavy metal (mg/kg)		Plants					
		Brachiaria eruciformis	*Polypogon monspeliensis*	*Phragmites australis*	*Cynodon ductylon*	*Prosopis farcta*	*Typha Domingensis*
Zn	Polluted Site	93.80** ± 6.24	164.20** ± 9.19	167.80** ± 10.39	207.80*** ± 12.96	227.40 ** ± 16.05	129.60*** ± 7.63
	Control Site	52.00 ± 3.67	85.02 ±11.25	89.20 ± 6.17	71.00 ± 8.73	124.20 ± 9.77	47.80 ± 5.13
Cr	Polluted Site	16.53*** ± 0.59	11.00*** ± 0.52	86.00*** ± 8.53	37.40** ± 4.01	16.80** ± 2.48	32.27** ± 3.75
	Control Site	8.60 ± 0.30	3.40 ± 0.11	7.48 ± 2.17	7.20 ± 1.03	2.80 ± 0.20	6.30 ± 1.17
As	Polluted Site	7.00** ± 0.52	8.40*** ± 0.41	10.00*** ± 0.46	6.60** ± 0.41	5.60*** ± 0.30	6.00*** ± 0.30
	Control Site	3.20 ± 0.11	3.00 ± 0.30	4.60 ± 0.41	2.80 ± 0.52	0.80 ± 0.11	1.80 ± 0.20
Cd	Polluted Site	0.43 * ± 0.03	0.60 * ± 0.04	0.46 * ± 0.06	0.44** ± 0.02	0.40 * ± 0.02	0.40 * ± 0.03
	Control Site	0.20 ± 0.05	0.28 ± 0.03	0.22 ± 0.02	0.26 ± 0.02	0.23 ± 0.03	0.26 ± 0.05
Hg	Polluted Site	2.30** ± 0.36	1.60** ± 0.20	2.93*** ± 0.32	2.60*** ± 0.23	2.90*** ± 0.26	2.00** ± 0.32
	Control Site	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Pb	Polluted Site	14.80 * ±1.97	14.07 * ± 1.78	15.60 * ± 1.32	16.20** ± 1.21	15.50 * ± 1.15	16.00 * ± 1.44
	Control Site	8.22 ± 1.31	8.02 ± 0.87	10.27 ± 0.91	9.53 ± 0.75	10.27 ± 0.93	9.20 ± 0.98

means of three replicates ± standard error Significant at $*p \le 0.05$, $** p \le 0.01$, $***p \le 0.001$

3.2. Correlation between heavy metals

The Pearson's correlation matrix, provided in Table 2, which shows a statistically significant correlation between metals present in all of the selected plants. Mercury has a strong positive correlation ($p < 0.001$) with zinc ($r = 0.733$), arsenic ($r = 0.735$) and lead ($r = 0.769$). A significantly positive relationship exists between lead and arsenic ($r = 0.613$) and arsenic and chromium ($r = 0.619$) as well.

Table 2: Pearson's Correlation (r) between Plant leaves heavy metal in polluted site

Heavy Metals	Zn	Cr	As	Cd	Hg	Pb
Zn	1					
Cr	0.396 *	1				
As	0.474 **	0.619 **	1			
Cd	0.432 **	0.313	0.544 ***	1		
Hg	0.733***	0.327	0.735 ***	470 **	1	
Pb	0.599 ***	0.518 **	0.613 ***	0.415 *	0.769 ***	1

*Correlation is significant at P ≤ 0.05 level **Correlation is significant at P ≤ 0.01 level
***Correlation is significant at P ≤ 0.001 level

3.3. Photosynthetic pigments and Carotenoids

The variation between chlorophylls and carotenoids observed in plants at both locations is presented in Table 3.

The chlorophyll a, chlorophyll b and total chlorophyll in all studied plants are significantly reduced in the polluted site as compared to the unpolluted site, with the exception of carotenoids. *Brachiaria eruciformis* shows a non-significant decrease in chlorophyll a. Also, the amount of chlorophyll a and b in *Typha domingensis* is not statistically significant. The maximum reduction percentage of chlorophyll a (26.88%) was found in *T. domingensis* while the minimum rate of it (8.92%) was found in *P. monspeliensis. The chlorophyll* b (59.54 %) and total chlorophyll (39.39 %) reduction percentages were highest in *P. monspeliensis.* The lowest reductions for T. chlorophyll (17.49 %) and chlorophyll b (12.59 %) were shown in *C. dactylon.* In this study, the level of carotenoids in all plants was increased in the polluted site in contrast to the control site.

Table 3: Chlorophyll a, chlorophyll b, total chlorophylls (mg/g) with their reduction percentages (R%) and carotenoids (mg/g) in plant leaves of polluted and control areas.

Leaf pigments (mg/g)		Plants					
		Brachiaria eruciformis	*Polypogon monspeliensis*	*Phragmites australis*	*Cynodon ductylon*	*Prosopis Farcta*	*Typha domingensis*
Chlorophyll a	Polluted Site	1.51 ± 0.12	1.43* ± 0.01	1.27** ± 0.01	1.34* ± 0.03	1.44* ± 0.03	0.68 ± 0.01
	Control Site	1.71 ± 0.08	1.57 ± 0.03	1.40 ± 0.08	1.57 ± 0.01	1.60 ± 0.02	0.93 ± 0.16
	R%	11.70	8.92	9.48	14.60	9.69	26.88
Chlorophyll b	Polluted Site	1.23 ** ± 0.04	0.96 * ± 0.13	1.21** ± 0.12	2.36 ± 0.08	0.95 *** ± 0.03	0.29 ± 0.01
	Control Site	2.24 ± 0.20	2.37 ± 0.29	2.22 ± 0.13	2.70 ± 0.18	2.24 ± 0.06	0.40 ± 0.08
	R%	45.09	59.54	45.57	12.59	57.65	27.50
Total Chlorophylls	Polluted Site	2.74 * ± 0.08	2.39 ** ± 0.14	2.48 ** ± 0.08	3.52 * ± 0.10	2.44 *** ± 0.08	0.97 * ± 0.04
	Control Site	3.95 ± 0.15	3.94 ± 0.25	3.62 ± 0.07	4.27 ± 0.12	3.84 ± 0.05	1.34 ± 0.05
	R%	30.69	39.39	31.61	17.49	36.51	27.24
Carotenoids	Polluted Site	1.92 * ± 0.14	2.16 ** ± 0.16	1.90 * ± 0.02	2.35 ± 0.18	1.82 * ± 0.19	1.09 ± 0.02
	Control Site	1.23 ± 0.06	1.29 ± 0.06	1.05 ± 0.06	1.39 ± 0.11	0.59 * ± 0.05	0.38 ± 0.07

Means of three replicates ± standard error
Significant at $*p \leq 0.05$, $** p \leq 0.01$, $***p \leq 0.001$

The correlation between heavy metals, chlorophylls, and carotenoid is displayed in Table 4. There was a negative correlation between all heavy metals and chlorophylls. Hg exhibits a significant negative correlation at a 0.05 level with chlorophyll a (r = -0.429).

The correlation between most analyzed heavy metals and carotenoids was positive except for cadmium, r = -0.187.

Table 4: Pearson's correlation between heavy metals and photosynthetic pigments, carotenoids and in leaves of studied plant.

Heavy Metals	**Chlorophyll a**	**Chlorophyll b**	**Total Chlorophyll**	**Carotenoids**
Zn	-0.170	-0.070	-0.096	0.174
Cr	-0.210	-0.223	-0.276	0.413
As	-0.108	-0.025	-0.046	0.398
Cd	-0.149	-0.154	-0.257	-0.187
Hg	- 0.429 *	-0.269	-0.310	0.475 *
Pb	- 0.207	-0.114	-0.125	0.183

*Correlation is significant at $P \leq 0.05$ level -negative correlation.

3.4. Total Nitrogen and Protein

The concentration of nitrogen and protein in both locations is revealed in Table 5. Both of them show a significant decrease in the polluted site. *Phragmites australis* has the highest nitrogen and protein reduction at 42.24 percent, followed by *Prosopis farcta* at 36.81 percent. *Brachiaria eruciformis* had the lowest nitrogen and protein content reductions, at 9.53 percent.

Table 5: Nitrogen and protein concentrations in plant leaves of contaminated and unpolluted site

Plants	Total Nitrogen %			Total Protein %		
	Polluted Site	Control Site	R%	Polluted Site	Control Site	R%
Brachiaria eruciformis	3.00 * ± 0.08	3.32 ± 0.04	9.53	18.81 * ± 0.50	20.79 ± 0.28	9.53
Polypogon Monspeliensis	2.40 ** ± 0.12	3.64 ± 0.20	33.93	15.04 ** ± 0.77	22.77 ± 1.26	33.93
Phragmites australis	1.89 ** ± 0.20	3.28 ± 0.15	42.24	11.87 ** ±1.29	20.54 ± 0.95	42.24
Cynodon dactylon	2.88 * ± 0.24	4.26 ± 0.28	32.50	18.00 * ± 1.53	26.67 ± 1.76	32.50
Prosopis farcta	1.26 ** ± 0.11	2.00 ± 0.07	36.81	7.91** ± 0.70	12.53 ± 0.81	36.81
Typha Domingensis	1.61* ± 0.18	2.17 ± 0.06	25.97	10.09 * ± 1.14	13.62 ± 0.38	25.97

Means of three replicates ± standard error Significant at $*p \leq 0.05$, $** p \leq 0.01$

Correlation between total nitrogen, protein, and heavy metals contained in Table 6. All studied heavy metals were negatively linked with total nitrogen and protein. As, Hg, and Zn have a significant impact on nitrogen and protein ($p < 0.01$). Chromium and lead ($p < 0.05$) and cadmium is not statistically significant.

Table 6: Pearson's correlation (r) between total nitrogen, total protein and heavy metals in the leaves of studied plants in the polluted site

Heavy Metals	**Total Nitrogen**	**Total Protein**
Zn	-0.454 **	-0.454 **
Cr	-0.404 *	-0.404 *
As	-0.495 **	-0.495 **
Cd	-0.300	-0.300
Hg	-0.491 **	-0.491 **
Pb	-0.409 *	-0.409 *

*Correlation is significant at P ≤ 0.05 level -negative correlation

**Correlation is significant at P ≤ 0.01 level

4. DISCUSSIONS

Zinc is an important micronutrient that has a role in many biological processes, even at low quantities. However, when it reaches a toxic level, it causes changes in the way of enzymes work, slows down growth and metabolism, stops roots and shoots from growing, and causes oxidative damage in plants (Gondal, 2021). Its toxicity in plants starts at 300 to 400 mg/kg, relying on the type of plant (Broadley et al., 2007). Based on this value, the Zn concentration of all plant species at both locations was below this threshold.In the same way, Doğanlar and Atmaca (2011) found higher Zn deposition in the leaf tissues of all plant species (*P. coccinea, N. oleander, P. orientalis, R. pseudo-acacia, M. azederach,* L. *nobilis,* and *A. negundo* L) in the polluted areas of the Antakya region compared to the control. Salih and Aziz (2019) found that the polluted area around the Erbil Steel factory had high levels of zinc 484.8 mg/kg in *Olea europaea* leaf and 912.33mg/kg in *Dadonaea viscosa.*

Chromium's toxicity affects plants growth and development severely. Cr is also regarded as a human carcinogen. It enters the body through breathing or eating foods that have been contaminated with Cr (Srivastava et al., 2021). Its permissible value in plants is 1.30 as prescribed by WHO (1996). According to a study performed by Tariq (2021), the amount of chromium in vegetables grown with untreated wastewater in the fields of the Turaq region, ranged from 39.42 mg/kg to 64.78 mg/kg, which is much more than allowable level. Bajraktari et al. (2022) worked on white willow bark from the Kosovo A and B thermal power plants exhibited a range of 0.85 to 1.89 mg/kg of chromium, with a mean value of 1.28 mg/kg.

Arsenic (As) is a highly poisonous substance that poses major threats to humans and the ecosystem. It's a mineral that is not essential for plant growth and is usually very toxic to plants (Bali and Sidhu, 2021). Sharma et al. (2021a) found a smaller amount of arsenic in the leaves of *Eclipta alba* (3.46 mg/kg) and *Alternanthera* *philoxeroide* (3.14 mg/kg), both of which grew on a contaminated site from pulp and paper industry.

Cadmium is a non-essential mineral; hence, plants have not evolved a specific absorption mechanism for it. Rather, Cd enters plants and is carried by different metal transporters through various membranes (Feng et al., 2017). The outcomes of our findings are in line with the study of Uka et al. (2021), who reported Cd in a range 0.21 ± 0.08 to 1.08 ± 0.55 mg/kg in tree species of Kumasi Metropolis, Ghana. Chaoua et al. (2019) found that vegetables can store high level of heavy metals in their roots and leaves on land that is watered with wastewater. The amount of Cd in their study was high, with the mean value of 0.63 mg/kg in the leaves of *Triticum turgidum* and 8.35 mg/kg in *Plantago major* leaves.

Hg occurs as a liquid metal at standard conditions of temperature and pressure, and it is one of the Earth's crust's most scarce minerals. Many plants can obtain Hg from both the ground and the atmosphere, and the amount of Hg differs in each tissue of a plant (Cosio et al., 2014). Various research has indicated that green portions (shoots) of certain plants assimilate mercury in higher concentrations than in their roots for instance *Phragmites australis, Panicum coloratum* and *Persicaria lapathifolia* (Mbanga et al., 2019). This finding agrees with the findings of Qian et al. (2018), who found a similar range of Hg of 1.7-2.5 mg/kg in *Brassica campestris* at wastelands composed of mine tailings in China.

Lead is a non-biodegradable metal occur in nature in relatively small quantities. Due to anthropogenic actions such as industrialization, mining, and burning fossil fuels, lead concentrations in the environment continue to rise. When people are subjected to unsafe levels of lead, it damages their bodies (Loh et al., 2016). The obtained results are close to those of Chaoua et al. (2019) , who reported Pb in foliage samples of *Vicia faba* with a mean content of 18.35 mg/kg in lands that use wastewater for irrigation. Cui et al. (2022) recorded at the rural locations of Tianjin, China almost the same results as our control site, which were 6.53 and 9.90 mg/kg for *Sabina chinensis* and *Platycladus orientalis* separately. These results are in agreement with the results of Aslam et al. (2012) who observed a higher concentration of lead in polluted site plants when compared with plants found in unpolluted place.

It has been observed that the availability of metals for plants varies. This is related to variations in the absorption routes of particular ions and competition for uptake among heavy metals. Various elements interact with each other at the root surface and inside the plant, which eventually influences their transportation and absorption (Sharma et al., 2007). Consequently, certain plants are able to endure high quantities of necessary and harmful elements in their tissues without undergoing detrimental effects (Pehoiu et al., 2020).

The results indicated that wild plants growing along roadways and in soils adjacent to wastewater flows accumulate considerable amounts of heavy metals. Hence, these species can be used to minimize soil and vehicular pollution near roadways and other damaged areas.

The relationship among selected elements were significantly positive. These results are in agreement with the findings of Anand and Ramamoorthy (2022), who found a positive relationship between the metals Pb, Cr, Zn, and Cd in the leaves of *Senna auriculata.*

Chlorophyll estimation determines the impact imposed by pollutants under stressful circumstances because it serves a key position in metabolic activities, thus, any change in chlorophyll concentration is tightly linked to plant growth (Yang et al., 2020). The amount of photosynthetic pigments in selected plants at contaminated places has been reduced, leading to a decline in chlorophyll at such sites. Chlorophyll is commonly converted to phaeophytin when magnesium ions are depleted as a result of a decrease in chlorophyll level (Pimple, 2017). This findings are consistent with the results of Salih and Aziz (2019) and Uka et al. (2021).

Carotenes are more resistant to the adverse consequences of heavy metals than chlorophyll. In a stressful environment, carotenoids are observed in greater concentration. It serves a defensive action through minimizing the degradation of chlorophyll molecules as well as other organic substances, thus reducing stress effects (Sharma et al., 2021b). Our outcomes are similar to the study of Mukherjee and Agrawal (2016) who show that the number of carotenoids in all studied species elevated in parks with more pollution.

Heavy metals with photosynthetic pigments have negative relationship. These relationships might have clarified the impact of specific metals on the biosynthesis of carotenoid and chlorophyll (Liu et al., 2007)

All living organisms require nitrogen (N), which is also a constituent of amino acids, nucleotides and other compounds. Total nitrogen comprises nitrate nitrogen, ammonium nitrogen, organic nitrogen, and all other types of nitrogen (Vázquez et al., 2008). Proposed explanations for the decreasing protein content in the contaminated plant include the accelerated rate of protein denaturation and its degradation to amino acids (Thambavani and Maheswari, 2014). There could be a role for degradative enzymes like proteases, which help break down polypeptide chains into amino acids so that the organism can handle the stress caused by pollutants (Dohmen et al., 1990). Our results are in conformity with the findings of Salih and Aziz (2019).

This reduction in plant pigments, total nitrogen, and protein is most likely due to antagonistic effects of heavy metal, as shown in (tables 4 and 6). Because of the toxic effects of these metals, they react with cellular components, thereby generating reactive oxygen species (ROS), which restricts plant development and productivity. Presence of large amount of heavy metals in plants promotes the formation of reactive oxygen species, leading to cellular membrane disruption, which in turn destroys chlorophyll and photosynthetic processes and inhibits the plant's overall growth (Cui et al., 2022).

5. CONCLUSIONS

Based on this research, it can be concluded that heavy metals influence a variety of biochemical parameters, including total nitrogen, proteins, chlorophyll, and carotenoids, in all examined plants. Decreasing and increasing levels of these parameters at both sites can be considered as an adaptation of the plant in that environmental condition to protect plants against pollution by heavy metals. All species exhibited decreased levels of nitrogen, protein, and chlorophyll in comparison to controls, and the presence of a negative correlation with heavy metals confirms that. In contrast, metal accumulation increased in the polluted area. According to this finding, the selected plants are both effective bioindicators and potential pollution monitors. This might prevent the distribution of heavy metals into the environment and contribute to the restoration of the ecosystem quality in areas exposed to pollution.

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Conflict of interest

The authors declare no conflict of interest

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