

## TRANSFER AND HEALTH RISK ASSESSMENT OF LEAD (Pb) ACROSS ENVIRONMENTAL AND BIOLOGICAL MATRICES IN WASTEWATER-IRRIGATED AGROECOSYSTEMS

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### Abstract

This research examined the distribution and transfer of lead (Pb) through environmental and biological matrices in agricultural areas of Bhalwal, Punjab (Pakistan), with special attention to the effects of their source of irrigation. Water, soil, cereal crops (*Triticum aestivum*, *Linum usitatissimum*, *Zea mays*, *Avena sativa*, and *Pennisetum glaucum*), and human blood samples were taken from sites irrigated with municipal wastewater (MWW), canal water (CW) and groundwater (GW). Pb concentrations were measured by atomic absorption spectrophotometry, and statistical analyses were used to explore spatial variability and correlation amongst the matrices. Pb concentrations in irrigation water were from 0.0002 to 0.016 mg/L and remained below the WHO permissible limit (0.5 mg/L). Pb concentrations in soil (24.1–32.5 mg/kg) were above the WHO limit (10 mg/kg), indicating significant contamination of wastewater-irrigated sites. Pb concentrations in cereal crops varied from 0.18 to 0.42 mg/kg and exhibited crop-specific and site-specific differences, with *L. usitatissimum* and *P. glaucum* accumulating higher Pb concentrations. Pb concentrations in human blood samples varied from 0.0003 to 0.006 mg/L, with the highest concentrations occurring at MWW sites. Pollution Load Index (PLI) values (2.97–3.99) were above unity, suggesting contamination, while Health Risk Index (HRI) values (0.48–0.62) were below 1.0, indicating no direct health risk.

**Key words:** Wastewater; Pollution; Heavy metal; Biomonitoring

### Introduction

Due to the variety of industrial and biological uses, metals have an important place in modern human society. Many metallic elements are essential micronutrients and participate in important biochemical and physiological processes, frequently playing roles as cofactors in enzymatic reactions, and are involved with maintaining cellular homeostasis (Xie *et al.*, 2018; Yi *et al.*, 2022). On the contrary, however, if present at excessive concentrations, metallic elements can cause toxicity, metabolic misregulation, and additional health disorders (Ugulu *et al.*, 2020).

Among the toxic metals, lead (Pb) is one of the most stable and abundant environmental pollutants (Siddique *et al.*, 2019). Lead occurs in nature as the mineral galena (PbS), and has been used in a variety of industrial products, including pipes, paints, batteries, and fuels. Lead remains in use because of its low melting point (327°C), high density, and corrosion resistance (Liang *et al.*, 2016). As a result of the amount of product produced using Pb, there is continuous release of lead into the environment. This continues to happen due to the presence of it in a variety of industrial effluents, vehicles, and improper waste disposal of products containing it, and collectively uses lead as a pathway in and to the atmosphere, soils, and water (Sahin *et al.*, 2016).

When exposed to the environment, Pb is highly persistent and mobile, allowing it to enter soil–plant–animal–human systems through complex biogeochemical channels (Khan *et al.*, 2020). In agricultural systems, the use of contaminated water sources for irrigation (such as municipal wastewater or industrial discharges) increases Pb input into soils where plants can take up Pb. Plants represent the first obvious entry point of heavy metals into the food web and can produce biomagnification at higher trophic levels (Nasreen *et al.*, 2021). Long-term exposure to Pb from contaminated food and water sources can cause serious adverse health effects, such as neurotoxic effects, nephrotoxic effects, and developmental delays in children (Liu *et al.*, 2023).

In Pakistan, the growing rate of water contamination from rapid urbanisation and industrialisation has heightened concerns about Pb contamination in agricultural systems. The Sargodha District in Punjab is a significant agricultural region where different irrigation sources, including municipal wastewater (MWW), canal water (CW), and groundwater (GW), are used for crop production. The quality variations of the irrigation sources affect the Pb uptake into the soils and cereal crops (Khan *et al.*, 2024).

The study presented in the following manuscript presents the distribution and bio-transfer of lead (Pb) in the soil-plant-human continuum in Sargodha District, Pakistan. Water, soil, and five common cereal crops (*Triticum aestivum*, *Linum usitatissimum*, *Zea mays*, *Avena sativa*, *Pennisetum glaucum*), as well as human tissue samples from the same region, were analysed for Pb concentrations. The study aimed to assess the levels of Pb contamination within these connected matrices and use the results to determine the level of health risk in humans from the consumption of Pb-contaminated crops and water. This research has important implications in determining potential pathways of contaminants in food webs, and highlights the environmental and public health risk within agricultural ecosystems that are receiving water from multiple irrigation sources.

## Material and Method

From April 2021 to May 2022, samples of water, soil, cereal crops, and human blood serum were collected from three agricultural sites in District Sargodha, Punjab, Pakistan. The selected sites (I, II, and III) were based on different irrigation sources, which were municipal wastewater (MWW) for site I, canal water (CW) for site II, and groundwater (GW) for site III. These three sources produced the majority of the irrigation in that district, and we wished to assess the potential impacts of that water quality on the accumulation of lead in the soil, plants, and subsequently into human tissue.

For cereal crop sampling, twenty-five samples of the crop species *Zea mays*, *Triticum aestivum*, *Linum usitatissimum*, *Avena sativa*, and *Pennisetum glaucum*, which are the five most widely raised cereal crop species, were sampled from each site. After careful collection into clean vessels at each site, the plants were washed thoroughly with deionised water to remove soil and dust, air dried at room temperature, and then oven dried at 75°C until achieving a constant weight. The dried plant material was finely ground using a stainless steel grinder, and circa two grams of the ground material was stored in air-permeable containers for future digestion.

Soil samples were gathered from the same locations at a depth of 0–15 cm using clean polyethene bags. Each sample of soil weighed approximately 2 grams. The samples were first air-dried for 72 hours at room temperature, followed by oven-drying at 80°C to remove residual moisture. Upon drying, the soil was gently ground, passed through a 2 mm mesh sieve and stored in sealed containers for digestion and analysis.

Human blood samples were collected from voluntary subjects living near the sampling locations. The study procedures were approved according to ethical standards based on the principles of the Helsinki Declaration. After being fully informed of the purpose and protocol of the study, the subjects signed an informed written consent form. Three millilitres of venous blood per subject were

collected in sterile syringes and placed into labelled tubes immediately. The blood samples were centrifuged at 3000 rpm for 15 minutes for serum separation, and the samples were stored in –20°C in polyethene tubes until digestion and analysis.

All of the collected samples underwent wet acid digestion based on the procedures by Qiu *et al.*, (2023) and Naseem & Muhammad (2022), modified slightly to improve digestion efficiency. The digestion resulted in the complete mineralisation of organic matter and the release of lead ions to solution. The digests were subsequently filtered through Whatman No. 42 filter paper and were taken to a known volume with deionised water before instrumental analysis.

Lead (Pb) concentrations in all matrices, i.e., water, soil, plant, and serum, were determined using an Atomic Absorption Spectrophotometer (AAS) with a hollow cathode lamp specific to Pb. The instrument was operated at a wavelength of 283.3 nanometers and slit width set to 0.7 nanometers and lamp current set to 6 milliamperes, with an air–acetylene flame during the atomization. Calibration of the AAS was conducted with standard Pb solutions formulated from certified reference materials (e.g., CEM 103 lead standard), and analytical accuracy was verified by analysing a blank and duplicate samples throughout the analysis.

**Statistical study:** The levels of Pb found in the samples were measured using ANOVA and SPSS software to determine correlations and variance (Khan *et al.*, 2023).

**Pollution load index (PLI):** Zhang *et al.*, (2023) calculated PLI by using this chemical formula:

$$PLI = \frac{\text{Value of metal (mg/kg) in the studied soil}}{\text{Metals reference values in soil}}$$

**Bioconcentration factor (BCF):** Cui *et al.*, (2004) used these calculations for BCF:

$$BCF \text{ soil cereal} = \frac{(\text{Metals content in cereals})}{(\text{Value of metals in soil})}$$

**Daily intake of metals:** The daily intake of metals (DIM) is calculated using the following formula:

$$DIM = M [\text{forage}] * CF * D \text{ food intake} / BW$$

whereas, M [fodder] = metal concentration. CF = Conversion factor.  $D_{\text{food intake}}$  = Daily ingested amount and BW = Average body weight (Chen *et al.*, 2021).

**Health risk index (HRI):** Estimation of HRI done using (Tegegne, 2015)

$$HRI = DIM / Rfd$$

**Enrichment factor (EF):** EF is determined by using following formula (Tegegne, 2015).

$$\text{Enrichment factor (EF)} = (\text{Metal content in cereals} / \text{Conc. of metal in soil}) \text{ sample} / (\text{Value of metal in crops} / \text{Conc. of metal in soil}) \text{ standard}$$

## Results

**Water Pb concentrations:** The Pb values in water samples showed a variation among the three sampling sites. The highest mean Pb concentration was observed at Site I, which was irrigated with municipal wastewater, with a mean value of  $0.016 \pm 0.0005$  mg/L (Table 1). The lowest concentration was detected at Site III, irrigated with groundwater, showing a mean value of  $0.002 \pm 0.0001$  mg/L. The trend of Pb concentration followed the order Site I > Site II > Site III. Statistical analysis indicated a highly significant difference among the sites (MS = 0.0221,  $p < 0.001$ ), confirming that the irrigation source influenced Pb levels in irrigation waters.

**Soil Pb concentrations:** The highest level of Pb at Site I, which was irrigated with municipal wastewater, was measured in *Linum usitatissimum* ( $32.55 \pm 0.38$  mg/kg), followed closely by *Avena sativa* ( $32.16 \pm 0.17$  mg/kg). The lowest value, however, was observed in *Zea mays* ( $20.70 \pm 0.39$  mg/kg) (Table 2). The sequence of Pb accumulation in soil at Site I was *L. usitatissimum* > *A. sativa* > *Triticum aestivum* > *Pennisetum glaucum* > *Z. mays*. At Site II, which was supplied with water from the canal, the Pb concentrations ranged from  $27.28 \pm 0.03$  mg/kg in *L. usitatissimum* to  $28.20 \pm 0.03$  mg/kg in *P. glaucum*. The order of decreasing Pb concentration at Site II was *P. glaucum* > *A. sativa* > *Z. mays* > *L. usitatissimum* > *T. aestivum*. Site III appeared to have lower levels of Pb in the soil relative to the two previous sites. However, *P. glaucum* still exhibited the maximum Pb concentration ( $25.24 \pm 0.10$  mg/kg) while *Z. mays* exhibited the minimum ( $24.13 \pm 0.14$  mg/kg). The sequence of order in concentration at Site III was *P. glaucum* > *T. aestivum* > *A. sativa* > *L. usitatissimum* > *Z. mays*. The Analysis of Variance (ANOVA) showed that the site and crop type had a significant effect on Pb accumulation in soils (Table 3). The mean squared (MS) values of the ANOVA Design for site were 183.06 ( $p < 0.001$ ), crop species were 1.879 ( $p < 0.001$ ), and site-plant interaction was 0.700 ( $p < 0.001$ ).

**Cereal Pb concentrations:** At Site I, supplied by municipal wastewater irrigation, *Pennisetum glaucum* had the maximum concentration of Pb ( $4.55 \pm 0.12$  mg/kg); followed by for *Zea mays* concentration of Pb ( $4.66 \pm 0.27$  mg/kg) and *Linum usitatissimum* ( $4.40 \pm 0.12$  mg/kg), while *Triticum aestivum* had the lowest Pb concentration ( $3.61 \pm 0.19$  mg/kg) (Table 4). Pb accumulation at Site I followed the order *P. glaucum* > *L. usitatissimum* > *Z. mays* > *Avena sativa* > *T. aestivum*. At Site II, where canal water was used for irrigation, Pb concentrations were lower (compared to Site I), from *A. sativa* ( $3.62 \pm 0.09$  mg/kg) to *L. usitatissimum* ( $2.37 \pm 0.12$  mg/kg) in the order of decreasing Pb concentrations order of *A. sativa* > *Z. mays* > *P. glaucum* > *T. aestivum* > *L. usitatissimum*. At Site III, being irrigated by groundwater had the overall lowest Pb concentrations in the crops sampled (again, there was an overall maximum concentration in *L. usitatissimum*  $2.53 \pm 0.09$  mg/kg to a minimum concentration in *A. sativa*  $1.33 \pm 0.05$  mg/kg). Pb accumulation followed the decreasing order of *L. usitatissimum* > *Z. mays* > *P. glaucum* > *T. aestivum* > *A. sativa* at Site III. ANOVA indicated that the factors of site and crop species were both significantly

different in Pb concentrations, with mean square (MS) square values of 19.604 ( $p < 0.01$ ) for site, 0.978 ( $p < 0.01$ ) for plant species, and 0.794 ( $p < 0.001$ ) for the site-plant interaction (Table 5).

**Table 1. Pb levels in water samples (mg/L) (Mean  $\pm$  S.E.).**

Site	Site I (MWW)	Site II (CW)	Site III (GW)
Mean Pb value $\pm$ S.E.	$0.016 \pm 0.0005$	$0.006 \pm 0.0003$	$0.002 \pm 0.0001$
MS	0.0221***		

\*, \*\*, \*\*\*= significant at 0.05, 0.01 and 0.001 level. ns = non-significant

**Table 2. Pb levels in soil samples (mg/kg) (Mean  $\pm$  S.E.).**

Crop	Site I (MWW)	Site II (CW)	Site III (GW)
<i>T. aestivum</i>	$22.73 \pm 0.45$	$17.23 \pm 0.30$	$16.09 \pm 0.24$
<i>L. usitatissimum</i>	$32.55 \pm 0.38$	$27.28 \pm 0.03$	$18.2506 \pm 0.01$
<i>Z. mays</i>	$20.70 \pm 0.39$	$27.30 \pm 0.03$	$24.13 \pm 0.14$
<i>A. sativa</i>	$32.16 \pm 0.17$	$28.03 \pm 0.22$	$25.45 \pm 0.17$
<i>P. glaucum</i>	$28.72 \pm 0.08$	$17.29 \pm 0.11$	$25.24 \pm 0.10$

**Table 3. ANOVA for Pb values in soil.**

Variables	Df	MS
Site	2	183.06***
Plant	4	1.879**
Site*Plant	8	0.700***
Error	30	0.429

\*, \*\*, \*\*\*= Significant at 0.05, 0.01 and 0.001 level. Ns = Non-significant

**Table 4. Pb levels in crop samples (mg/kg) (Mean  $\pm$  S.E.).**

Crop	Site I (MWW)	Site II (CW)	Site III (GW)
<i>T. aestivum</i>	$3.61 \pm 0.19$	$2.38 \pm 0.19$	$1.62 \pm 0.31$
<i>L. usitatissimum</i>	$4.40 \pm 0.12$	$2.37 \pm 0.12$	$2.53 \pm 0.09$
<i>Z. mays</i>	$4.66 \pm 0.27$	$3.22 \pm 0.40$	$2.44 \pm 0.16$
<i>A. sativa</i>	$3.70 \pm 0.13$	$3.62 \pm 0.09$	$1.33 \pm 0.05$
<i>P. glaucum</i>	$4.55 \pm 0.12$	$2.49 \pm 0.07$	$1.65 \pm 0.07$

**Table 5. ANOVA for Pb values in crops.**

Variables	Df	MS
Site	2	19.604**
Plant	4	0.978**
Site*Plant	8	0.794***
Error	30	0.108

\*, \*\*, \*\*\*= Significant at 0.05, 0.01 and 0.001 level. Ns = Non-significant

**Human blood Pb concentrations:** Lead (Pb) levels found in blood serum samples were clearly reduced across the three sampling sites (Fig. 1). Site I indicated the highest mean Pb concentration (0.006 mg/L), where municipal wastewater was used for agricultural irrigation, while the lowest Pb concentration was observed in Site III (0.0003 mg/L), which utilised groundwater. Pb concentrations at Site II, which received canal water, were intermediate between Sites I and III. The overall order of Pb concentrations in human blood serum was Site I > Site II > Site III, closely representing the calculated contamination gradient of Pb in irrigation water, soil and cereal crops.

Despite differences in mean values, correlation analysis indicated no evidence of statistically significant relationships between Pb concentrations in soil, cereal crops, or blood serum at any of the monitored sites. The coefficient of correlation values of soil and cereal samples were 0.152, 0.039, and -0.452 for sites I, II, and III, respectively. Correlation values of cereal crops and blood serum were -0.501, -0.209, and -0.025 were all non-significant ( $p > 0.05$ ) (Table 6). These findings suggest that, though environmental Pb exposure varies from site to site, there was no strong evidence of direct transfer from contaminated soil or crops into the blood serum of the examined individuals.

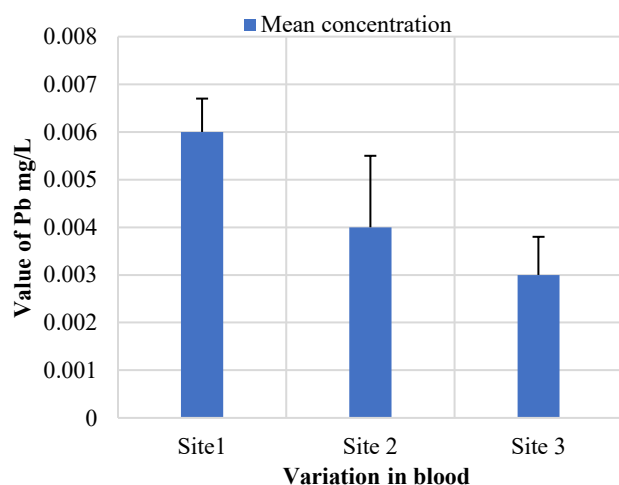


Fig. 1. Pb values in blood serum samples.

**Table 6. Correlation between samples of soil, cereal and blood serum.**

Site	Soil X Cereal	Cereal X Blood
I	0.152 <sup>ns</sup>	-0.501 <sup>ns</sup>
II	0.039 <sup>ns</sup>	-0.209 <sup>ns</sup>
III	-0.452 <sup>ns</sup>	-0.025 <sup>ns</sup>

\*, \*\*, \*\*\*= Significant at 0.05, 0.01 and 0.001 level. ns = Non-significant

**Table 7. Pollution load index of soil for Pb.**

Crop	Site I (MWW)	Site II (CW)	Site III (GW)
<i>T. aestivum</i>	3.89	3.34	3.07
<i>L. usitatissimum</i>	3.99	3.34	2.97
<i>Z. mays</i>	3.76	3.35	2.96
<i>A. sativa</i>	3.94	3.43	3.12
<i>P. glaucum</i>	3.89	3.47	3.09

**Table 8. Bioconcentration factor of Pb.**

Crop	Site I (MWW)	Site II (CW)	Site III (GW)
<i>T. aestivum</i>	0.11	0.08	0.06
<i>L. usitatissimum</i>	0.13	0.08	0.10
<i>Z. mays</i>	0.15	0.11	0.10
<i>A. sativa</i>	0.11	0.12	0.05
<i>P. glaucum</i>	0.14	0.08	0.06

**Table 9. Enrichment Factor of Pb.**

Crop	Site I (MWW)	Site II (CW)	Site III (GW)
<i>T. aestivum</i>	0.31	0.24	0.18
<i>L. usitatissimum</i>	0.37	0.24	0.28
<i>Z. mays</i>	0.42	0.32	0.28
<i>A. sativa</i>	0.31	0.35	0.14
<i>P. glaucum</i>	0.39	0.24	0.18

**Table 10. Daily intake of metal Pb.**

Crop	Site I (MWW)	Site II (CW)	Site III (GW)
<i>T. aestivum</i>	0.001	0.00126	0.0008
<i>L. usitatissimum</i>	0.002	0.0012	0.0012
<i>Z. mays</i>	0.0024	0.0017	0.0013
<i>A. sativa</i>	0.0019	0.0019	0.00071
<i>P. glaucum</i>	0.002	0.0013	0.0008

**Table 11. Health Risk Index of Pb.**

Crop	Site I (MWW)	Site II (CW)	Site III (GW)
<i>T. aestivum</i>	0.48	0.31	0.21
<i>L. usitatissimum</i>	0.58	0.315	0.33
<i>Z. mays</i>	0.62	0.42	0.32
<i>A. sativa</i>	0.49	0.48	0.17
<i>P. glaucum</i>	0.60	0.33	0.22

**Pollution load index:** Maximum PLI for soil was observed in *L. usitatissimum* (3.99 mg/kg), and the lowest concentration was found in *Z. mays* (2.97 mg/kg) (Table 7). At site I, trend of PLI was *L. usitatissimum* > *A. sativa* > *T. aestivum* > *P. glaucum* > *Z. mays*. At site II, the command of PLI was *P. glaucum* > *A. sativa* > *Z. mays* > *L. usitatissimum* > *T. aestivum*. At site III, the instruction of PLI was *A. sativa* > *P. glaucum* > *T. aestivum* > *L. usitatissimum* > *Z. mays*.

**Bioconcentration factor:** Maximum BCF for soil was observed in *Z. mays* (0.15) and the lowest level was found in *T. aestivum* (0.064) (Table 8). At site I the directive of BCF was *Z. mays* > *P. glaucum* > *L. usitatissimum* > *A. sativa* > *T. aestivum*. At site II, the trend of BCF was *A. sativa* > *Z. mays* > *P. glaucum* > *T. aestivum* > *L. usitatissimum*. At site III, the demand of BCF was *L. usitatissimum* > *Z. mays* > *P. glaucum* > *T. aestivum* > *A. sativa*.

**Enrichment factor:** The highest EF value for soil was observed in *Z. mays* (0.42), and the lowest level was found in *T. aestivum* (0.180) (Table 9). At site I, the trend of EF was *Z. mays* > *P. glaucum* > *L. usitatissimum* > *A. sativa* > *T. aestivum*. At site II, the trend of EF was *A. sativa* > *Z. mays* > *P. glaucum* > *T. aestivum* > *L. usitatissimum*. At site III, the directive of EF was *L. usitatissimum* > *Z. mays* > *P. glaucum* > *A. sativa* > *T. aestivum*.

**Daily intake of metal:** The highest level of DIM for soil was observed in *Z. mays* (0.002), while the lowest concentration was found in *T. aestivum* (0.0008) (Table 10). At site I, the order of EF was *Z. mays* > *P. glaucum* > *L. usitatissimum* > *A. sativa* > *T. aestivum*. At site II, the edict of EF was *A. sativa* > *Z. mays* > *P. glaucum* > *T. aestivum* > *L. usitatissimum*. At site III, the order of EF was *Z. mays* > *L. usitatissimum* > *P. glaucum* > *T. aestivum* > *A. sativa*.

**Health risk index (HRI):** Maximum HRI for soil was observed in *Z. mays* (0.62). Minimum concentration was found in *T. aestivum* (0.48) (Table 11). At site I, the directive of HRI was *Z. mays* > *P. glaucum* > *L. usitatissimum* > *A. sativa* > *T. aestivum*. At site II, the order of HRI was *A. sativa* > *Z. mays* > *P. glaucum* > *T. aestivum* > *L. usitatissimum*. At site III, the direction of HRI was *L. usitatissimum* > *Z. mays* > *P. glaucum* > *T. aestivum* > *A. sativa*.

## Discussion

The findings of this study indicate clear spatial differences in Pb levels in environmental and biological materials, demonstrating a role of irrigation sources in the accumulation of Pb and the potential for transfer through the food chain. The Pb level in the water used for irrigation varied from 0.0002 to 0.016 mg/L, which is less than the observations made by Ahmad *et al.*, (2020) of 0.14 to 0.30 mg/L in similar agricultural systems. All of the concentrations measured were below the WHO acceptable levels for drinking water (0.5 mg/L). However, all measured Pb concentrations showed a consistent trend of higher levels in sites irrigated with municipal wastewater.

The concentrations of Pb in soils varied between 24.1 and 32.5 mg/kg, which is significantly higher than findings by Kanwal *et al.*, (2020), who found Pb concentrations in agricultural soils of Punjab were found to be 2.08-16.57 mg/kg. The present soil Pb values were also above the WHO permissible limit of 10 mg/kg, which gives evidence of significant Pb contamination in sites examined, especially for sites irrigating with municipal wastewater. The issue with elevated soil Pb concentrations is that they can disrupt soil fertility and contribute to metal accumulation in plants, which ultimately has consequences on product quality and safety (Ugulu, 2015). Chronic exposure to Pb through contaminated soil and subsequently food has been linked to several health-related issues, including anaemia virus neurological malfunction, and kidney injuries (Chen *et al.*, 2021; Zhu *et al.*, 2022). The European Union (EU, 2002) reported a maximum Pb concentration of 300 mg/kg as the critical limit for heavily polluted soils, while the consistently high values observed in this study reflect localised contamination pressure from urban emissions and wastewater irrigation as opposed to background levels associated with naturally occurring soils (Ross, 2009).

The Pb concentration in cereals ranged from 0.18 to 0.42 mg/kg, which is lower than the concentrations reported for edible crops in previous studies carried out around industrial sites. Elevated levels of Pb in edible crops near industries have been attributed to direct atmospheric deposition and contaminated irrigation (Ahmad *et al.*, 2020). Current results support previous findings that the level of Pb in cereals is affected by the source of irrigation and the levels of Pb in associated soil. *Linum usitatissimum* and *Pennisetum glaucum* accumulated more Pb than *Triticum aestivum* and *Avena sativa*, suggesting that cereals differ in their absorption and translocation capabilities for heavy metals. This may relate to physiological characteristics, including root structure, transpiration rate, and root affinity for Pb and components of root exudates and cell walls, respectively (Munir *et al.*, 2019). The increased level of Pb within grains as influenced by using wastewater suggests an additional pathway for Pb to enter the human food chain, even in instances where the Pb concentration within the water is below regulatory levels.

Blood serum Pb concentrations in our study were found to be between 0.0003 to 0.006 mg/L, lower than human blood Pb concentrations found by López *et al.*, (2008), which were greater in blood samples from populations with exposure to industries through their environments. This relatively low level of blood Pb suggest that some, although not greatly, Pb exposure from the environment may be occurring; and food and nutrition variability and physiological conditions coupled with minimal intakes of locally grown cereals may limit Pb bioaccumulation. Zhao *et al.*, 2024 reported that, the established trend in blood Pb, where samples tested had higher Pb levels from those living in wastewater-irrigated areas, supports our working hypothesis of a gradual process of Pb transfer to humans through the soil–plant–human continuum.

The Pollution Load Index (PLI) values in this study, which had a range of 2.97 to 3.99, are above the critical value of 1, which indicates that the soils and crops from the site are contaminated with Pb. Since PLI values above 1 indicate cumulative metal accumulation representing potential ecological risk, and values below 1 indicate low contamination (Rai *et al.*, 2019), the elevated PLI values in this study corroborate anthropogenic input of Pb in the agricultural space. Similarly, the calculated Health Risk Index (HRI) values for Pb were also less than the critical value of 1.0 (ranging from 0.48 to 0.62), which indicates that the current risk from Pb exposure through annual consumption of cereals does not present an immediate health risk to the local population at this time (Liang *et al.*, 2016). That being said, Pb measured at a detectable level from the samples of all the matrices studied (water, soil, cereal, and blood) indicates ongoing exposure and signifies the possibility of bioaccumulation from long-term exposure through time.

## Conclusion

The findings of this study underscore the interconnected nature of Pb contamination in agricultural systems. Municipal wastewater irrigation was identified as the primary source of Pb input, leading to elevated soil concentrations and subsequent accumulation in cereals and human tissues. Although the present Pb levels in water and crops are within permissible limits, the consistent contamination gradient from water to soil and crops points toward a cumulative effect that could intensify over time. Sustainable irrigation management, regular monitoring of heavy metals, and implementation of wastewater treatment measures are therefore essential to safeguard environmental and public health in the Sargodha and comparable ecosystems.

**Conflict of Interest:** All participants declare that there is no conflict of interest

**Data Availability:** All the generated data of this study are included in the manuscript.

**Author Contribution:** K.S: experimental work, Z.I.K.: supervisor, K. A: resource management, F. Z.: manuscript writing, N.R: manuscript editing, A.A: tables and figures editing, I.R. N.: software and statistical analysis, M.I.A: manuscript revision, I.U: data validation

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