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Assessment of Roadside Potentially Toxic Metal Contamination Using *Linum usitatissimum* L. as a Bioindicator in Sargodha, Pakistan

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Abstract

In this study, *Linum usitatissimum* L. (flax), a wild plant species predominantly growing in roadside habitats, is investigated with regard to contamination and accumulation of potentially toxic metals (PTMs). For this purpose, samples of soil and Linum were collected from Sargodha-Bhalwal road of Sargodha City. These samples of soil & plant were tested for the following HMs: Zn, Cu, Co, Mn, Mo, Fe, Pb, Cd using Flame Atomic Absorption Spectroscopy. Physico-chemical analysis of Bhalwal Roadside Locations (BRL) soils exhibited higher EC (11.42 dS/m), pH (7.8), organic matter (2.73 mg/kg), and PTM concentrations, with BRL1 showing maximum levels of Zn (30.88 mg/kg), Cu (17.16 mg/kg), Co (2.98 mg/kg), and Mn (97.94 mg/kg) than off-road sites. Linum samples also showed higher metal accumulation at Bhalwal Roadside Locations, with the highest Zn (33.48 mg/kg) and Fe (32 mg/kg) recorded at Bhalwal Roadside Location 1. Correlation analysis revealed strong positive relationships between Cd-Zn (r = 0.9992), Pb-Mn (r = 0.9789), and Zn-Cu (r = 0.9663), suggesting shared uptake mechanisms. Bioaccumulation factors were highest at Bhalwal Roadside Location 1, particularly for Co (0.37), Zn (1.08), and Cu (0.40), indicating greater metal uptake from roadside contamination. The present study reveals that the roadside vegetation, particularly *L. usitatissimum* L. has a great potential as bioindicator for the potentially toxic metals pollution in the urban environment. The findings enhance our understanding of soil-plant metal transfer mechanisms and serve as a foundation for the development of sustainable environmental monitoring strategies.

Keywords: Roadside pollution, Bioindicator, *Linum usitatissimum*, Sargodha city.

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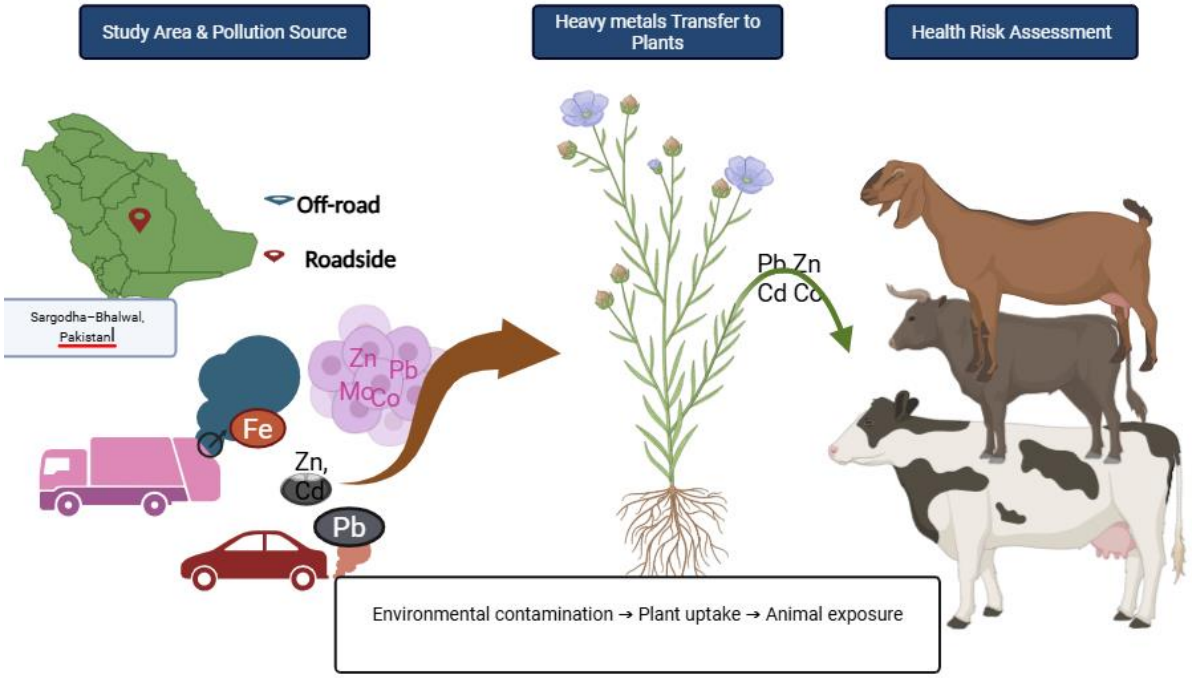
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Graphical abstract



Roadside Potentially Toxic Metal (PTM) Contamination and Bioaccumulation in *Linum usitatissimum L*.

1. Introduction

Environment pollution by potentially toxic metals is becoming a big issue especially in urban and peri urban areas where the impacts of population pressure and development activities are strongly felt (An *et al.*, 2022, Lanjwani *et al.*, 2024). Metals, especially the PTMs cause severe impacts on the environment and people, and the organisms due to their toxic and non-degradable properties and their ability to accumulate themselves (Antonenko *et al.*, 2021, Abubakar *et al.*, 2024).

Runoff from automobile exhausts, industries, and inadequate control over their wastes make significant contribution to pollution from the PTMs in the habitats at the sides of the roads (Asif *et al.*, 2020, Ullah & Khan, 2022, Anjum *et al.*, 2024). The transportation of PTMs from the roads to the soil and then to the plants growing along the roadsides, remains a key environmental issue, especially in the urban and peri-urban areas (Uka *et al.*, 2021).

These pollutants collect on the topsoil layer, and the concentration of these elements is found to reduce as one move away from the road. It is used as a source of heavy metal pollution of plants through their root systems (Nikolaeva *et al.*, 2021). Potential Soil factors include, pH, organic matter content and Specific uptake mechanisms involved in the plant species have an impact on the bio availability of these metals (Han *et al.*, 2020; Bui *et al.*, 2023).

Once entered into plants, HMs tend to bioaccumulate in plant tissues; parts of roots, stems, leaves or even edibles parts of plants that are potentially dangerous to plant health, ecology, and food chain (Yang *et al.*, 2020; Korotchenko *et al.*, 2023). This makes it significant to prevent potentially toxic metals , and to evaluate the repercussions in the ecological and health assemblies (Jisha & Sadiya, 2021; Stinshoff *et al.*, 2024).

Roadside environments, especially in such growing cities of Sargodha Pakistan, have become susceptible to potentially toxic metals (Men *et al.*, 2025). They are usually subjected to vehicular emissions, dust particles and industrial effluents which in turn discharge potentially toxic metals into the ground (Uka *et al.*, 2021). So far, even though the use of roadside vegetation as a bioindicator of potentially toxic metals has been examined in many studies, most research has centered on the study of well-known species and little has been done on area specific or unstudied plants. Specifically, the use of *Linum usitatissimum* L. as a bioindicator species in the ecosystems adjacent to roads has not been well studied, particularly in the environment of Pakistan. The literature has largely focused on the accumulation of potentially toxic metals in general vegetation without incorporating the dynamics of soil-plant transfer, correlation analysis of metals and quantitative bioaccumulation using bio-concentration factors (BCF) into one literature (Bartkowiak *et al.*, 2024; Wan *et al.*, 2024).

Linum usitatissimum L., (Flax) is the wild plant species found in Pakistan which is adaptable to different types of environment. Because of its proficiency to germinate on polluted soils, and also it serving as a biomarker of potentially toxic metals accumulation, the organism is also ideal for use in environmental assessment. Since *L. usitatissimum* is wild species it was in direct contact with the soil as shown by its capacity to reflect the contamination prevailing in its environment (Sabir *et al.*, 2022; Zhakypbek *et al.*, 2024).

Linum usitatissimum L. is an ideal candidate for roadside biomonitoring as it is a natural inhabitant of disturbed and roadside habitats, widely distributed in Pakistan, and has capability to absorb and accumulate metals from contaminated soils through its root system. Despite these features, this species has been comparatively less studied as a bioindicator in roadside ecosystems, especially in the context of Pakistan. Therefore, its systematic evaluation would be timely and scientifically important.

The current work aims to assess the potential of *L. usitatissimum* for use as a bioindicator species for assessing PTMs contamination. Thus, the current research will (i) measure the potentially toxic metals in roadside and off road soils, (ii) determine the accumulation of these metals in *Linum usitatissimum*, (iii) examine soil-plant transfer with the help of bioconcentration factors and (iv) test the applicability of this species as a bioindicator of road side pollution. This paper fills the gap in existing knowledge by evaluating potentially toxic metals dynamics in urban road side ecosystem.

Moreover, the results will be useful for policymakers and urban planners to realize the scale of pollution in roadside habitats and offer insights for creating strategies of habitat remediation and management of sustainable urban development.

2. Materials and Methods

2.1. *Study area*

Sargodha is the twelfth largest city in Pakistan with regard to population; the city is located in the Punjab province. It also makes it one of the few planned cities in Pakistan today. The city has developed on flat grounds with only slight elevation at Kirana Hills. Sargodha enjoys a good climate and the land is proved to be very fertile because the area plays an important role in agriculture of Pakistan.

The present study is planned to quantify the effects of potentially toxic metals (PTMs)at Sargodha-Bhalwal road (32°06'51.3" N and 72°44'30.3" E) due to vulnerability of emissions from vehicular movements and industrial activities. (**Figure 1**).

2.2. *Sampling Design & collection*

The sampling design to be used was a gradient-based one to assess the spatial distribution of potentially toxic metals. In order to represent different levels pf traffic derived pollutants, current study sites were stratified according to their distances from the road as reported by previous studies (Foroughi & Weil, 2025).

- **Roadside (RS):** 1–30 m from the road
- **Off-road (OR):** 40–70 m from the road
- A total of **six locations** were selected: three roadside (Bhalwal road Locations: BRL1, BRL2, BRL3) and three off-road (Bhalwal Off-road Locations: BOL1, BOL2, BOL3) (**Table 1**).
- At each location:
- At each location, three composite samples of *Linum usitatissimum* were prepared, each pooled from 30 randomly collected individual samples. Soil samples were collected from where the plant samples had been collected.

This design ensured reliable comparison between contaminated and relatively uncontaminated zones.

Soil samples were collected from the top 0-20 cm layer using a soil auger, while plant samples were gathered from the same site. After measuring the weights of samples, both soil and plant samples were stored in labeled polyethylene and paper bags, respectively, and transported to the laboratory.

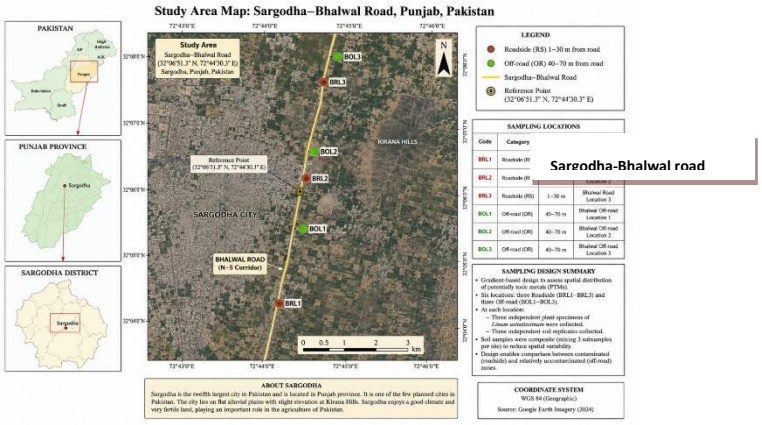


Figure 1. Study area; Sargodha Bhalwal-road.

2.3. *Sample preparation*

Soil samples were air dried and sieved as well as ground in a wooden mortar and pestle whereas Plant samples were oven dried at 70°C till the weight became constant. These dried samples were thus crushed into fine powder form using a mechanical grinder.

2.4. *Analytical procedure*

One gram of soil sample and 0.5 gram of plant sample were separately transferred to digestion vessels and treated with a mixture of concentrated nitric acid (HNO_3, 65%) and perchloric acid ($HClO_4$, 70%) at a ratio of 4:1 (v/v) with 8 mL and 2 mL, respectively. The samples were pre-digested at room temperature for 30 min, and then heated in a digestion block at 120 °C for 2 h and finally the temperature was increased to 200 °C until a clear colourless solution was achieved. The digested sample was filtered and made up to a certain volume with distilled water. The filtrate was then used to determine the various heavy metals (Zn, Cu, Co, Mn, Mo, Fe, Pb, Cd) by Atomic Absorption Spectrophotometer (Shimadzu AA-7000, Shimadzu, Japan). The instrumental parameters used for AAS in determination of these HMs are summarized in **Table 2.**

Table 1. Location Sites and Sample types.

Samples	Sites	Locations	Locations No.
Soil, Plant	Off-road (OR)	Bhalwal Off-road Locations	BOL1
			BOL2
			BOL3
Soil, Plant	Roadside (RS)	Bhalwal Roadside Locations	BRL1
			BRL2
			BRL3

Table 2. Instrumental parameter for AAS.

Metal	Wavelength (nm)	Slit Width (nm)	Lamp Current (mA)	Type of Flame
Cd	228.8	0.7	8	Air acetylene
Cu	324.8	0.7	10	Air acetylene
Co	240.7	0.2	30	Air acetylene
Fe	248.3, 258.3	0.2	30	Air acetylene
Mn	279.5	0.2	10	Air acetylene
Zn	213.9	1	10	Air acetylene
Pb	217, 283.3	0.7	10	Air acetylene
Mo	313.3	0.7	10	Air acetylene

References: (Gogoi et al., 2024)

Table 3. Permissible limits of HM in soil and plants.

Heavy metals	Permissible limits in soil ($mgkg^{-1}$)	Permissible limits in plants ($mgkg^{-1}$)
Cd	1.5	0.05
Cu	50	40
Co	20	0.02-10
Fe	Not regulated	42.5
Zn	200	2.3-50
Pb	50	0.1-0.3
Mn	500	10-50
Mo	2	0.3-0.6

References: (Carrasco Cabrera & Medina Pastor, 2021; OMS, 2019)

Table 4. Analysis of physiochemical properties of soil at roadside (BRL) and off-road (BOL) locations along Sargodha–Bhalwal road.

Sites	Locations	EC (dS/m)	pH	Organic Matter (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)	Saturation (%)
Off-road (OR)	BOL1	6.77±0.005	7.6±0.05	2.41±0.005	20.2±0.05	557±0.5	29±0.5
	BOL2	5.86±0.005	7.2±0.05	2.28±0.005	18.6±0.05	524±0.5	26±0.5
	BOL3	4.07±0.005	6.8±0.03	1.29±0.008	15.9±0.08	463±0.5	25±0.5
Roadside(RS)	BRL1	11.42±0.005	7.7±0.05	2.73±0.005	34.2±0.05	635±0.5	43±0.5
	BRL2	10.87±0.005	7.8±0.05	2.63±0.005	31.5±0.05	577±0.5	40±0.5
	BRL3	8.27±0.005	7.1±0.05	2.57±0.005	24.2±0.05	564±0.5	32±0.5

2.5. *Quality Control and Assurance (QA/QC)*

To make the analysis accurate and reliable: Standard solutions of each metal were then used to prepare calibration curves. Contamination was checked by the use of analytical blanks. The analysis of all samples was carried out in three replicates. The recovery rates were kept at 90-110 percent. The precision of the analytical procedure was checked by repeating the analysis of the samples and the results were found to be within the acceptable range of 90-110% as per the quality assurance procedures described by Anjum *et al.* (2024), for similar roadside heavy metal studies in Pakistan. The accuracy of the instrument was validated using the certified reference material NIST SRM 2711a (Montana II Soil). In the present work, the recovery rates were also maintained between 90–110% in all the replicates, thus confirming the reliability of the analytical procedure (Anjum et al., 2024). The instrument detection limits (IDL) of Shimadzu AA-7000 were Zn (0.001), Cu (0.003), Co (0.005), Mn (0.001), Mo (0.010), Fe (0.003), Pb (0.010) and Cd (0.001) mg/kg. All concentrations of samples were well above these detection limits.

2.6. *Data analysis*

The results obtained from HM analysis were processed statistically to understand the relationship between heavy metal concentrations in soil and weeds. Data processing was performed using Minitab, Graph Pad prism, PAST software. The concentrations of HM in soil and plant samples were calculated. These concentrations were then compared with permissible limits set by international standards (**Table 3**).

2.7. *Bioconcentration Factor (BCF)*

The bioaccumulation potential of *Linum usitatissimum* was evaluated using the bioconcentration factor (BCF), calculated as:

$$BCF = \frac{C(plants)}{C(soils)} \quad (1)$$

Where **C(plants)** is the concentration of a heavy metal in plant tissue (mg/kg dry weight) and **C(soil)** is the concentration of the same heavy metal in the soil (mg/kg dry weight).

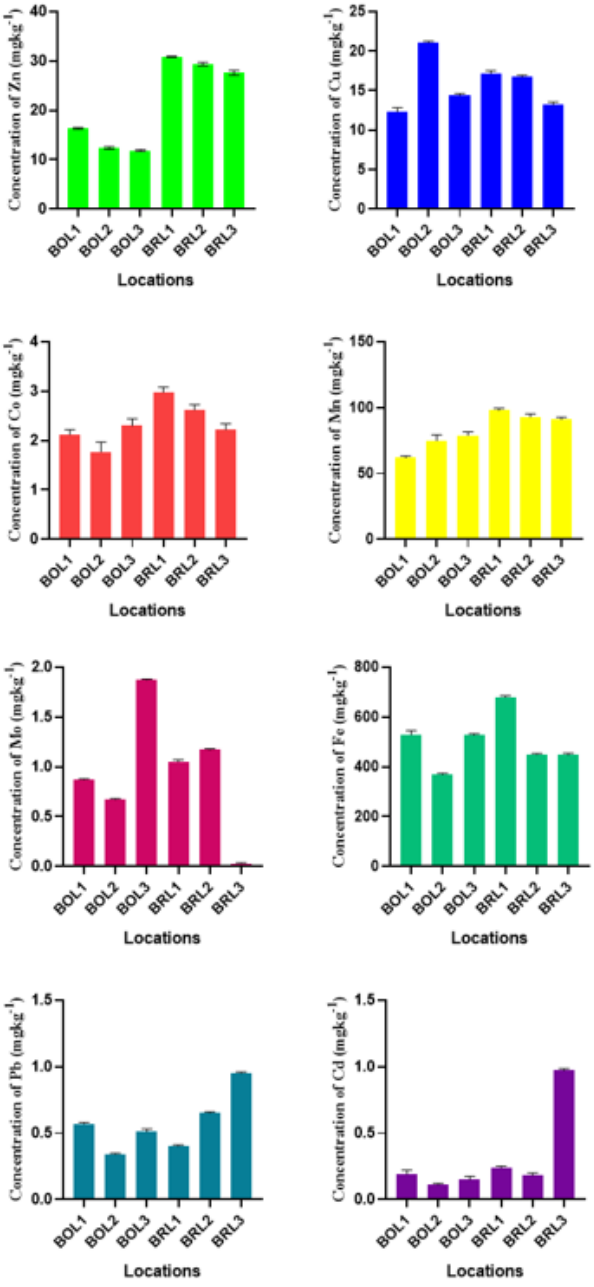


Figure 2. Comparison of HM concentrations in soil samples at roadside (BRL) and off-road (BOL) locations along Sargodha–Bhalwal road.

3. Results

3.1. *Physicochemical analysis of soil samples*

Higher electrical conductivity (11.42 dS/m) were recorded at BRL1, while highest pH (7.8) were recorded at BRL2, highest organic matter (2.73 mg/kg) were recorded at BRL1, phosphorus (67.0 mg/kg), and potassium (533 mg/kg) were recorded at the roadside site BRL2, while the lowest values for these parameters were found at off-road locations, particularly BOL2 and BOL3. Soil saturation was highest at BOL2 (37%) and lowest at BRL1 (20%) **(Table 4)**.

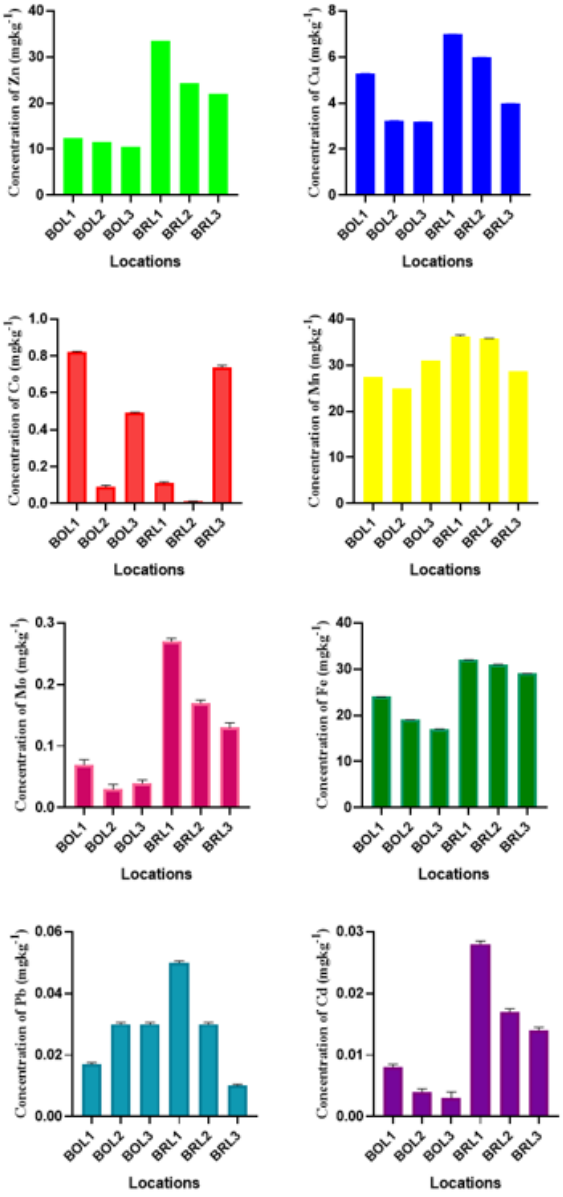


Figure 3. Comparison of HMs concentrations in *Linum* samples at roadside (BRL) and off-road (BOL) locations along Sargodha–Bhalwal road.

3.2. *Heavy metal analysis of soil samples*

Analysis of Variance showed that the concentration of heavy metals varies significantly by location, particularly for zinc, copper, cobalt, molybdenum, iron, lead, and cadmium, with many differences also noted across sites. Zinc, manganese, and cadmium exhibit non-significant variance by site. The error terms indicate substantial variability within samples. Overall, both site and location significantly influence heavy metal concentrations in soil samples.

HM concentrations (mg/kg) were higher in BRL soil samples compared to BOL sites. The highest levels of Zn (30.88), Cu (17.16), Co (2.98), Mn (97.94), Pb (0.40), and Cd (0.24) were recorded at BRL1. In contrast, off-road locations showed lower concentrations, with BOL3 having the lowest levels of Zn (21.05), Cu (6.97), Co (0.87), Mn (59.48), Pb (0.35),

and Cd (0.13). These results indicate that roadside soils experience higher HM contamination, likely due to traffic emissions and anthropogenic activities (**Figure 2)**.

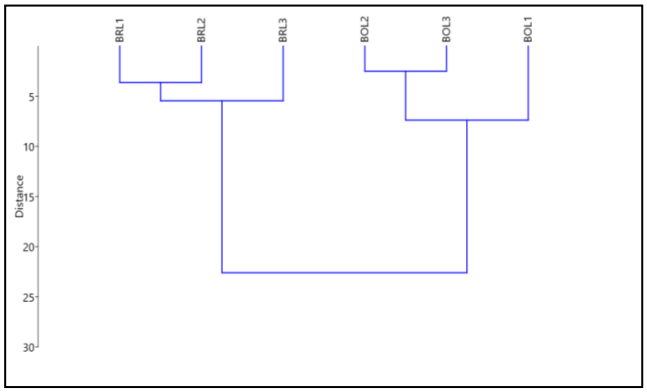


Figure 4. Dendrogram showing HMs concentrations in *L. usitatissimum* samples at roadside (BRL) and off-road (BOL) locations along Sargodha–Bhalwal road.

3.3. *Concentration of heavy metals in samples of L. usitatissimum*

Analysis of variance (ANOVA) for the concentrations of heavy metals (Zn, Cu, Co, Mn, Mo, Fe, Pb, Cd) in Linum across different sites and locations showed that significant differences were observed in zinc ($P < 0.05$) and iron ($P< 0.01$) concentrations among sites, while locations showed highly significant variations for zinc ($P < 0.0001$) and iron ($P < 0.0001$). For other metals like copper and cobalt, no significant differences were detected between sites, but they were significant across locations. HM concentrations in *L. unsitatissimum* samples were notably higher at BRL compared to BOL locations. The order of HM concentration (mg/kg) at BRL was as follows: Fe (32) > Mn (36.30) > Zn (33.48) > Cu (6.98) > Co (0.11) > Mo (0.27) > Cd (0.028) > Pb (0.05). Zn and Fe peaked at BRL1. BOL showed lower levels, with Zn ranging from 9.59–14.40 and Fe from 13.15–17.68. Cd levels were highest at BRL1. **(Figures 3, 4).**

3.4. *Correlation between concentrations of HMs in L. usitatissimum*

Pearson correlation analysis was performed for all six sampling locations (n = 6) where each sampling location was the mean of three replicates, giving a total of 18 data points per metal. The highest correlation was between Cd and Zn (r = 0.9992), indicating a shared uptake mechanism. Pb and Mn (r = 0.9789) also showed a strong relationship linking their co-absorption in soil. Cu and Mo (r = 0.8757) also exhibited simultaneous uptake. Additional correlations, such as Zn-Cu (r = 0.9663), Co-Mn (r = 0.9896), and Fe-Cd (r = 0.7821), further confirm complex metal interactions affecting plant absorption **(Table 5 and Figure 5).**

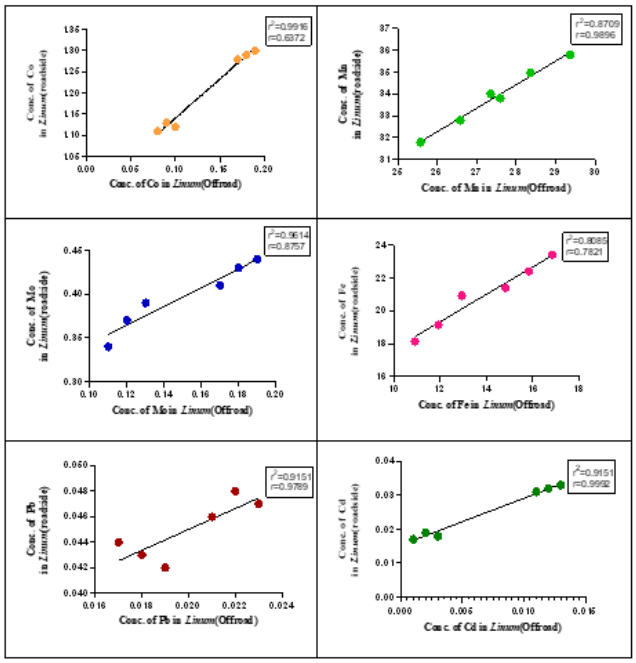


Figure 5. Correlation between HMs concentrations in *Linum samples* at roadside (BRL) and off-road (BOL) locations along Sargodha–Bhalwal road.

Table 5. Correlation between concentrations of HMs in *Linum* at roadside (BRL) and off-road (BOL) locations along Sargodha–Bhalwal road

Metal	Zn	Cu	Co	Pb	Mn	Mo	Fe	Cd
Correlation Value	0.9663	0.8639	0.6372	0.9789	0.9896	0.8757	0.7821	0.9992

Table 6. BCF of heavy metals in *L. usitatissimum* samples at roadside (BRL) and off-road (BOL) locations along Sargodha–Bhalwal road

Sites	Locations	Zn	Cu	Co	Mn	Mo	Fe	Pb	Cd
Off-road	BOL1	0.76	0.43	0.39	0.44	0.01	0.04	0.03	0.04
(OR)	BOL2	0.88	0.15	0.05	0.33	0.04	0.05	0.08	0.04
	BOL3	0.88	0.22	0.21	0.39	0.02	0.03	0.06	0.02
Roadside	BRL1	1.08	0.4	0.37	0.37	0.26	0.05	0.12	0.12
(RS)	BRL2	0.96	0.26	0.29	0.37	0.15	0.07	0.07	0.09
	BRL3	0.87	0.3	0.27	0.32	0.33	0.06	0.03	0.02

3.5. *BCF of HMs in L. usitatissimum samples*

The values of BCF express the ability of *L. usitatissimum* to accumulate HMs from the surrounding soil. The BCF values were consistently higher at BRLs compared to BOLs, indicating greater metal uptake from contaminated soils. Among roadside locations, BRL1 recorded the highest BCFs for Zn (1.08), Cu (0.4), Co (0.37), Mn (0.37), Mo (0.26), Fe (0.05), Pb (0.12), and Cd (0.12). Off-road sites showed lower BCFs, with the lowest observed at BOL3 for most metals, such as Fe (0.01) and Mo (0.02). Co showed the

highest BCF across all locations, indicating greater mobility and uptake in *L. usitatissimum*, particularly at BRL1 and BRL3 (0.35) (**Table 6**).

4. Discussion

The elevated concentrations of heavy metals at roadside sites (BRL1-BRL3) can be attributed to different sources of traffic-related and anthropogenic emissions(Khan *et al.*, 2026). Residues of leaded fuel and additives of lubricating oils are continuously depositing fine metal-laden particles along road corridors, and vehicular exhaust and incomplete combustion of fuel are well-established sources of Pb and Cd in roadside soils (Anjum *et al.*, 2024 and Kaur *et al.* (2022). Zn is mainly contributed by tire wear as zinc oxide is a common vulcanizing agent in tire manufacturing, which releases Zn-rich particles to road surfaces via mechanical abrasion (Bartkowiak *et al.*, 2024). Similarly, Cu- and Pb-rich dust particles are generated by brake wear as a consequence of the metallic composition of brake pads that accumulate in the topsoil layer closest to the road (Gajbhiye *et al.* 2022). These metals are further dispersed by atmospheric deposition of traffic generated particles, while surface runoff and wind driven resuspension contribute to contamination of the surrounding soil profile (Antonenko *et al.*, 2021, Ma *et al.*, 2026).

In addition to vehicular sources, the local industrial activities in the Sargodha region also contribute to the overall metal load through aerial emissions and effluent discharge and increase the Fe, Mn and Co concentrations at BRL sites (Asif *et al.*, 2020).

The physicochemical properties of the soil measured at the BRL sites were important in influencing the bioavailability and uptake of potentially toxic metals by *L. usitatissimum*. The alkaline pH range of 7.1–8.2 observed in roadside locations generally results in a reduction of metal solubility as metals tend to form insoluble hydroxides and carbonates under alkaline conditions thereby restricting their immediate availability for root absorption (Khan, Liu, *et al.*, 2023). However, total metal concentrations were always higher at BRL than BOL sites and this was enough to lead to measurable accumulation in *L. usitatissimum* tissues, suggesting that even partially bioavailable metal fractions in alkaline conditions can be detected and taken up by this species. Moreover, the organic matter content in BRL sites (2.57–2.73 mg/kg) is higher than in off-road sites (1.29–2.41 mg/kg), which may increase the retention of metals in soil by organic complexation. At the same time, organic matter may preserve a fraction of metals in soluble forms available to plant roots. Therefore, the combined effect of alkaline pH and high organic matter at BRL sites represents a soil environment in which metal bioavailability is affected by natural soil chemistry, resulting in higher metal concentrations in *L. usitatissimum* at roadside sites.

The redistribution of potentially toxic metals in roadside habitats is further influenced by surface runoff, resuspension of contaminated dust, and direct deposition.

Mir *et al.* (2020), identified the highest concentrations of nitrogen (197.7), phosphorus (17.89), and potassium (92.56) in saffron fields located beside roadways. Comparable trends have been documented in research by Aydin & Pakyürek (2020), Skorbiłowicz *et al.* (2021) and Zhakypbek *et al.* (2024) linking elevated roadside nutrient concentrations to vehicular emissions, road dust, and anthropogenic factors, whereas diminished saturation indicates potential soil compaction due to heavy traffic (Asiminicesei *et al.*, 2024; Chen *et al.*, 2023).

Similar to BRL1 (Zn: 30.88, Pb: 0.40), Popek *et al.* (2024) recorded Zn levels reaching 68 and Pb exceeding 5 close to roadways. Similar to BRL1 (0.24), Bouida *et al.* (2024) and Kaur *et al.* (2022) found Cd levels in urban roadside soils as high as 1.2. The high amount of these elements along road side soils highlight environmental challenges related with roadside contamination and may require routine surveillance of these areas.

In contaminated soil the high bioavailability of metals and presence of active soil-plants exchange are shown by high level of heavy metals in *L. usitatissimum* at road side location. The intake of metal by plants is regulated by internal physiological processes like root adsorption capability and transport of metal through plant tissues and external processes like soil characteristics and concentration of metal ions. The results shows consistency with study performed by Fidos & Rutkowska (2023) that found Zn accumulation in plants along the road side with values 32.8, which is also related to current study in BRL1 (33.48).

Study performed by Ebru Tuna *et al.* (2022) showed that Fe concentration in plants close to road ways is up to 2 mg/kg, which is also related with BRL1 (32). Siraj *et al.* (2022) reported Cd level (0.06-0.08) in plants along road side which also is similar to present BRL1 study value (0.028). Gajbhiye *et al.* (2022) connected Cu and Pb accumulation in plants to pollution related to traffic which is also in line with Cu (6.98) and Pb (0.05) at BRl1. These results, support the conclusion of this present study as they highlight heavy metal contamination related to traffic in road side habitats.

The correlation analysis highlights that there is a strong interdependence between heavy metal in *L. usitatissimum,* which shows that potential co-accumulation. Maximum value of r is 0.9992 between Zn and Cd, showed a common uptake process that is most probably effected by vehicle emission (Ahmad *et al.*, 2020).In addition, Pb and Mn had a significant correlation (r = 0.9789). This is again consistent with current study showing a link between their co absorption in soil. Cu and Mo (r = 0.8757) also showed related uptake which may depend on organic matter and soil pH. The presence of complex metal interaction affecting the absorption by plants is further confirmed by Zn- Cu(r = 0.9663), Co-Mn (r = 0.9896) and Fe-Cd (r = 0.7821)(Qader & Salih, 2023).

The BCF values of Co and Zn along the road side is significantly higher with highest BRL1 (0.37 for Co and 1.08 for Zn). This showed the capability of *L. usitatissimum* to use it as a biomarker for certain metal ions.The results are in similar with Akbar *et al.* (2024) and Upahi *et al.* (2021), suggested that plants grown at road side locations

exhibited high BCF values for Cd (0.42) and Zn (0.51). Plants grown at highway showed high bioconcentration of Cd (0.37) and Pb (0.29) as observed by Anand & Ramamoorthy (2022), Hozhabralsadat et al. (2022) and Martsev et al. (2022). With the exception of Zn at off-road locations (BOL1 = 0.76, BOL2 = 0.88, BOL3 = 0.88), BCF values across all metals and all sites remained below 1, indicating that *Linum usitatissimum* accumulates potentially toxic metals from soil into its tissues but does not meet the threshold criterion for hyperaccumulation, which requires BCF values exceeding 1 alongside tissue concentrations above 1000 mg/kg for Zn and Mn or 100 mg/kg for Cd and Pb (Anjum *et al.*, 2024).This pattern is ecologically significant because it confirms that *L. usitatissimum* functions as a bioindicator rather than a phytoremediator , it sensitively reflects the metal contamination status of the surrounding soil without accumulating metals to levels that would remove meaningful quantities from the environment. *L. usitatissimum* did not remove and concentrate metals in a harvestable biomass at the concentrations recorded in this study, so it cannot be considered a true phytoremediator to reduce soil contamination. Thus, its value is its potential to reflect spatial gradients of soil metal pollution as clearly shown by the consistently higher BCF values at the BRL sites than at the BOL sites, making it a reliable and cost-effective tool for environmental biomonitoring of roadside contamination in urban areas of Pakistan.

Regional evidence from Punjab supports results of the present study. Khan *et al.* (2023) reported that heavy metals can accumulate gradually in the organs of the body with the passage of time due to long half-life and cause adverse effects. Khan *et al.* (2023) observed that the concentration of Cu was significantly higher in the soil of the SW-irrigated site than in the soil of other irrigation sources in the Sargodha District. The PLI value of Cu was greater than one in all the samples which shows that they could cause health hazards with higher PLI values for SW than CW and TW. The bioconcentration factor was less than one which shows that a very small amount of Cu was accumulated in the edible part of the plant. This pattern of elevated PLI alongside sub-unity BCF values closely mirrors the findings of the present study, where total metal loading at BRL sites was consistently higher than at BOL sites, yet BCF values for *L. usitatissimum* remained below 1 across all metals and sites (Khan, Haider, *et al.*, 2023).

Overall, the results show that *L. usitatissimum* can effectively monitor the degree of environmental pollution and is sensitive to changes in soil metal. It is a potential bioindicator species for assessing the levels of heavy metal contamination in the environment along the roadside, according to its accumulation, correlation, and BCF values. However, as different metal ions have different capacities to accumulate, it is more satisfactory to check certain elements like Zn, Co and Cd.

5. Conclusions

The current study showed that the roadside soils of Sargodha–Bhalwal road were significantly contaminated with potentially toxic metals than off-road soils, mainly due to vehicular emissions and anthropogenic activities. Among the metals studied, Zn, Mn, Cu, Co, Pb and Cd were significantly higher in roadside sites with BRL1 having the highest levels. *L. usitatissimum* accumulated higher metal concentrations at roadside sites, particularly Zn, Mn and Fe, suggesting its sensitivity to spatial gradients of soil metal pollution.

Its ability to absorb and store some metals like Co and Zn also confirmed by bioconcentration factor (BCF) study, this makes it an excellent bioindicator species. The strong interaction among metals indicates that metals are commonly absorbed by plants and its uptake and bioavailability is mainly dependent on soil properties. Considering all thing, combining plant-based analysis and soil analysis is a reliable way to measure environmental pollution in roadside ecosystem.

Bioconcentration factor (BCF) analysis also confirms its ability to uptake and store metals mainly Co and Zn, which makes it as a good bioindicator species. The strong correlation among metals suggest the widespread uptake by metals and impact of soil properties on metal uptake by plants and their bioavailability. Overall study suggest that the combining plant-based analysis and soil analysis is a sound method to measure environmental pollution in roadside ecosystems. However, it is important to recognize that the employment of three replicate samples per site is a relatively small sample size that may limit the generalisability of the findings and future studies should incorporate larger sample sizes across multiple road types and seasons to strengthen the conclusions.

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Supplementary File

Table 1. LOD and LOQ for all 8 heavy metals — soil and plant (Linum) matrices (n = 10 blank replicates):

Metal	Wavelength (nm)	Soil LOD (mg/kg)	Soil LOQ (mg/kg)	Plant LOD (mg/kg)	Plant LOQ (mg/kg)	R^2
Zn	213.9	0.05	0.17	0.02	0.07	0.999
Cu	324.8	0.02	0.07	0.01	0.03	0.999
Co	240.7	0.005	0.017	0.003	0.010	0.999
Mn	279.5	0.03	0.10	0.02	0.07	0.999
Mo	313.3	0.005	0.017	0.003	0.010	0.999

Fe	248.3	0.50	1.67	0.10	0.33	0.999
Pb	217.0	0.003	0.010	0.002	0.007	0.999
Cd	228.8	0.001	0.003	0.0003	0.001	0.999

Table 2 Analysis of variance of concentration of heavy metals in soil samples

Source of Variance	DF	Zn	Cu	Co	Mn	Mo	Fe	Pb	Cd
Sites	2	8.80 ns	5.641 *	0.297 ns	45.158 ns	6.0025 ***	40952 *	0.364 ns	0.38234 ***
Locations	3	8139.65 ***	181.98 ***	23.3772 ***	280.562 ***	1.3456 ***	103985 **	71.3462 ***	9.09023 ***
Sites * Locations	6	111.65 ns	6.743 *	9.641 ***	0.212 ns	1.1025 ***	576 ns	4.396 ns	0.6058 ***
Error	32	70.44 ***	1.264 ***	0.5636 ***	17.934 ***	0.07324 ***	9502 ***	1.5562 ***	0.0245 ***

*ns= non-significant; **0.01 level of significant; *0.05 significant level; ***0.0001 level of significant*

Table 3. Concentration of heavy metals (mg/kg)in soil samples at roadside (BRL) and off-road (BOL) locations along Sargodha–Bhalwal road.

Sites	Locations	Zn	Cu	Co	Mn	Mo	Fe	Pb	Cd
Off-road	BOL1	16.38±0.05	12.32±0.005	2.12±0.01	61.94±0.01	0.87±0.01	529±0.8	0.57±0.01	0.19±0.005
(OR)	BOL2	12.38±0.05	21.08±0.005	1.77±0.01	74.95±0.02	0.67±0.01	369±0.02	0.34±0.008	0.11±0.005
	BOL3	11.88±0.05	14.38±0.005	2.3±0.01	78.95±0.02	1.87±0.01	527±0.02	0.51±0.02	0.15±0.005
Roadside	BRL1	30.88±0.05	17.16±0.008	2.98±0.02	97.94±0.01	1.05±0.02	679±0.02	0.40±0.01	0.24±0.005
(RS)	BRL2	29.38±0.05	16.73±0.005	2.62±0.01	92.95±0.03	1.17±0.01	449±0.02	0.65±0.01	0.18±0.005
	BRL3	27.58±0.05	13.22±0.005	2.22±0.01	91.00±0.03	0.03±0.008	447±0.02	0.95±0.01	0.97±0.005

Table 4. Analysis of variance of concentration of heavy metals in samples of *Linum.*

Source of Variance	DF	Zn	Cu	Co	Mn	Mo	Fe	Pb	Cd
Sites	2	46.69 *	0.819 ns	0.0529 ns	9.965 ns	0.000278 ns	43.45 **	0.000036 ns	0.000036 ns
Locations	3	3001.21 ***	50.7656 ***	4.8841 ***	909.224 ***	0.453378 ***	490.992 ***	0.000136 ns	0.003025 **
Sites * Locations	6	1.07 ns	6.275 ns	0.01 ns	31.547 *	0.001344 ns	38.172 **	0.01 ns	0.01 ns
Error	32	9.1 ***	1.8402 ***	0.10535 ***	5.129 ***	0.015249 ***	4.955 ***	0.000102 ***	0.000292 ***

*ns= non-significant; **0.01 level of significant; *0.05 significant level; ***0.0001 level of significant.*

Table 5. Concentrations of heavy metals in samples of *Linum* at roadside (BRL) and off-road (BOL) locations along Sargodha–Bhalwal road.

Sites	Locations	Zn (mg/kg)	Cu (mg/kg)	Co (mg/kg)	Mn (mg/kg)	Mo (mg/kg)	Fe (mg/kg)	Pb (mg/kg)	Cd (mg/kg)
Off-road (OR)	BOL1	12.48 ± 0.008	5.28 ± 0.008	0.82 ± 0.005	27.48 ± 0.005	0.07 ± 0.008	24 ± 0.005	0.017 ± 0.0005	0.008 ± 0.0005
	BOL2	11.58 ± 0.008	3.23 ± 0.008	0.09 ± 0.01	24.86 ± 0.005	0.03 ± 0.008	19 ± 0.005	0.03 ± 0.0005	0.004 ± 0.0005
	BOL3	10.48 ± 0.008	3.18 ± 0.008	0.49 ± 0.005	30.95 ± 0.005	0.04 ± 0.005	17 ± 0.005	0.03 ± 0.0005	0.003 ± 0.001
Roadside	BRL1	33.48 ± 0.008	6.98 ± 0.005	0.11 ± 0.008	36.30 ± 0.3	0.27 ± 0.005	32 ± 0.005	0.05 ± 0.0005	0.028 ± 0.0005
(RS)	BRL2	24.28 ± 0.008	5.98 ± 0.005	0.01 ± 0.003	35.83 ± 0.005	0.17 ± 0.005	31 ± 0.005	0.03 ± 0.0005	0.017 ± 0.0005
	BRL3	22.08 ± 0.008	3.98 ± 0.005	0.74 ± 0.01	28.72 ± 0.005	0.13 ± 0.008	29 ± 0.005	0.01 ± 0.0005	0.014 ± 0.0005