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EMPIRICAL TRACKING OF HEAVY METAL TRANSFER FROM SEWAGE-IRRIGATED SOILS TO SPINACH (*SPINACIA OLERACEA* L.) AND IT'S BIOACCUMULATION IN HUMAN CONSUMERS IN SARGODHA, PAKISTAN

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Abstract

Vegetables are crucial dietary sources of vitamins, proteins, and carbohydrates, important for human health. Plants growing in soil were irrigated with sewage water are the health threats for human beings. Research was under taken to evaluate the metal contamination levels in soil, irrigation water, and spinach (*Spinacia oleracea* L.) cultivated along Faisalabad and Lahore roads in Sargodha, Pakistan. The water, soil, and vegetable samples were collected from canal water (CW1, CW2) and water sites (SW1, SW2). Electrical conductivity and sodium absorption ratio levels were considerably higher while residual sodium carbonate values rose in sewage water samples showing poor water quality. Water type SW2 showed the highest levels of heavy metals especially copper, iron, zinc, manganese, cadmium, cobalt, lead, and molybdenum. Soil treated with sewage water had very high salt content, poor nutrient quality, and high metal concentrations than soil irrigated through canals. The assessment tools including Daily Intake of Metals (DIM) and Health Risk Index (HRI) show that sewage water caused harmful effects because of high contamination with heavy metals. The research shows heavy metal pollution needs control through better irrigation methods and water treatment plus strong regulatory oversight

Key words: Vegetables; Heavy metal contamination; Sewage water irrigation; Spinach; Sargodha

Introduction

Vegetables are considered the protective foods and their sources of vitamins and minerals, carbohydrates and proteins (Prajapati *et al*., 2024). Several vegetables are known for their therapeutic effects, particularly in reducing the impact of diseases (Ahmed *et al*., 2021, Ahmad *et al*., 2026). Intake of vegetables within Pakistani population is 400 g/capita/day (Chen *et al*., 2022). The yield of vegetables is still low compared to other countries (Nimgarri *et al*., 2023). More vegetables are eaten because they contain some nutrients factors that are considered essential for human being and are classified under protective foods because these foods are believed to help prevent diseases among human beings (Yahia *et al*., 2017). The vegetables cultivated in the fields are infected by the heavy metals and nearby the sources of heavy metals contamination may possibly contain more than the other vegetables (Zwolak *et al*., 2019). Therefore, heavy metals are quickly deposit on the surface of vegetables and spread to different tissues of vegetables. It was relatively easier to the study about the role of leafy vegetables, where root and leaf contain a higher concentration of heavy metals than stem and fruits (Leblebici *et al*., 2020).

Spinach (*Spinacia oleracea* L.) is a vegetable in the Amaranthaceae family, first used by the Chinese in 647 A.D. It is classified into smooth-leaf, savoy, and semi-savoy types, with smooth-leaf commonly used in salads and processing, and savoy and semi-savoy for cooking (Ramaiyan *et al*., 2020). Spinach is rich in water (91.4%) and low in calories, with 2.9% protein, 3.6% carbohydrates, and 0.4% fat. Around 82 varieties exist, including Amsterdam Giant, Bloomsdale, and Viroflay. China is the leading producer, accounting for 90% of global production, followed by the U.S., Japan, Turkey, Indonesia, and France. Despite being the second-largest producer, U.S. consumption remains low compared to other leafy greens like lettuce and broccoli (Kumar *et al*., 2020: Nazar *et al*., 2026). In 2019, the average U.S. per capita consumption was 0.91 g/day of fresh spinach and 0.49 g/day of processed spinach, growing from 0.81 kg per person in 1970 to 1.17 kg in 2019.

Heavy Metals as specifically defined are metals that have a density of more than 5 gcm^{-3}. These are the most toxic food pollutants and have emerged with importance due to their detrimental effect to nature (Khalef *et al*., 2022). The toxicities of varying heavy metals could be attributed to the ability of the heavy metal to form stable coordinated compounds with various organic and inorganic ligands which then entail biological hazards even with very little concentrations (Egorova & Ananikov, 2017). The plants are heavy metals accumulators and excluders (Napoli *et al*., 2019). Phytore- mediation technologies such as phytoextraction, phytostabilization, rhizofiltration and phytovolatilization have been used to take of heavy metal by plants (Nedjimi, 2021). Information that is publicly

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accessible indicate that the form of Cd that travels in the xylem sap may bind with small molecules such as organic acids (Sterckeman & Thomine, 2020).

The metals may dissolve in the surface and ground water, soil and other crops plants (Bello *et al*.,2023). Through the variation of pigment concentration, water content, dry weight and growth, plants respond differently in relation to the nutrient and metal concentration (Naser *et al*., 2018). Heavy metals have attracted Global concern as they are accumulated in living organisms (Mosalem *et al*., 2024). This fact makes it one of the most severe environmental problems worldwide when present in agricultural soils and vegetables (Rahman & Singh, 2019: Ghafar *et al*., 2022). Some of the heavy metals get accumulated in the kidneys and livers of humans, once people consume contaminated foods (Pandey & Madhuri, 2014).

The primary necessity of life is water. By 2050, the generation and discharge of industrial wastewater to the environment will be doubled and this will put more pressure on availability of fresh water source (Hassan *et al*., 2023: Rahimoon *et al*., 2025). According to another report, the demand of water in the global scene will further grow up to a maximum of 55% in 2050. Most of these water sources are being degraded, and according to statistic nearly a quarter of the earth aquatic systems are over exploited (Gallo *et al*., 2016). This population growth could have many major effects on the fresh water and its availability and use to several related sectors (Lytton *et al*., 2021). Technical water resources for irrigation may get to irrigate forty million hectares of land area that is 15% of the area of cultivated land (Duarte *et al*., 2022). However, the current average annual water supply per capita in the Indus Basin is below 1000 cubic meters per year, so the problem of water scarcity in this basin may worsen (Habib, 2021). Because of the acute energy crisis, groundwater pumping for soil irrigation is expensive and unpredictable (Lytton *et al*., 2021).

The majority of the groundwater in different areas of Pakistan is of low quality because of elevated levels of soluble salts and harmful metal elements (Ghafar *et al*., 2021: Rashid *et al*., 2022). The presence of hazardous metals is the main issue with using wastewater for land irrigation (Kesari *et al*., 2021). Toxic metal levels in wastewaters have increased as a result of the widespread use of these metals in many household items and industrial processes nowadays. Research has outlined the potential risks associated with the accumulation of hazardous metals in topsoil that is watered by wastewater (Mahfooz *et al*., 2020). In general, using wastewater enhances the organic matter and structure of the soil, which raises its hydraulic conductivity (Ghafar *et al*., 2019: Ibrahimi *et al*., 2022).

The objective of this study is to measure heavy metal concentrations in soil and water in Sargodha City, examine heavy metal accumulation in selected vegetable crops, assess the physical and chemical characteristics of soil and water samples and evaluate the potential risks.

Materials and Methods

Study area: The study was conducted along the Faisalabad (Southwest) and Lahore Road (Northwest) in Sargodha, Pakistan. Sargodha, located at latitude 32.08586 and longitude 72.67418 in Pakistan, is known for its agricultural significance (Fig. 1).

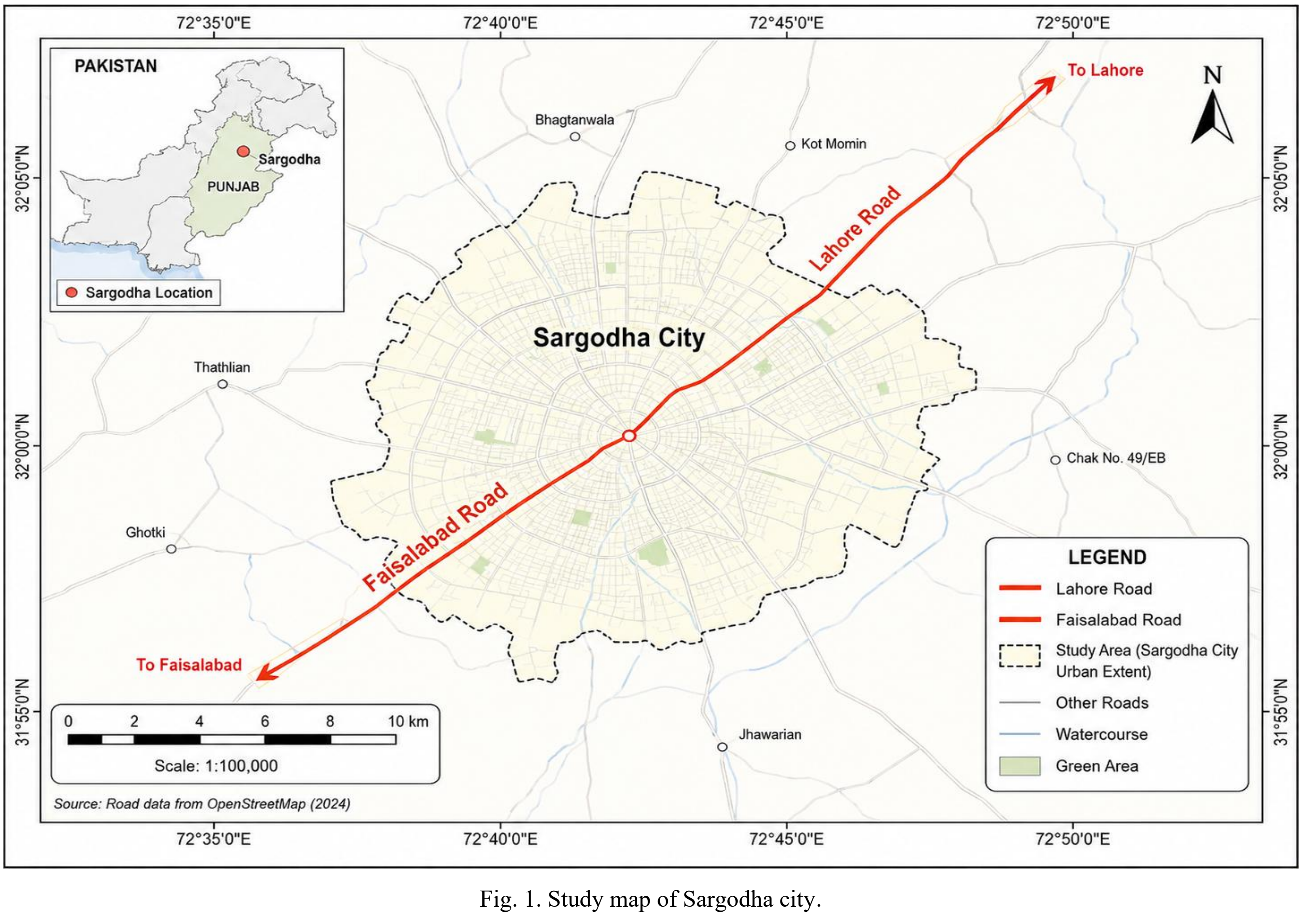

Fig. 1. Study map of Sargodha city.

Sample collection: Water, soil, and spinach samples were collected from different locations for analysis (Table 1). Water, Soil and Vegetable Sampling.

Table 1. Area of study sites for sample collections.

Site	Tehsil	Location	Irrigation source
CW 1	Sargodha	Lahore Road	Canal water
SW 1	Sargodha		Sewage water
CW 2	Sargodha	Faisalabad Road	Canal water
SW 2	Sargodha		Sewage water

Four water samples (5 ml each) were taken from canal and sewage water, stored in labeled plastic bottles at room temperature. Soil samples were gathered from two irrigated sites (canal and sewage water) with each sample weighing 1 kg. Three replicates of each soil sample were air-dried and then oven-dried at 70-75°C for five days to remove moisture, crushed into fine powder, and stored in 1 g polythene bags for testing. Vegetable samples were collected from two locations, totaling 4 samples with three replicates from each site. The vegetables were washed with distilled water, air-dried, oven-dried at 70-75°C for three days, ground into a fine powder, and stored in 1 g containers for examination. Human Blood was collected from sites of collection from the local populations using spinach for cooking.

Drying and grinding of the soil and vegetable samples: The collected soil and vegetable samples were dried and ground for further analysis following standard protocols. Soil samples were air-dried in wooden trays, then ground with a wooden pestle and mortar to reduce heterogeneity and enhance surface area for chemical reactions. The ground soil was sieved through a 2 mm mesh to obtain a uniform texture and stored in labeled plastic zipper bags. Vegetable samples were air-dried in a well-ventilated area at room temperature to remove moisture, then oven-dried at low temperature until a constant weight was achieved. The dried vegetables were ground using a mortar and pestle, although a mechanical grinder or mill. The processed samples were weighed and stored in labeled plastic zipper bags for analysis (Ryan, 2024).

Heavy metal analysis and Statistical analysis: Both soil and plant samples were digested using a standard acid digestion method. Flame atomic absorption spectrophotometer (FAAS) model AA 6300 Shimadzu, was employed for measuring specific heavy metals. A total of 8 heavy metals (Zn, Cu, Co, Mn, Mo, Fe, Pb and Cd) were analyzed in each sample. Statistical analyses, including mean comparisons and correlation coefficients, were performed using SPSS, Minitab, and Graph Pad prism, PAST software. The results were compared with permissible limits set by international standards (Table 2).

Analysis of indices: Several indices were calculated and interpreted to evaluate contamination risk, metal contamination levels and potential toxicity in the study area (Halawani *et al*., 2022). The indices used for this assessment are listed below:

Daily intake of metals (DIM)

According to Shahid *et al*., (2015), there are a variety of methods for heavy metals to accumulate in Individual body, including through skin contact, inhaling, and eating contaminated vegetables. The daily Consumption of heavy metals is calculated using the formula below:

$$\text{DIM} = \frac{\text{C x F x D food intake}}{\text{W}}$$

C represents the concentration of metal in plants, F represents conversion factor, D represents vegetables intake per day, W is Average body weight of humans.

Daily intake of metal.

$D_{\text{food intake}}$	Conversion factor	Average body weight
31.5 g/person/day*	0.085**?	70 kg*

(Jan *et al*., 2018)

Health risk index (HRI): In this investigation, HRI was utilized to estimate the potential metal exposure that might happen if humans ate vegetable samples. The daily metal intake (DIM) in vegetable products divided by the oral reference dose is used to determine the HRI (Shehu *et al*., 2023).

$$\text{HRI} = \frac{\text{Daily intake of metals (DIM)}}{\text{Oral reference dose (RfD)}}$$

Contamination factor (CF): Based on the contaminant quantities in the sample's contents and their background concentration, the contamination factor (Cf) can be utilised to measure the pollution status of contaminants within the soil (Rahman *et al*., 2022).

The Cf was calculated according to the following formula:

$$C_f = \frac{C_m \text{ Sample}}{C_m \text{ Background}}$$

Where; 'C_m sample' indicate to the metal content in the soil 'C_m background' is the metal concentration from a natural Source.

Table 2. Permissible limits of heavy metals in water, soil and plants.

Heavy metals	Permissible limits in water (mgL^{-1})	Permissible limits in soil ($mgKg^{-1}$)	Permissible limits in plants ($mgKg^{-1}$)
Cd	0.1	3-6	0.47
Cu	2	100	25.8
Co	0.05	750	0.46
Cr	0.1	11	2.74
Pb	0.1	200	2.8
Mn	0.2	100-300	137.3
Ni	2	50	5.02
Zn	5	300	13.5
Fe	5	50,000	968.25

Result

Physicochemical properties of water from two sites used for spinach cultivation: Electrical conductivity reached its minimum value at CW1 with a reading of 310.97 µS/cm yet CW2 recorded 417.95 µS/cm. The water quality measurements at SW1 and SW2 demonstrated higher values through electrical conductivity levels reaching 1370.95 µS/cm and 1454.62 µS/cm respectively. Wastewater-irrigated sites displayed increased levels of calcium and magnesium (Ca^{+2}, Mg) where SW1 reached 6.45 meq/L and SW2 measured 6.75 meq/L yet CW1 and CW2 possessed lower readings of 2.47 meq/L and 2.72 meq/L respectively. Na levels displayed significant differences between CW1 and CW2 because they held 0.38 meq/L and 0.42 meq/L but SW1 and SW2 contained much higher concentrations reaching 12.38 meq/L and 12.65 meq/L.

The measurement of bicarbonate (HCO_3) showed CW1 (2.38 meq/L) and CW2 (2.65 meq/L) contained lower concentrations than SW1 (4.65 meq/L) and SW2 (4.75 meq/L). The analysis of Chloride (Cl) revealed that CW1 contained the smallest amount at 0.02 meq/L while CW2 had 0.35 meq/L followed by SW1 and SW2 with substantial concentrations at 11.48 meq/L and 12.15 meq/L. Sodium adsorption ratio values were among the lowest at CW1 (0.27) and CW2 (0.31) but SW1 (11.92) and SW2 (12.4) showed substantial risks due to sodium hazards. The residual sodium carbonate (RSC) values at CW1 and CW2 were 0.02 meq/L and 1.13 meq/L respectively while SW1 and SW2 showed higher values of 3.65 meq/L and 2.65 meq/L respectively (Table 1).

Physicochemical properties of soil collected from two sites for spinach cultivation: Soil analyses conducted at two spinach cultivation sites exhibited major differences within their physiological and chemical attributes. Soil electrical conductivity measurements from sewage water-irrigated sites (SW1 and SW2) reached 16.66±0.06 mS/cm and 16.36±0.06 mS/cm while measurements from canal water-irrigated sites (CW1 and CW2) remained at 3.55±0.07 mS/cm and 3.83±0.06 mS/cm. Soil P ranged from 7.4±0.1 mg/kg at CW1 up to 8.65±0.12 mg/kg at SW2.

The evaluation of potassium levels demonstrated that sites irrigated with sewage water contained elevated potassium with SW2 levels exceeding others at 126.95±1.15 mg/kg yet CW2 recorded the lowest concentration at 70.95±1.15 mg/kg. The soils receiving sewage water irrigation displayed elevated organic matter levels where SW1 and SW2 collectively showed 1.38±0.01% and 1.29±0.01% respectively while the conventional water irrigation sites had lower values 0.72±0.01% and 0.79±0.01%. The saturation percentages of the soils demonstrated parallel trends as SW1 and SW2 exhibiting 46.95±1.15% and 41.95±1.15% whereas CW1 and CW2 displayed lower saturation values at 37.95±1.15% and 32.62±1.76%. Soil properties experience noticeable changes under sewage water irrigation which may influence the growth of spinach crops (Fig. 3; Table 3).

Pearson correlation analysis of heavy metals in irrigation water showed sound association patterns representing common sources and geochemical behavior. Strong positive correlations were observed among Cu, Co, Mn, Zn, and Cd ($r \approx 0.86$–1.00), suggesting a shared anthropogenic activity, primarily linked to wastewater discharge and urban runoff. Iron showed moderate to strong positive correlations with Zn and Mn ($r \approx 0.69$–0.83), reflecting mixed control from both anthropogenic inputs and natural geogenic processes. In contrast, Pb exhibited weak to moderate negative correlations with most metals, indicating a distinct source or geochemical behavior; however, Pb showed a strong positive correlation with Mo ($r \approx 0.82$), implying a separate but common contamination pathway. Generally, two dominant metal groups were identified: a Cu–Zn–Mn–Co–Cd cluster associated with wastewater-derived contamination and a Pb–Mo association representing an independent source, showing that were consistent with PCA and HCA results and highlight sewage irrigation as the major driver of heavy-metal enrichment in the study area.

Generally, the correlation structure indicates two dominant metal associations: (i) a Cu–Zn–Mn–Co–Cd cluster associated with wastewater-derived contamination (ii) a Pb–Mo association representing a separate contamination pathway. These results are consistent with the multivariate analysis (PCA and HCA), which further confirmed the role of irrigation source, particularly sewage water, as the primary driver of heavy-metal enrichment in the studied area (Fig. 5).

Metal concentration (mg/kg) in soil samples: Data findings showed that heavy metals were present at higher concentrations in sewage water-irrigated (SW) soil compared to canal water-irrigated (CW) soil. The concentrations of 8 heavy metals including Cu, Fe, Zn, Mn, Cd, and Pb proved to be substantially higher in SW soils as indicated by SW1 exhibiting 1.993 mg/kg of Cu while CW1 displayed 0.094 mg/kg. Fe content reached 1.962 mg/kg the highest value in agricultural lands which received SW irrigation but CW agricultural sites measured only 0.93 mg/kg of Fe. The SW soils exhibited higher concentrations of Zn, Mn and Pb compared to CW soils with SW1 presenting Pb levels at 2.748 mg/kg that exceeded double those of CW soils. The SW soils possessed the highest Cd concentrations reaching 1.099 mg/kg whereas the CW soils contained the lowest Cd level of 0.005 mg/kg in CW2. The concentrations of Co and Mo were detected in minimal amounts but subtle variations were observed in Co among the different sites (Fig. 2; Table 4).

Metal concentrations (mg/kg) in spinach samples: Our results showed that plants grown with sewage water irrigation (SW) accumulated substantially higher amounts of heavy metals than those receiving canal water irrigation (CW). The copper content in SW1 (1.747 mg/kg) and SW2 (1.558 mg/kg) exceeded the levels in CW1 (0.077 mg/kg) and CW2 (0.087 mg/kg). Studies revealed that SW sites contained iron concentrations between 1.581 mg/kg which exceeded the range of CW site measurements that reached only 0.31 mg/kg. Spinach from SW sites contained greater concentrations of Zn and Mn than CW sites according to analytical results SW spinach with 1.091 mg/kg Zn and 0.02 mg/kg Mn compared to CW spinach. The concentration of Cd in SW water samples reached up to 0.093 mg/kg while remaining at only 0.019 mg/kg in CW water samples. The analysis showed that SW sites contained higher cobalt concentrations reaching up to 0.086 mg/kg while CW spinach displayed minimal cobalt levels starting at 0.008 mg/kg. The levels of Lead (Pb) exceeded other metals in sewage-irrigated spinach with SW1 showing results of 0.935 mg/kg while CW1 tested at 0.155 mg/kg. A trace amount of Molybdenum (Mo) occurred at all sampling locations throughout the study (Fig. 4; Table 5).

Analysis of heavy metals in spinach exhibited several strong to moderate positive correlations, representing a common source of contamination and similar uptake pathways. Specifically, metals such as Pb, Cd, Cr and Ni exhibited strong positive correlations, suggesting their simultaneous absorption from contaminated soil and irrigation water. Cu and Zn also revealed a positive association, showing their shared geochemical behavior and plant physiological roles. Weak or negative correlations observed for certain metal pairs showed selective uptake or differential mobility within plant tissues. In conclusion, the correlation pattern suggests that spinach accumulates multiple heavy metals concurrently, primarily influenced by environmental exposure (Fig. 6).

Heavy metal concentration (mg/L) in human blood: The research demonstrated that heavy metal found higher in blood samples taken from individuals who consumed SW spinach compared to those in the blood of CW spinach consumers. Consumers of SW showed extremely high copper amounts exceeding 1.00 mg/kg while CW consumers showed minimal copper under 0.002 mg/kg. The blood of SW consumers showed higher levels of Iron at 0.0064–0.0072 mg/kg compared to CW consumers at 0.0028–0.003 mg/kg.

Blood tests revealed that Southwest consumers of spinach had significantly higher concentrations of Zinc (Zn) ranging from 0.0715 to 0.0785 mg/kg while the Northwest consumers demonstrated lower levels between 0.0255 and 0.0285 mg/kg. The levels of manganese (Mn) mirrored those of copper (Cu), as SW consumers possessed higher amounts (0.0015–0.0019 mg/kg) than CW consumers (0.0002–0.0004 mg/kg). Samples from SW consumers maintained higher levels of Cadmium (0.013–0.016 mg/kg) compared to those from CW consumers who had (0.006–0.008 mg/kg). A high level of Cobalt (Co) at 0.0377 mg/kg was recorded in SW1 compared to SW2 which was measured at 0.0007 mg/kg and 0.0074 mg/kg in SW3. SW consumers exhibited elevated lead levels at 0.0109–0.013 mg/kg which significantly surpassed CW consumer concentrations at 0.0013–0.0018 mg/kg. The levels of Molybdenum (Mo) were extremely low throughout the study with SW1 having the highest measure of 0.0024 mg/kg while CW2 recorded the lowest at0.0004 mg/kg (Table 6).

Correlation matrix for heavy metals in blood showed significant positive correlations among toxic metals, particularly Pb, Cd and Ni which imply common exposure routes, most likely through dietary intake. Moderate correlations between Cr, Cu and Zn showed their involvement in metabolic and physiological processes, alongside environmental exposure. Some weak or inverse correlations suggested differences in absorption, distribution and excretion rates of metals within the human body. The studied associations verified that multiple heavy metals coexisted in blood due to combined dietary and environmental sources rather than isolated exposure (Fig. 7).

Health risk index of spinach: Evaluation of spinach heavy metals at multiple sites was performed for copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), cadmium (Cd), cobalt (Co), lead (Pb), and molybdenum (Mo). The Health Risk Index survey at CW1 revealed concentrations of 0.0008663 Cu, 0.3336429 Fe, 0.24 Zn, 0.0192857 Mn, 0.0342 Cd, 0.0973256 Co, 0.0049821 Pb, and 0.07 Mo. SW1 heavy metal tests generated higher reading values compared to others with 0.0196538 Cu, 1.0164857 Fe, 1.6371 Zn, 0.0417857 Mn, 0.0837 Cd, 0.8947674 Results from CW2 HRI testing yielded lower readings wherein Cu showed 0.0009788 and Fe displayed 0.1992857 and Zn measured 0.1845 and Mn registered at 0.0182144 followed by Cd at 0.0171 while Co showed 0.0805814 and Pb reached 0.0030536 and Mo reached 0.045.

The HRI is defined as the ratio of the estimated daily intake of a metal to its reference oral dose (RfD), the maximum daily exposure to a substance considered to be nonhazardous to health. The heavy metal reading indices (HRI) was 0.0175275 for Cu and 0.7702264 for Fe in SW2 where values of Zn and Mn 1.6275 and 0.0642857 respectively for Cd it was at 0.06939 and Co 0.5598837 and Pb reached 0.0273214 while Mo reached 0.23. Spinach irrigated with SW1 and SW2 sewage water showed elevated HRI values exceeding CW1 and CW2 canal water-treated plants which enhances the health risks that stem from using sewage water as an irrigation source (Table 7). Health Risk Index (HRI) and Daily Intake of Metals (DIM) are computed for determining the health hazards resulting from the ingestion of polluted food crops.

Daily intake of metal of spinach: The assessments of daily metal intake of spinach at different locations were performed for copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn), cadmium (Cd), cobalt (Co), lead (Pb), and molybdenum (Mo). At CW1 the Daily Intake of Metal (DIM) was Cu at 0.03465 and Fe at 0.23355 and Zn at 0.072 and Mn at 0.0027 and Cd at 0.0171 and Co at 0.004185 and Pb at 0.06975 and Mo at 0.00063. SW1 displayed much higher metal content levels compared to CW1 according to the Daily Intake of Metal (DIM) values which included 0.78615 for Cu, 0.71154 for Fe, 0.49113 for Zn, 0.00585 for Mn, 0.04185 for Cd, 0.038475 for Co, 0.42075 for Pb and 0.00216 for Mo.

The DIM analysis of CW2 showed decreased values when measured for Cu (0.03915), Fe (0.1395), Zn (0.05535), Mn (0.00255), Cd (0.00855), Co (0.003465), Pb (0.04275), and Mo (0.000405). The testing of SW2 produced raised DIM scores ultimately reaching 0.7011 for Cu and 0.5391585 for Fe and 0.48825 for Zn and 0.009 for Mn as well as 0.034695 for Cd and 0.024075 for Co and 0.3825 for Pb and 0.00207 for Mo. Heavy metal exposure in daily spinach consumption surpasses water from sewage irrigation (SW1 and SW2) compared to water from the canal (CW1 and CW2). The obtained data indicates potential health threats to people who eat these spinach products (Table 8). From the principal component analysis (PCA), the samples of irrigation water, soil, and spinach were found to be distinctly segregated on the basis of irrigation source and location of collection. With regard to water samples, PC1 and PC2 accounted for 94.84% and 4.78%, respectively, of the total variation (Fig. 8a), while for soil and spinach (Fig. 8b), the figures were 99.56% and 0.33%, respectively, for PC1, and 93.47% and 6.44%, respectively, for PC2. The treatments of sewage water were all found to lie on the positive side of PC1, while the treatments of canal water lay on the negative side (Fig. 8c). The replicates had been observed to cluster together in both the water and soil samples, while the spinach samples were found to have more scatter within groups.

Table 1. Physicochemical properties of water collected from two sites for spinach cultivation.

Sites	EC (µS/cm)	$Ca^{+2}Mg$ (meq/L)	Na (meq/L)	HCO_3 (meq/L)	Cl (meq/L)	SAR	RSC (meq/L)
CW1	310.97 ± 1.17	2.47 ± 0.13	0.38 ± 0.03	2.38 ± 0.09	0.02 ± 0.01	0.27 ± 0.01	0.02 ± 0.01
SW1	1370.95 ± 1.15	6.45 ± 0.12	12.38 ± 0.09	4.65 ± 0.12	11.48 ± 0.09	11.92 ± 0.01	3.65 ± 0.12
CW2	417.95 ± 1.15	2.72 ± 0.12	0.42 ± 0.01	2.65 ± 0.12	0.35 ± 0.12	0.31 ± 0.01	1.13 ± 0.01
SW2	1454.62 ± 1.45	6.75 ± 0.12	12.65 ± 0.12	4.75 ± 0.12	12.15 ± 0.12	12.4 ± 0.01	2.65 ± 0.12

Table 2. Heavy metal concentrations in water samples from two sites for spinach cultivation.

Sites	Cu (mg/Kg)	Fe (mg/Kg)	Zn (mg/Kg)	Mn (mg/Kg)	Cd (mg/Kg)	Co (mg/Kg)	Pb (mg/Kg)	Mo (mg/Kg)
CW1	0.046 ± 0.0029	4.673 ± 0.0029	0.442 ± 0.0029	0.06 ± 0.0029	0.012 ± 0.0003	0.032 ± 0.0003	0.102 ± 0.0003	0.003 ± 0.0001
SW1	0.251 ± 0.0029	6.043 ± 0.0029	0.679 ± 0.0029	0.213 ± 0.0029	0.015 ± 0.0003	0.072 ± 0.0003	0.106 ± 0.0003	0.007 ± 0.00029
CW2	0.061 ± 0.00289	5.886 ± 0.00289	0.553 ± 0.00289	0.071 ± 0.00289	0.015 ± 0.00029	0.035 ± 0.00093	0.011 ± 0.00029	0.001 ± 0.00005
SW2	0.362 ± 0.00289	6.05 ± 0.02354	0.789 ± 0.00289	0.252 ± 0.00289	0.025 ± 0.00029	0.096 ± 0.00029	0.031 ± 0.00029	0.002 ± 0.00029

Table 3. Physicochemical properties of soil collected from two sites for spinach cultivation.

Sites	EC (mS/cm)	pH	P (mg/Kg)	K (mg/Kg)	OM (%)	Saturation (%)
CW1	3.55 ± 0.07	7.7 ± 0.06	7.4 ± 0.1	76.95 ± 1.15	0.72 ± 0.01	37.95 ± 1.15
SW1	16.66 ± 0.06	8.23 ± 0.09	8.35 ± 0.12	112.95 ± 1.15	1.38 ± 0.01	46.95 ± 1.15
CW2	3.83 ± 0.06	7.4 ± 0.06	7.55 ± 0.17	70.95 ± 1.15	0.79 ± 0.01	32.62 ± 1.76
SW2	16.36 ± 0.06	8.7 ± 0.06	8.65 ± 0.12	126.95 ± 1.15	1.29 ± 0.01	41.95 ± 1.15

Table 4. Heavy metal concentrations in soil samples from two sites for spinach cultivation.

Sites	Cu (mg/Kg)	Fe (mg/Kg)	Zn (mg/Kg)	Mn (mg/Kg)	Cd (mg/Kg)	Co (mg/Kg)	Pb (mg/Kg)	Mo (mg/Kg)
CW1	0.094 ± 0.005	0.93 ± 0.005	0.782 ± 0.005	0.009 ± 0.005	0.047 ± 0.0003	0.23 ± 0.0003	1.005 ± 0.005	0.006 ± 0.0012
SW1	1.993 ± 0.005	1.962 ± 0.005	1.425 ± 0.005	0.047 ± 0.005	1.098 ± 0.0003	0.26 ± 0.0002	2.748 ± 0.005	0.008 ± 0
CW2	0.082 ± 0.003	0.84 ± 0.003	0.894 ± 0.003	0.007 ± 0.003	0.005 ± 0	0.241 ± 0	1 ± 0.003	0.007± 0
SW2	1.923 ± 0.003	1.851 ± 0.003	1.313 ± 0.003	0.057 ± 0.003	1.099 ± 0	0.261 ± 0	2.432 ± 0.003	0.01 ± 0

Table 5. Heavy metal concentrations in spinach samples from two sites.

Sites	Cu (mg/Kg)	Fe (mg/Kg)	Zn (mg/Kg)	Mn (mg/Kg)	Cd(mg/Kg)	Co (mg/Kg)	Pb (mg/Kg)	Mo (mg/Kg)
CW1	0.077 ± 0.0029	0.519 ± 0.0029	0.16 ± 0.0029	0.006 ± 0.0029	0.038 ± 0.0029	0.009 ± 0.0003	0.155 ± 0.0029	0.001 ± 0.0003
SW1	1.747 ± 0.0029	1.581 ± 0.0029	1.091 ± 0.0029	0.013 ± 0.0029	0.093 ± 0.0029	0.086 ± 0.0003	0.935 ± 0.0029	0.005 ± 0.0003
CW2	0.087 ± 0.0029	0.31 ± 0.0029	0.123 ± 0.0029	0.006 ± 0.0023	0.019 ± 0.0029	0.008 ± 0.0003	0.095 ± 0.0029	0.001 ± 0.0003
SW2	1.558 ± 0.0029	1.198 ± 0.3446	1.085 ± 0.0029	0.02 ± 0.0029	0.077 ± 0.0029	0.054 ± 0.0003	0.85 ± 0.0058	0.005 ± 0.0003

Table 6. Heavy metal concentrations in human blood samples after consumption of spinach sites.

Sites	Cu (mg/Kg)	Fe (mg/Kg)	Zn (mg/Kg)	Mn (mg/Kg)	Cd (mg/Kg)	Co (mg/Kg)	Pb (mg/Kg)	Mo (mg/Kg)
CW1	0.0014±0.00029	0.003±0.00006	0.0285±0.00029	0.0004±0.00003	0.008±0.00058	0.0047±0.00179	0.0018±0.00006	0.0006±0.00012
SW1	1.002±0.00029	0.0072±0.00006	0.0785±0.00029	0.0019±0.00003	0.016±0.00058	0.0377±0.01717	0.013±0.00058	0.0024±0.00012
CW2	0.001±0.00029	0.0028±0.00006	0.0255±0.00029	0.0002±0.00003	0.006±0.00058	0.0007±0.00012	0.0013±0.00012	0.0004±0.00012
SW2	1.0014±0.00029	0.0064±0.00006	0.0715±0.00029	0.0015±0.00003	0.013±0.00058	0.0074±0.00582	0.0109±0.00006	0.0017±0.00012

Table 7. Health risk index of spinach.

Indices	Site	Cu	Fe	Zn	Mn	Cd	Co	Pb	Mo
HRI	CW1	0.0008663	0.3336429	0.24	0.0192857	0.0342	0.0973256	0.0049821	0.07
	SW1	0.0196538	1.0164857	1.6371	0.0417857	0.0837	0.8947674	0.0300536	0.24
	CW2	0.0009788	0.1992857	0.1845	0.0182144	0.0171	0.0805814	0.0030536	0.045
	SW2	0.0175275	0.7702264	1.6275	0.0642857	0.06939	0.5598837	0.0273214	0.23

Table 8. Daily intake of metal of spinach.

Indices	Site	Cu	Fe	Zn	Mn	Cd	Co	Pb	Mo
DIM	CW1	0.03465	0.23355	0.072	0.0027	0.0171	0.004185	0.06975	0.00063
	SW1	0.78615	0.71154	0.49113	0.00585	0.04185	0.038475	0.42075	0.00216
	CW2	0.03915	0.1395	0.05535	0.00255	0.00855	0.003465	0.04275	0.000405
	SW2	0.7011	0.5391585	0.48825	0.009	0.034695	0.024075	0.3825	0.00207

Table 9. Contamination factor of spinach.

Indices	Site	Cu	Fe	Zn	Mn	Cd	Co	Pb	Mo
CF	CW1	0.0072481	0.0163445	0.01564	4.225E-05	0.0006831	0.0606053	0.0271486	0.0051862
	SW1	0.1545271	0.0344833	0.028492	0.0002207	0.0159091	0.0684737	0.0742703	0.0068919
	CW2	0.0063721	0.0147627	0.01788	3.286E-05	6.686E-05	0.0635	0.0270162	0.0065075
	SW2	0.1490388	0.0325343	0.026264	0.0002676	0.0159333	0.0687455	0.0657297	0.0087838

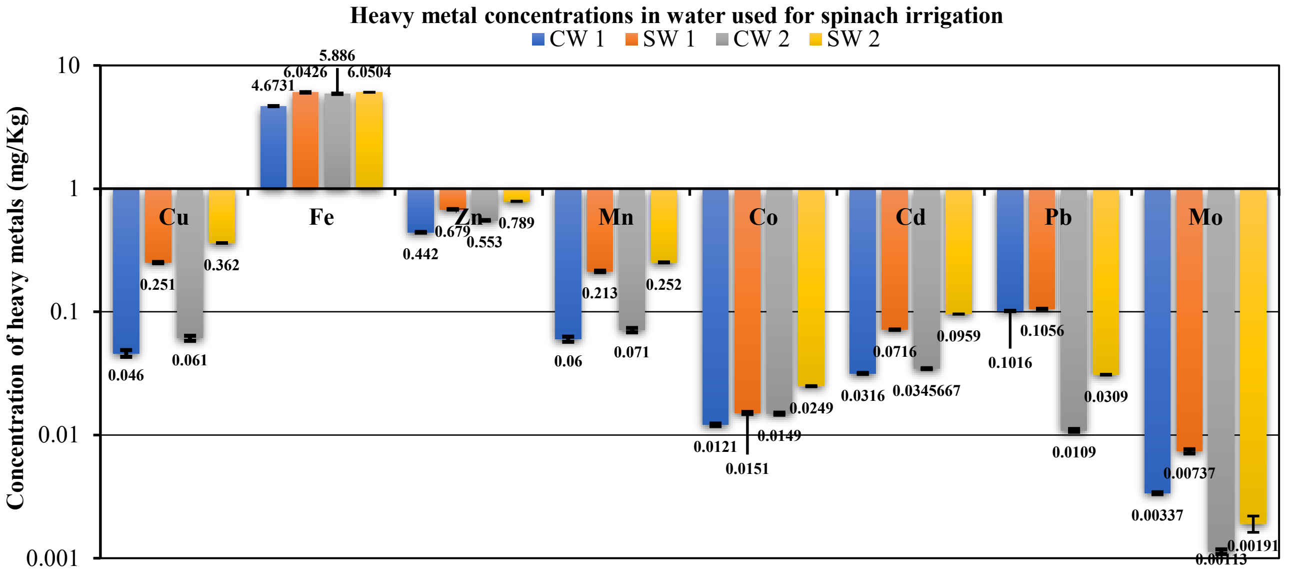

Fig. 2. Heavy metal concentrations in water used for spinach irrigation.

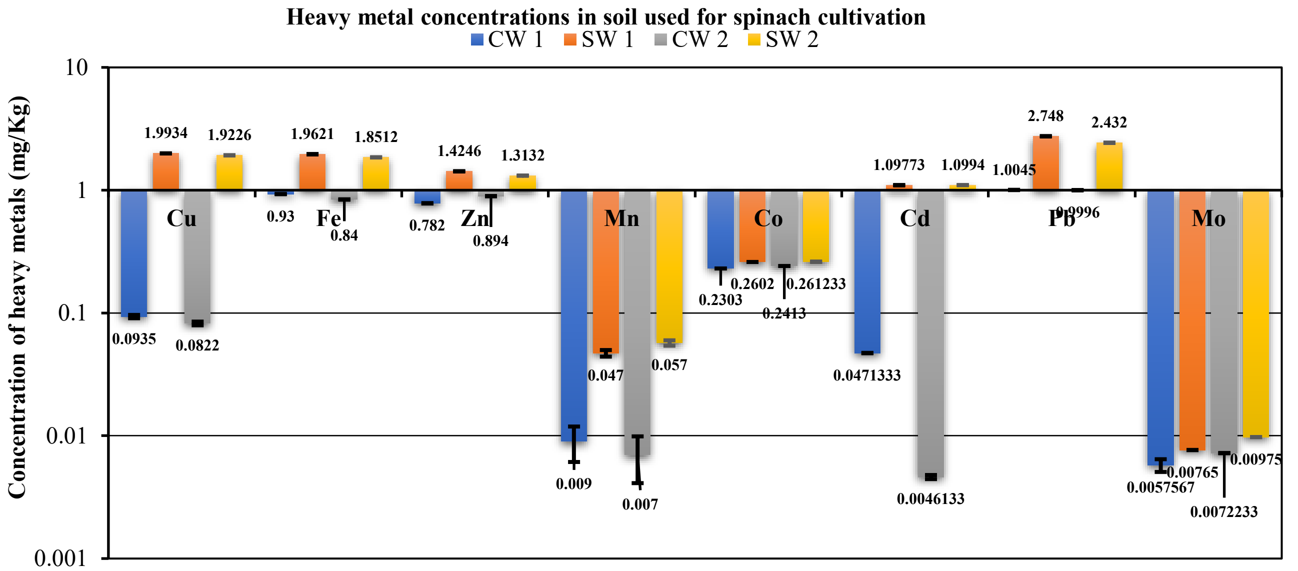

Fig. 3. Heavy metal concentrations in soil used for spinach cultivation.

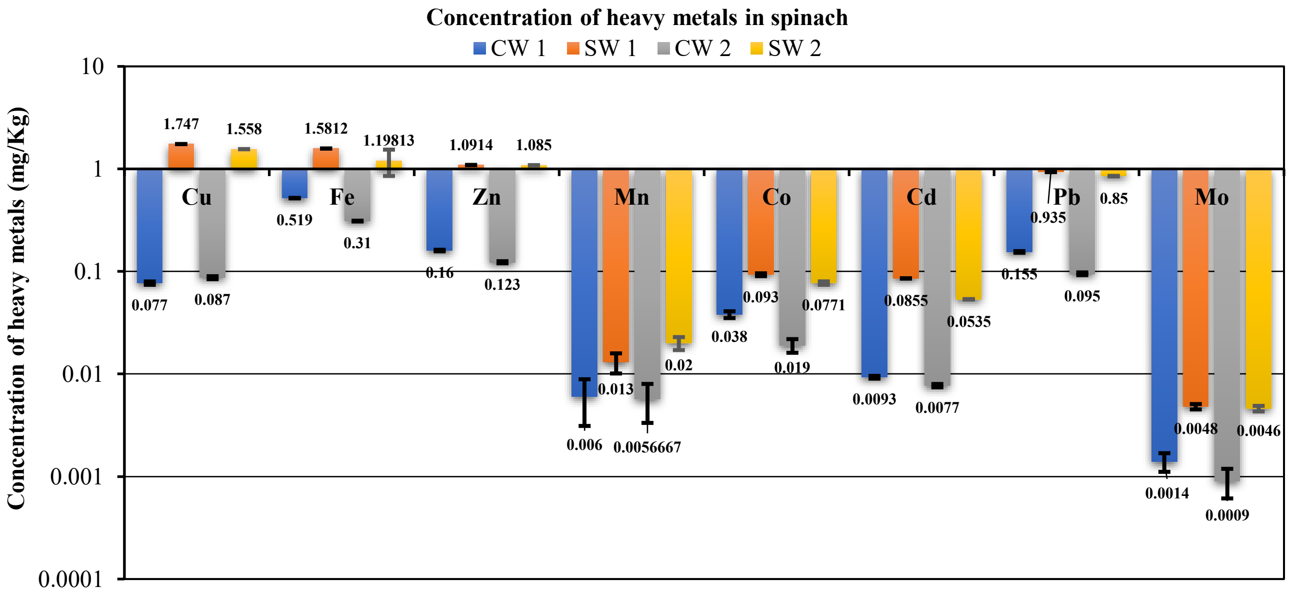

Fig. 4. Concentration of heavy metals in spinach.

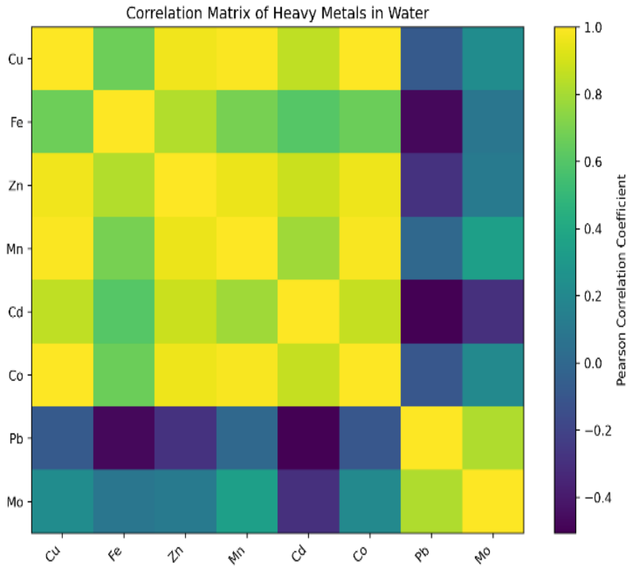

Fig. 5. Pearson correlation analysis of heavy metals in irrigation water.

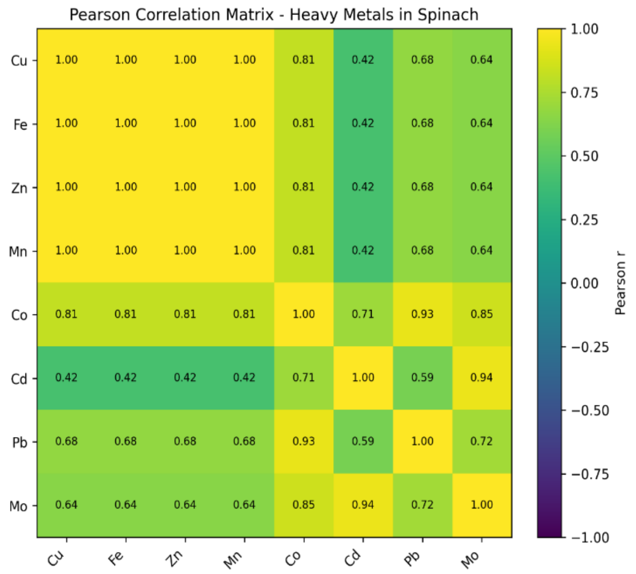

Fig. 6. Pearson correlation analysis of heavy metals in spinach.

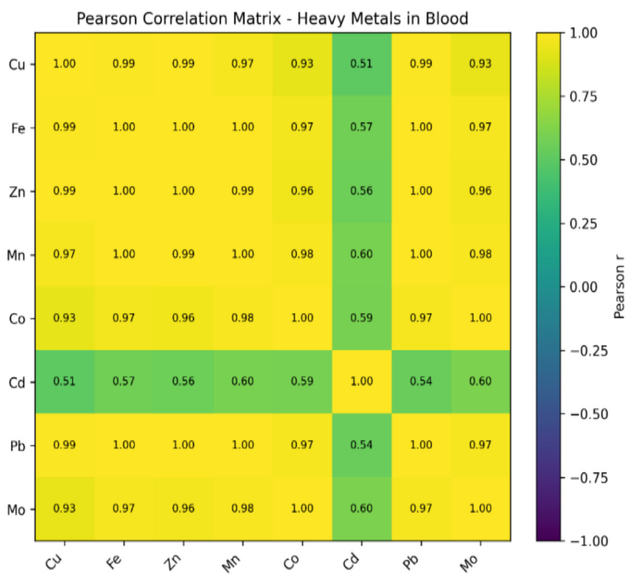

Fig. 7. Pearson correlation analysis for heavy metals in blood of animals.

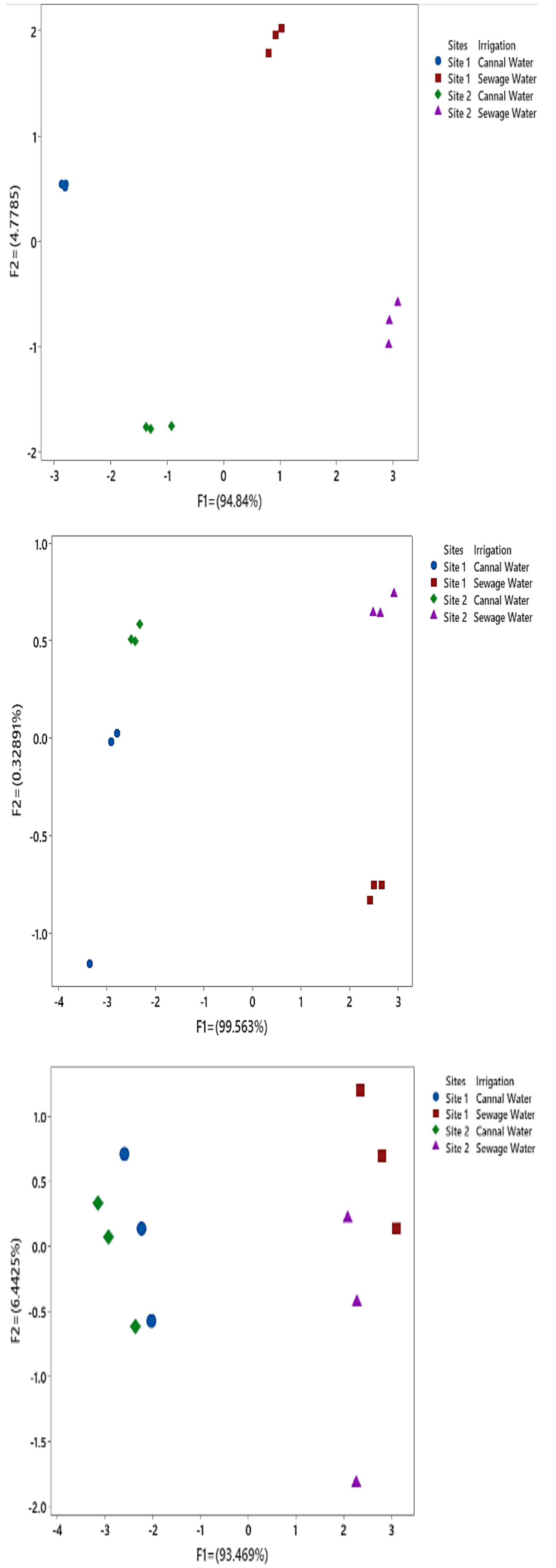

Fig. 8 Principal component analysis score plot showing (a) Heavy metal analysis in H_2O (b) Soil and (c) spinach samples.

Contamination factor (CF): The Contamination Factor (CF) analysis examines metal pollutions in spinach samples grown across various locations by using base concentrations as a reference point (Table 9). The data consisted of two control sites using canal water for testing called CW1 and CW2 as well as two sites using sewage water under test conditions SW1 and SW2. The study examined Cu, Fe, Zn, Mn, Cd, Co, Pb and Mo. The study area consisted of two research sites: Site 1 at Lahore Road together with Site 2 situated at Faisalabad Road. The contamination factor values for CW1 (canal water at Site 1) remained relatively small compared to other data in the table since cobalt reached a maximum level of 0.0606053 and lead reached a maximum of 0.0271486.

Discussion

Ma *et al*., (2021) investigated different water quality effects mainly on agricultural physicochemical characteristics. Abdelaal *et al*., (2022) evaluated the relationship between water solution sodium levels and their impact on spinach plant development. Laboratory solutions containing high sodium concentrations inhibit seed germination and cause impairment to developmental vitality of tested growth specimens. The availability of nutrients in spinach declines due to excessive electrical conductivity levels in irrigation water (Lu *et al*., 2022). Ahmed *et al*., (2023) proved how wastewater chloride poisonsed hurt greens by making them toxic to production levels. Soil pH levels and nutrient availability are affected by elevated bicarbonate concentrations according to Goutam *et al*., (2022) explained that high bicarbonate levels would create difficulties with soil alkalinity during spinach production. The sodium adsorption ratio values in irrigation water lead to soil structure depletion (El-Sharkawy *et al*., 2022). There are two detrimental effects from these conditions: water holding capacity is reduced while roots have to struggle more to penetrate into the soil. Research evidence demonstrates that water quality measurement must be performed to enhance agricultural production while maintaining crop health.

Studies demonstrate that heavy metals in irrigation water are dangerous for agricultural along with environmental sustainability issues. The study by Othman *et al*., (2021) established that wastewater irrigation practices raised copper and lead metal content followed by the built up of metals in soil together with food products. Khan *et al*., (2023) conducted analyses of iron concentrations between wastewater and canal water while microbial sewage water samples were taken from Lahore Road and Faisalabad Road confirmed elevated iron levels in wastewater. Fuentes-Rivas *et al*., (2023) toxic levels of zinc and manganese. Aslam *et al*., (2024) supported the requirement for intensive water quality checks because they demonstrated how irrigating water containing cadmium and cobalt impacted plants and soil system health. Natasha *et al*., (2020) determined that lead contamination constituted a hazard of using sewage water for irrigation operations. The crucial need exists for ongoing testing of heavy metal concentration testing of irrigation water to stop negative effects on both crop production and human wellness.

The quality of soil along with its influence on plant health received academic attention by researchers who specialized in spinach cultivation. The elevated electrical conductivity in soil decreases plant salt stress tolerance particularly when soils receive sewage irrigation (Aly *et al*., 2023). The excessive alkalinity in sewage-irrigated soils with pH levels above 8 produced severe micronutrient imbalances that decreased crop production Gupta *et al*., 2022; Nawab *et al*., 2024. Liu & Wang (2024) showed that phosphorus concentrations were higher in soils irrigated with wastewater although this abundance of nutrients led to decreased plant absorption from wastewater pollutants. The study conducted by Ahmed & colleagues (2023) shows that high potassium levels through sewage irrigation lead to healthier plants but extreme potassium levels result in imbalance. It has been reported that sewage-irrigated soils have better fertility alongside improved structure yet scientists warn about needing controlled management of chemical pollutants Singh *et al*., (2024) and Khokhar *et al*., (2024). According to Cui *et al*., (2015) and Iqbal *et al*., (2021) sewage irrigation results in better soil water retention yet it presents the risk of waterlogging in areas with poor drainage. My research results show that agricultural production along with spinach growth is strongly affected by the combination of irrigation practices and soil physical and chemical properties.

Multiple scientific researches were carried out to study the heavy metals in soil along with their effects on cultivated crops that use irrigation with wastewater. The analysis by Chen *et al*., (2022) showed spinach plants grown in sewage-irrigated fields had elevated heavy metal content of copper and lead as poor growth. Sarwar *et al*., (2020) demonstrated that waste water irrigation increased iron and zinc content in soils beyond the level what was detected in canal water irrigation zones because sewage water showed higher metal concentrations along Lahore Road and Faisalabad Road. Study by Ahmed *et al*., (2023) supports previous findings regarding the harmful impact of heavy metal pollution on plant health and food quality for metal-absorbing plants such as spinach according to Saeed *et al*., (2023). Agricultural soil contamination by high levels of cadmium and cobalt creates severe threats to both soil fertility and agricultural productivity (Rashid *et al*., 2023). Hussain & Qureshi (2020) emphasize that monitoring heavy metal levels in irrigation water and soil helps reduce health dangers from consuming polluted vegetables. The comprehensive evidences from different investigations support the need to establish proper irrigation management practices aimed at reducing heavy metal accumulation in spinach-growing agricultural soils.

A large number of researchers efforts examined heavy metal amounts in spinach along with other agricultural crops and their association with water salinity. Chen *et al*., (2022) reported that heavy metals were significantly increased especially of copper and lead, in spinach when grown using sewage water for irrigation supporting the present research. The work of Sarwar *et al*., (2020) showed that spinach plants irrigated with wastewater gained both iron and zinc at notably elevated levels thus confirming our findings that the high metal content in spinach grown from Lahore Road and Faisalabad Road biological waste water. Saeed *et al*., (2023) cautioned people about health risks from eating crops grown in polluted soils because cadmium represents one of the toxic metals of concern. Rashid *et al*., (2023) investigated leafy vegetable metal accumulation under different irrigation approaches to validate the risks of consuming sewage-contaminated spinach. Hussain & Qureshi (2020) highlighted the need for comprehensive studies of heavy metal levels in agricultural outputs when dealing with health threats that become prominent in areas that utilize wastewater for irrigation. Studies demonstrate the fundamental necessity of managing irrigation water quality because it protects both agricultural foods from dangerous metal pollution and protects consumers from harm (Nur *et al*., 2023; Mobeen *et al*., 2026).

There are quite a number of studies analyzing the health effects of heavy metals deposited in human blood which are caused by the consumption of crops especially spinach grown with the water contaminated with the above-mentioned heavy metals. For example, Khaliq *et al*., (2022) surveyed the concentrations of heavy metals in the blood of persons consuming spinach collected from aqueous solutions? containing untreated sewage water in urban Pakistan. Their studies showed that the consumers had highly raised blood levels of Lead (Pb) and Cadmium (Cd), in their blood which are in preparation with the rising levels found in spinach samples of Lahore Road and Faisalabad Road in this study. Gupta *et al*., (2023) also reported high-level influence from water-borne heavy metal in people consuming the leafy greens which were grown with the affected water.

Following elements in the blood of the patients were also analyzed for Copper (Cu) and Zinc (Zn), which results corresponded to the present analysis of SW1 and SW2 where Cu was observed at a much higher concentration (Mishra *et al*., 2023; Ghafar *et al*., 2026). Another study by Gupta *et al*., (2021) concentrated on heavy metals in the blood from participants who ingested vegetables, especially spinach, grown from the untreated sewage. They noted that the Iron (Fe) and Cobalt (Co) concentrations were high compared to pre-irrigation levels and are with the results of the blood samples of the people who took sewage-irrigated spinach from both of the sites.

Conclusion

This study compares spinach irrigated with sewage water (SW) versus canal water (CW) through a drastic evaluation of soil characteristics and heavy metal levels and corresponding health risks. Sites that receive irrigation with sewage water show serious degrees of contamination through increased levels of electrical conductivity and sodium and calcium sodium and magnesium sodium and heavy metals such as Cu, Fe, Zn, Mn, Cd, Co, Pb and Mo. The excessive heavy metals detected in soil from SW also had increased concentrations of these pollutants in spinach crops. Heavy metals in spinach samples from SW sites showed dangerous levels that reach a maximum of 1.747 mg/kg Cu and 1.581 mg/kg Fe while reaching 1.091 mg/kg Zn and 0.02 mg/kg Mn and 0.093 mg/kg Cd alongside 0.086 mg/kg Co and 0.935 mg/kg Pb and minimal Mo concentrations. The levels of heavy metals measured at SW sites surpass concentrations recorded at CW sites because irrigation methods influence the accumulation of these pollutants. The evaluations of health risks comprising Health Risk Index (HRI) and Daily Intake of Metal (DIM) and Contamination Factor (CF) demonstrate SW spinach presents elevated threats to human consumers. The HRI values at SW sites exceed established safety levels to such an extent that they present chronic risks which may cause organ damage and developmental delays along with heavy metal intoxication. Laboratory tests of blood collected from SW spinach consumers revealed extreme metal concentrations of Cu, Fe, Zn, Mn and Pb which indicates the harmful effects on human health.

Recommendations

The strict enforcement of wastewater discharge rules to agricultural lands combined with regular safety standards checks protects against non-compliance. Irrigating fields with treated water or safer water sources will help reduce contamination risks. Soil remediation through phytoremediation and other methods helps lower the availability of heavy metals in the soil. Community members and farmers need education about the risks from non-treated sewage water use in irrigation systems while learning about the health dangers from consuming contaminated vegetables. Spreading nutritional variety in food choices helps reduce combined heavy metal accumulation when consuming affected spinach.

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Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

Ethical Approval

Ethical Statement: The Institutional Human Ethics Committee of University of Sargodha (Approval No.25-A18 IEC UOS) has allowed all the protocols used in this experiment. All the experimental methods of this study have followed all the appropriate guidance and regulations including NRC standards.

Availability of data and materials: Data and material are available for research purpose and for reference.

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Authors' Contribution: Zafar Iqbal Khan came up with the idea for the experiment, and Muzna Shahid carried out the methodology for it. Muhammad A. Ghafar and Asma Ashfaq used software and statistics; Kafeel Ahmad did the validation; Muzna Shahid did the formal analysis; Muhammad A. Ghafar did the investigation; Ijaz Rasool Noorka provided the resources; Adnan Rab Nawaz Helps in reviewing the draft and editing it. Naila Riaz and Faiza Zameer did the visualization; All authors have reviewed and consented to the published version of the manuscript.

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