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COMPARATIVE TOXICOLOGICAL EVALUATION OF HEAVY METALS IN ROOT VEGETABLES (CARROT AND TURNIP) IRRIGATED WITH WASTEWATER IN SARGODHA, PAKISTAN

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Abstract

The metabolism of food crops irrigated with untreated wastewater leads to heavy metal exposure in edible plant components, and even at extremely low doses, the consumption of toxic elements can pose serious health threats to humans. This problem is alarmingly striking in Pakistan, where wastewater irrigation for agriculture has become extensive in urban and peri-urban areas due to water scarcity. The present study integrates investigations on two commonly consumed root vegetables, carrot (*Daucus carota* L.) and turnip (*Brassica rapa* L.), cultivated in Sargodha and Sillanwali during 2023–2024 using sewage water, canal water, and tube well water. The study aimed to examine the levels of copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), nickel (Ni), cadmium (Cd), lead (Pb), and chromium (Cr) in soil, water, crops, and human blood samples, and to evaluate the potential health risks associated with their consumption. Physico-chemical analysis of soil and water samples was carried out, and heavy metal contamination was assessed using contamination factor (CF), enrichment factor (EF), bioconcentration factor (BCF), estimated daily intake (EDI), and hazard quotient (HQ). The uptake of metals from soil into the edible parts of carrot and turnip was also evaluated. Results showed that metal concentrations were highest at sewage water sites (SW1) and lowest at tube well sites (TW2). Comparative assessment revealed species-specific uptake patterns, with carrot showing relatively higher accumulation of Fe and Mn, while turnip exhibited comparatively higher Zn and Pb concentrations. However, all index values and HQ remained below 1, indicating that metal concentrations were within permissible limits. This study adds weight to existing evidence suggesting that open cultivation under untreated wastewater should be discouraged and highlights the urgent need for wastewater treatment and continuous monitoring to ensure food safety.

Key words: Carrot; Turnip; Wastewater irrigation; Heavy metals; Contamination factor; Food safety

Introduction

Globally, carrot is one of the most grown root vegetables. The consumption of carrots and carrot-derived products is ever on the rise due to the natural nutrients they have, including carotenoids and dietary fibers (Dadan, 2021). The edible carrot (*Daucus carota* L.) belongs to those crops cultivated all over the world whose output and fresh or processed consumption activity are very promising. This is because there are more than 428 million tons of world carrot production in a year, and the growing area is said to be around 11.5 million hectares (Ahmad *et al*., 2019).

Carrots (*Daucus carota L.*) are ranked tenth in the value of their nutrients. Hence, they are increasingly consumed due to the carotenoids (vitamin A), which are very useful for the eyes (Djoufack *et al*., 2023). This vegetable has a wide scope of production in the world, with yields exceeding 40 million tons per hectare (FAOSTAT, 2020). Carrot is a pivotal source of minerals, polyacetylene, carotenoid content and vitamins, which show that it is a healthy and nutritious crop. This vegetable, which is grown and eaten almost all over the globe, ranks seventh by volume among all eighty types of vegetables worldwide. Carrots have high beta-carotene content and moderate amounts of phosphorus, iron, vitamin B, calcium and folate. In addition, it is usually consumed as a salad along with tons of other culinary applications. Carrots are the most cultivated crop after rice and wheat in the world due to their consumption both in raw and cooked dishes. It is also recognized and greatly appreciated for its use (Ghani *et al*., 2021).

Turnip (*Brassica rapa* L.) is also an important root vegetable, a subspecies of the family Cruciferae (Jia *et al*., 2020). This is one of the most used species amongst the

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Brassica species consumed all over the world; thus, it has a major significance in the diet of humans (Shehata *et al*., 2019). Turnip contains high levels of calcium, magnesium, iron, zinc, vitamin B1, vitamin B2 and dietary fiber, along with bioactive compounds. It's an energy-rich nutritious food with very low fat and calories. According to Paul *et al*., (2019), The turnip is one of the oldest and most important cultivated vegetables, having been eaten since prehistoric times, and was believed to possess medicinal qualities, being a traditional remedy for many ailments.

Studies on turnip phytochemical constituents have revealed that glucosinolates, isothiocyanates, flavonoids, and volatiles are among the main constituents identified. The plant has numerous bioactivities, such as antioxidants, anti-tumor, anti-diabetic, anti-inflammatory, anti-microbial, hypo-lipidemic, cardioprotective, hepatoprotective, nephron-protective, and analgesic properties (Paul *et al*., 2020: Akram *et al*., 2026). Besides, it is used in different cuisines worldwide, particularly the root part that can be eaten raw and also cooked, boiled, or steamed. Turkish cuisine is loaded with turnip dishes, which have been going on since the times of the Seljuks and Ottomans, including soups, appetizers, meat dishes, vegetables, fruits, grains, pastries, and drinks (Badem, 2020: Mahmood *et al*., 2021). The preparation of turnips is culturally and regionally diverse, and many recipes utilize raw, boiled, or steamed leaves and roots. In some areas, pickled turnip also forms a common preparation, usually combined with several meats, herbs, and vegetables (Badem, 2021).

As per estimates, over 7.7 billion people globally report a lack of access to clean drinking water (Boretti & Rosa, 2019). The absence of targeted clean and fresh water resources can indeed be a major constraint in agricultural production, as well as an insufficient food supply. Previously, it has been reported that the agricultural sector consumes more water than any other sector on the planet. By the year 2025, it is anticipated that the global water demands in agriculture will increase by more than 60% (Ghafar *et al*., 2025: Velasco-Muñoz *et al*., 2019).

This increase in water demand and the limited availability of freshwater resources have made it imperative for the farming community to use untreated sewage water for the cultivation of food crops all over the world. Irrigation with wastewater is generally practiced in regions facing severe water scarcity. Furthermore, the fact that wastewater has some essential nutrients that reduce the cost of production by 10-20% when wastewater is used for irrigation is an additional reason for poor farmers to use wastewater (Khalid *et al*., 2018). Wastewater, which contains significant amounts of nutrients such as Ca, N, Cu, P, Zn, K, S, and Mn, is beneficial to crop productivity. However, the use of wastewater contradicts its benefits since it is harmful to human health because it contains harmful metallic contents (Khan *et al*., 2019: Hassan *et al*., 2025).

Wastewater also often contains several metals, which are health hazards when released into water and soil (Khan *et al*., 2021). Regular application of treated and untreated sewage water can elevate the level of toxic metals in agricultural soil and edible crops, while such metals may enter the food chain and affect the health of the population (Ahmad *et al*., 2019). Metals with a density greater than 5 g/cm^3 are generally referred to as heavy metals. This class of metals is toxic, non- biodegradable, and does not serve any biological purpose, five times denser than water. They are extremely poisonous even in minute amounts, should be treated with utmost care as they are hazardous to human life cause oxidative stress upon ingestion due to both natural causes and those that are manmade (Oyugi *et al*., 2021; Paithankar *et al*., 2021).

Animal and human health suffer tremendously as a result of consuming vegetables and fruits that are contaminated with heavy metals. In their turn, this may be due to the consumption of contaminated vegetables. Particularly, Cadmium (Cd), Lead (Pb), and Arsenic (As) metals are carcinogenic and also known to induce several neurological problems, kidney, bone, and cardiovascular diseases. Other heavy metals like Copper (Cu) and Zinc (Zn) are non-carcinogenic, but when taken in excessive proportions, may pose a threat to one's health by causing liver failure, stomach pains, and altering the immune system (Baghaie & Fereydoni, 2019).

In Pakistan, with a persistent shortage of available fresh water from the twentieth century onward, agricultural practices have included the use of contaminated wastewater. Due to the nutrients and organic matter contained in wastewater, its application would enhance soil fertility; however, the presence of heavy metals, which are no doubt harmful to the soil as well as the vegetables grown in it. Heavy metals are also taken up by the crops grown through the contaminated soil. Therefore, it is critical to assess how contaminated vegetables are a health risk. The present findings were a directed evaluation of health risks due to metal contamination in food crops, including carrots and turnips.

Material and Methods

Study area: The present investigation occurred at two locations (Sargodha and Sillanwali) within the District Sargodha, located in the Punjab province in Pakistan. Sargodha is a cultivating and business center in the northwest part of the country. Most of the area is flat, fertile plains with a few minor hills on the outskirts. There are maximum temperatures up to 50 C and minimum temperatures below freezing in the summer. The area was mostly dry, with the possibility of precipitation exceeding transpiration. Most of the rainfall occurs during the monsoon in August when the region experiences up to 250 mm of rainfall (Zahid *et al*., 2019).

Study sites: This study was divided into three locations, each located at different places in two sites of the Sargodha district, having different irrigation sources, i.e., irrigated with sewage water (SW), canal water (CW) and tube-well water (TW).

Sampling design: To collect the samples Randomized Complete Block Design (RCBD) was used. There were constant interactions between variables in RCBD. Sites comprising the RCBD were the source of soil irrigation in the current study. There were four crops at two sites (site 1 and site 2); each site had three locations (sewage water, canal water, and tube-well water), and there were three replicates of each crop sample (2x2x3x3=36). Crop samples were collected during the 2023-2024 crop season.

Collection of samples: The three types of water samples (sewage, canal and tube well) were taken from two tehsils in the Sargodha district. Before being transported to the lab, all samples were collected in plastic bottles prewashed with 1% nitric acid and stored at 4 degrees Celsius until analysis. The selected areas were dug up to 12 to 15 cm deep with a stainless-steel spade, partially incorporating all soil layers. Before being placed in a forced air oven for 47 hours, all samples were air-dried. The samples were gathered and stored in labeled, packed paper bags in an incubator at 70°C for 05 days. Randomly selected portions of selected food crops were sampled from fields irrigated with sewage water, canal water, and tube well water. Dust and other airborne contaminants were removed, and food crop samples were rinsed in distilled water. The sun-dried samples were then oven-dried for 5 days at 70 C. After being removed from the oven, all the samples were crushed into a fine powder using a piston mortar. The Jugular method was employed to collect blood samples, which were then placed into heparinized tubes. We gathered three identical and 18 blood samples. The blood samples were collected using disposable 10cc syringes and then placed into ADTA-K3 tubes. After collection, samples were spun at 2500 rpm for 2 minutes to obtain plasma. The plasma was transported to the lab in a chilly container. The lab kept the serum frozen for analysis.

Samples digestion: Using 5 ml of H_2O_2 and 3 ml of H_2SO_4, 5 ml of water was added to a glass container and subsequently processed for 30 minutes within the chamber that was being utilized. The water-based samples were removed from the chamber when the emissions stopped evaporating. Until the color of the solution fades, continue to add 2 ml of H_2O_2. After cooling, 50ml of water that was twice distilled was poured into the tank's capacity. The resulting solution was stored in polyethylene bottles. A wet digestion technique was used to digest organic matter. The digestion chamber used 4 ml of H_2SO_4 and 8 ml of H_2O_2 to process 1 gram of soil in a glass container for 30 minutes. After the evaporation ended, 2 ml of H_2O_2 was poured into the container. Unless the sample completely disappeared all its color, the procedure was repeated. Whatman No. 42 filter paper was used to filter the material that was digested. The ultimate volume of the samples was increased to 50ml by adding double-distilled water to the mixture and storing them in plastic bottles with labels. The blood samples were digested for 15 to 30 minutes with 2 ml of H_2SO_4 and 4 ml of H_2O_2 after being placed in two different flasks. After the flask had dried, samples were taken out, and 2 ml of H_2O_2 was added again until the sample color disappeared. The final volume of the sample was brought up to 50 ml by adding double-distilled water and filtering it through the Whatman No. 42 filter paper.

Preparation of standard solutions for metals: Standard curves for Zn, Cr, Fe, Pb, Cd, Ni, Cu and Mn were drawn during the mineral examination. Dissolving determined amounts of iron sulfide, copper acetate, cadmium chloride, lead chloride, zinc acetate, nickel bromide, and manganese acetate yielded stock solutions of 1000 ppm. To make a solution of each element with 100 parts per million, mix a small container with 10 parts of the stock solution and 90 parts of deionized water. This solution was used to make standard solutions (Table 1).

Table 1. Limits for atomic absorption spectrophotometer.

Metal	Wavelength	Slit width	Lamp current	Type of flame
Cu	324.8	0.5	6	Acetylene-air flame
Fe	248.3	0.2	12	Acetylene-air flame
Mn	234.2	0.4	10	Acetylene-air flame
Zn	213.9	1.0	8	Acetylene-air flame
Cd	228.8	0.7	8	Acetylene-air flame
Cr	236.7	0.6	11	Acetylene-air flame
Ni	232	0.2	12	Acetylene-air flame
Pb	283.3	0.7	10	Acetylene-air flame

Mn (Shahlaei & Pourhossein, 2014), Pb, Cr (Jafarian & Alehashem, 2013), Ni (Gadzhieva, 2014), Cd (Bortoleto *et al*., 2004), Fe (Gadzhieva, 2014)

Procedure for analysis of metals: After digestion, all samples were examined for metals utilizing an atomic absorption spectrophotometer and afterward diluted. A flame photometer was also used to identify the mineral concentration. Water samples were examined for sodium (Na^+) and potassium (K^+) utilizing a flame photometer (Jenway PFP-7). A calibration curve was drawn before sample analysis by running the standard metal solution. Metals concentration was identified by utilizing an atomic absorption spectrophotometer (Shimadzu Co., Ltd., Japan).

Metal determination: An atomic absorption spectrophotometer (Shimadzu Co., Ltd., Japan) was used to determine the metal content in water, soil and food crop samples. The National Institute of Standard Technology's Standard Reference Material (SRM 1570) for Mn, Cr, Cu, Fe, Zn, Pb, Cd, and Ni metals was used to verify the precision and accuracy of the analysis. Each sample contained metals that were detected in triplicate in each sample. All the outcomes were in line with international standards.

Assurance of quality control: To ensure that the study findings were acceptable, the following quality standards were used: The experiment used analytical grade chemicals from Sigma Aldrich, Merck, and BDH. This experiment used Pyrex glassware. After being washed with liquid detergent (Max) and dried in the oven for an hour, the glassware was completely cleaned. This study employed an atomic absorption instrument (Shimadzu Co. Ltd., Japan). Each sample contained three replicates containing different metals. The findings were in line with international standards.

Indices to evaluate heavy metal contamination

Contamination factor (CF): The contamination factor can be used to measure the pollution status of contaminants within the soil based on the quantities in the sample's contents and their background concentration.
The Cf is calculated by the following formula:

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

The metal content in the soil is represented by the Cm sample.

The metal concentration from a natural source is called the background Cm (Table 2).

Bioconcentration factor (BCF): The Bioconcentration factor (BCF) investigated metal value in edible parts of plants that transferred from the soil. Cui *et al*., (2004) equation determines its value by following the formula;

$$BCF = \frac{MV_{Plant}}{MV_{Soil}}$$

Metal concentration in the plant
Metal concentration in soil

Table 2. Background value of heavy metals in soil.

Metals	Background value (mgkg^{-1})
Cu	12.9
Fe	56.9
Mn	213
Zn	50
Cd	69
Cr	40
Ni	9.5
Pb	37

Enrichment factor (EF): A unit of measurement known as the soil EF has been developed to determine the quantity of contaminants collected in soil from agriculture and food crops (Barbieri *et al*., 2015). Standard concentrations of metals in soil and food crops given in Table 3.

$$EF = \frac{\text{(Metal concentration in food crop / Metal concentration in soil) sample}}{\text{(Metal concentration in food crop / Metal concentration in soil) standard}}$$

Table 3. Standard concentration of metals in soil and food crops.

Metals	Soil (mgkg-1)	Food crops (mgkg-1)
Cu	3.5d	12.2b*
Fe	50-55h	425c*
Mn	46.75f	500 d*
Zn	16e	99.4-100e*
Cd	0.76b	0.2a*
Cr	9.07g	2.3g
Ni	2.6c	5 g*
Pb	8.15a	3f*

a*,c*,d*,e*(Khan *et al*., 2018b), b*(Hussain *et al*., 2022), f*(Ahmad, 2022), g*(Bashir *et al*., 2020), a,b,c,d,e (Vodyanitskii, 2016), f,g (Reza & Singh, 2010), g (Kayode *et al*., 2021),h (Ogunlana *et al*., 2021)

Estimated daily intake (EDI): An estimate of a person's daily intake of a substance that is not expected to have a significant impact on their well-being. The EDI is expressed in mg/kg/day using the following formula (Amarh *et al*., 2023). Estimated daily intake of metal is given in Table 4.

$$EDI = \frac{C \times DI \times C.F}{BW}$$

C = Metal conc. In food crop in mgkg^{-1}; C.F = Conversion factor; DI = Daily intake; BW = Reference body weight**:**

Table 4. Daily intake of metal.

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

*(WHO, 2003) ,**(Jan *et al*., 2010)

Hazard of quotient (HQ): The proportion of risk associated with an impact at a level beyond which no harmful effects are anticipated.

$$HQ = \frac{EDI}{RfD}$$

EDI = Estimated daily intake (mg/kg/day) of metals
RfD = Measured Reference dose of metals (mg/kg/day)

Table 5. Oral reference dose of heavy metals.

Metals	Reference dose
Cu	0.040f
Fe	0.07g
Mn	0.14 h
Zn	0.300e
Cd	0.01a
Cr	1.5c
Ni	0.020d
Pb	0.04b

f, g, h, e, a, c, d, b (Table 5) (Orisakwe *et al*., 2017)

Results and Discussion

Physico-chemical properties of water and soil: The physico-chemical analysis of water samples from different irrigation sources revealed marked variations across sites. Sewage water (SW) sites consistently exhibited the highest values for electrical conductivity (EC), sodium (Na^+), calcium and magnesium (Ca^{2++} Mg^{2+}), bicarbonate, chloride, residual sodium carbonate (RSC), and sodium adsorption ratio (SAR), indicating a high salinity and mineral load. This suggests that crops irrigated with sewage water were exposed to elevated dissolved salts and nutrients, potentially influencing metal mobility and uptake. Canal water (CW) samples had intermediate values for these parameters, reflecting moderate water quality with some degree of mineral enrichment. Tube well (TW) water sites consistently showed the lowest EC, Na^+, Ca^{2++} Mg^{2+}, bicarbonate, chloride, RSC and SAR levels, reflecting minimal anthropogenic contamination and relatively cleaner water (Table 6). These differences indicate a strong influence of irrigation water type on the chemical environment of soils and crops. Notably, TW sites had very low bicarbonate and chloride concentrations, indicating reduced alkalinity and salinity, which favors better soil structure and lower risk of metal solubilization. The high RSC and SAR in SW water may contribute to sodicity issues in soil and promote preferential uptake of certain metals by plants.

Physico-chemical properties of soils, including pH, organic matter content, EC, potassium (K), phosphorus (P), and saturation, varied across sites and contributed to differential metal retention. Soils with higher organic matter and EC in SW sites showed higher metal concentrations, likely due to metal complexation and retention (Table 7).

Metal concentrations in soil: Analysis of soil samples demonstrated that sewage water irrigation led to significantly elevated concentrations of metals, including copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb), compared to tube well and canal water sites (Fig. 1). Among the sites, SW2 consistently showed the highest accumulation of almost all metals, indicating localized hotspots of contamination. Canal water-irrigated soils had intermediate metal levels, while TW-irrigated soils contained the lowest concentrations, often reflecting natural background values. This pattern demonstrates the strong influence of wastewater irrigation on metal enrichment in agricultural soils. ANOVA results confirmed highly significant differences ($p<0.001$) in metal concentrations between sites and locations, supporting the observed spatial variability in soil contamination.

Table 6. Physico-chemical properties of water samples collected from different sites of turnip fields.

Sites	Bicarbonate	SAR	RSC	E.C	Chloride	$Ca^{2+}Mg^{2+}$	Na^{2+}
SW1	10.19 ± 0.12	6.33 ± 0.008	3.23 ± 0.01	1845.0 ± 1.45	6.13 ± 0.008	7.70 ± 0.11	11.55 ± 0.01
CW1	9.76 ± 0.08	4.26 ± 0.005	2.85 ± 0.01	1267.3 ± 1.20	3.66 ± 0.008	5.87 ± 0.01	7.71 ± 0.32
TW1	2.59 ± 0.01	0.33 ± 0.008	0.16 ± 0.01	329.33 ± 1.45	0.53 ± 0.01	3.03 ± 0.12	0.70 ± 0.05
SW2	9.85 ± 0.008	5.85 ± 0.005	2.60 ± 0.05	1853.3 ± 1.76	6.50 ± 0.11	7.40 ± 0.11	10.55 ± 0.01
CW2	9.25 ± 0.008	4.95 ± 0.01	5.05 ± 0.008	1450.4 ± 0.01	3.15 ± 0.01	5.99 ± 0.01	7.89 ± 0.01
TW2	3.56 ± 0.08	0.34 ± 0.01	0.04 ± 0.008	313.33 ± 1.76	0.80 ± 0.05	2.76 ± 0.008	0.62 ± 0.02
Physico-chemical properties of water samples collected from different sites of carrot fields							
CW1	8.50 ± 0.05	4.47 ± 0.01	2.77 ± 0.01	1261.7 ± 0.88	3.59 ± 0.008	5.60 ± 0.05	7.06 ± 0.008
SW1	9.95 ± 0.005	6.47 ± 0.005	3.36 ± 0.005	1850.8 ± 0.30	6.27 ± 0.005	7.23 ± 0.08	11.59 ± 0.01
TW1	2.45 ± 0.008	0.38 ± 0.005	0.05 ± 0.008	352.0 ± 1.15	0.46 ± 0.005	2.63 ± 0.08	0.40 ± 0.05
CW2	8.13 ± 0.008	4.86 ± 0.008	3.15 ± 0.01	1237.4 ± 1.26	3.46 ± 0.14	5.00 ± 0.15	7.36 ± 0.02
SW2	8.34 ± 0.01	7.64 ± 0.01	3.33 ± 0.08	1638.0 ± 2.63	6.90 ± 0.17	4.69 ± 0.10	11.51 ± 0.02
TW2	2.63 ± 0.14	0.42 ± 0.008	0.05 ± 0.01	354.33 ± 2.33	0.63 ± 0.08	3.00 ± 0.17	0.53 ± 0.08

Table 7. Physico-chemical properties of soil samples collected from different irrigated sites of turnip fields.

Sites	Phosphorous	Potassium	Saturation	Organic Matter	E.C	pH
SW1	46.00 ± 1.53	405.00 ± 1.76	35.77 ± 0.02	1.24 ± 0.01	7.83 ± 0.01	8.87 ± 0.01
CW1	14.79 ± 0.01	216.33 ± 1.15	38.75 ± 0.008	0.79 ± 0.01	3.67 ± 0.01	8.10 ± 0.02
TW1	11.46 ± 0.33	208.66 ± 1.76	37.35 ± 0.008	0.74 ± 0.01	2.68 ± 0.01	7.43 ± 0.008
SW2	38.24 ± 0.01	425.34 ± 1.45	37.30 ± 0.05	1.26 ± 0.005	7.56 ± 0.12	8.36 ± 0.02
CW2	6.92 ± 0.02	216.66 ± 1.45	46.73 ± 1.13	1.11 ± 0.01	3.73 ± 0.01	7.88 ± 0.04
TW2	7.39 ± 0.01	1899.34 ± 1.45	33.48 ± 0.01	0.88 ± 0.01	2.20 ± 0.01	7.65 ± 0.01
Physico-chemical properties of soil samples collected from different irrigated sites of carrot fields						
CW1	15.26 ± 0.005	225.44 ± 0.57	38.25 ± 0.01	0.84 ± 0.005	3.76 ± 0.005	8.35 ± 0.005
SW1	44.66 ± 0.88	415.47 ± 0.01	36.69 ± 0.01	1.73 ± 0.005	7.99 ± 0.005	8.96 ± 0.008
TW1	12.16 ± 0.01	215.44 ± 0.57	39.24 ± 0.005	0.82 ± 0.005	2.71 ± 0.005	7.53 ± 0.005
CW2	5.31 ± 0.02	296.72 ± 0.02	34.85 ± 0.008	0.75 ± 0.01	4.55 ± 0.01	8.14 ± 0.01
SW2	35.40 ± 0.03	365.35 ± 0.01	38.37 ± 0.02	1.55 ± 0.01	7.45 ± 0.008	8.83 ± 0.20
TW2	9.46 ± 0.14	209.38 ± 0.02	35.04 ± 1.52	0.67 ± 0.008	1.85 ± 0.01	7.15 ± 0.01

Metal concentrations in crops

Carrot (*Daucus carota*): Crops irrigated with sewage water showed increased accumulation of Fe and Mn compared to those irrigated with canal or tube well water. Cu and Zn concentrations were moderate, and Cd and Ni remained below permissible limits, indicating minimal risk of toxic exposure. Highest metal accumulation in carrot was generally observed at SW1 and SW2 sites, reflecting the corresponding metal enrichment in sewage water and soils.

Turnip (*Brassica rapa*): Turnip showed a tendency to accumulate higher levels of Zn and Pb, particularly at SW1 and SW2, compared to tube well sites. Fe and Mn accumulation were lower than in carrot, highlighting species-specific uptake patterns. Cd and Ni levels in turnip remained within safe limits for human consumption (Fig. 2).

Both crops reflected the influence of irrigation source and soil properties on metal uptake. Tube well-irrigated crops consistently had the lowest metal concentrations, while sewage water irrigation increased metal loading in edible tissues. ANOVA analysis of crop metal concentrations indicated highly significant differences ($p<0.001$) across sites, emphasizing the role of both irrigation water type and site-specific soil conditions in determining crop metal content.

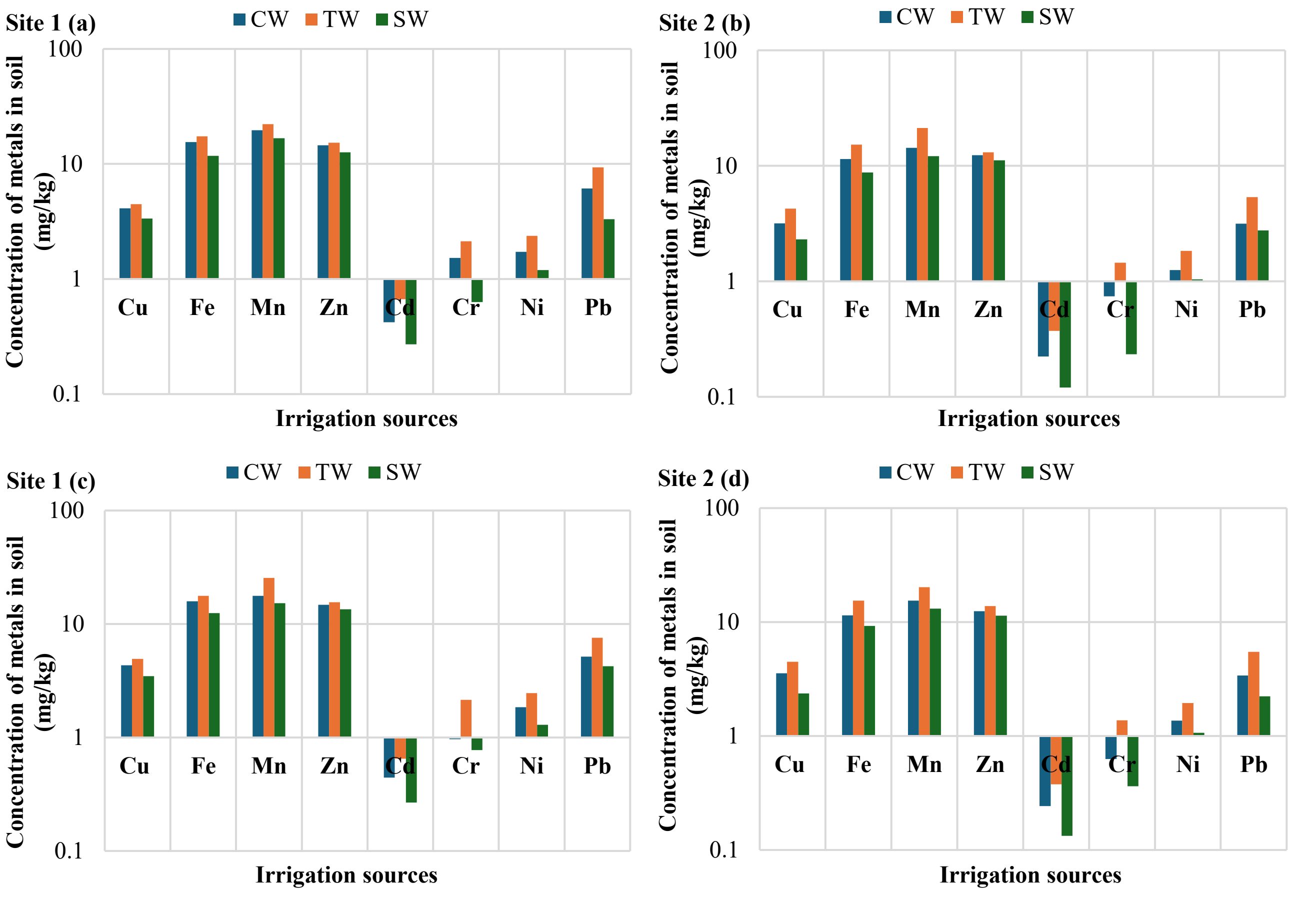

Fig. 1. Variation of metals concentration (mgKg^{-1}) in soil at three different locations of site 1 & 2 of turnip (a,b) & carrot (c,d) growing fields.

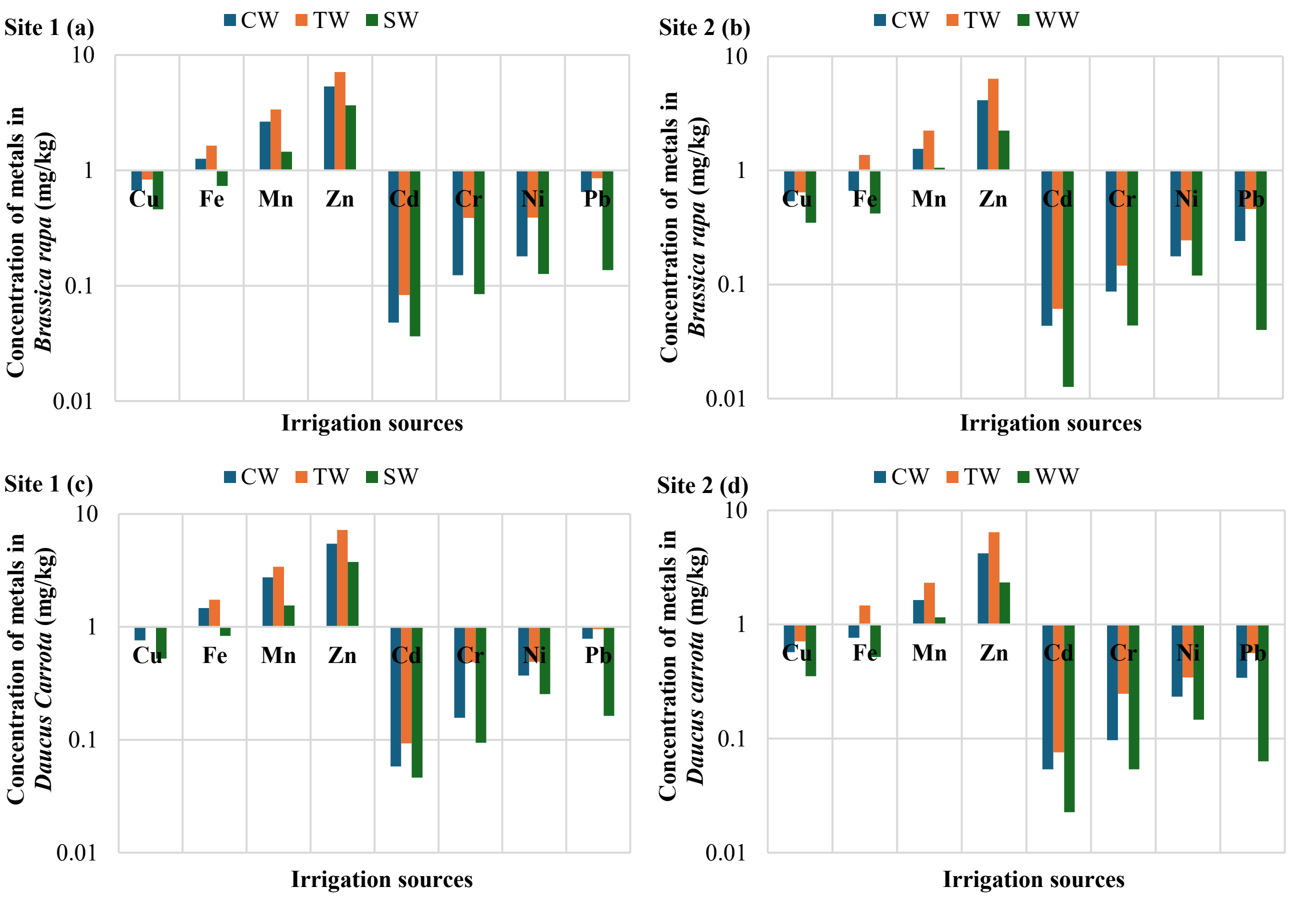

Fig. 2. Variation of metals concentrations (mgKg^{-1}) in *Brassica rapa* (a,b) & *Daucus carrota* (c,d) at three different locations of site 1 & 2.

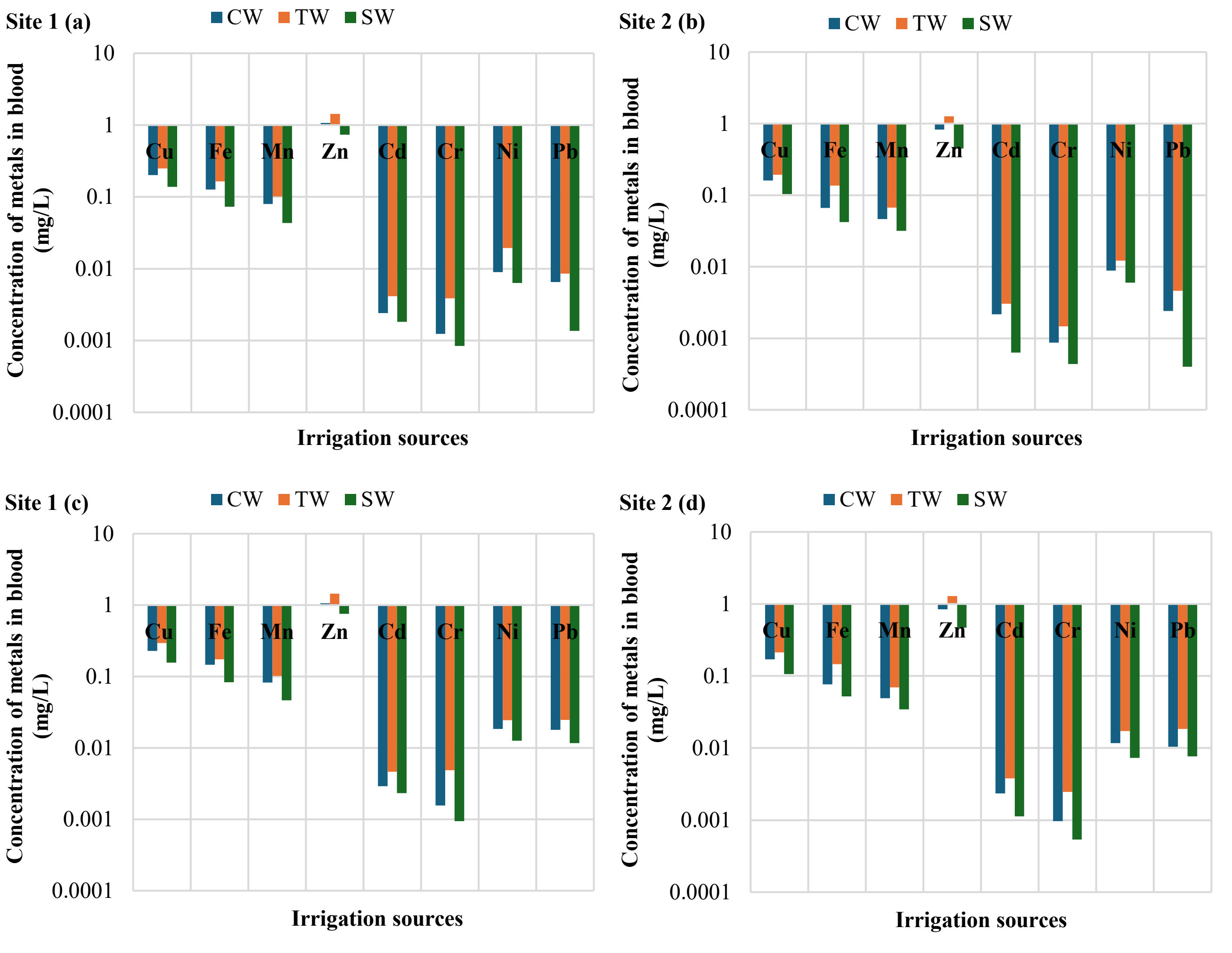

Fig. 3. Variation of metals concentration (mgL^{-1}) in blood at three different locations of site 1 & 2 using turnip (a,b) & carrot (c,d).

Blood serum analysis: Blood serum samples from humans consuming crops from different sites revealed variable metal concentrations that reflected trends observed in crop and soil analyses. Individuals consuming crops from sewage water sites had elevated levels of Zn, Cu and Pb compared to those consuming crops from tube well-irrigated sites. Fe and Mn concentrations in human serum largely remained within permissible limits, while Cd and Ni were below toxic thresholds in all samples, suggesting limited systemic exposure (Fig. 3).

The patterns of metal correlation in blood mirrored those observed in crops and soils, indicating that dietary intake is the primary pathway for these metals into the human system. Positive correlations among Cu, Fe and Zn suggested co-occurrence and potential synergistic absorption, while negative correlations for Cd and Pb suggested competitive interactions during uptake. ANOVA results confirmed that both site and location significantly influenced metal levels in blood serum, with strong interactions for most metals, highlighting the direct impact of irrigation water and crop metal content on human exposure.

Pollution Indices

Contamination factor (CF): CF values indicated moderate contamination in sewage water-irrigated sites for most metals, with Fe, Mn, and Zn showing the highest contamination levels in soils and crops. Tube well-irrigated sites showed minimal contamination (Table 8).

Enrichment factor (EF): EF analysis revealed species-specific uptake patterns. Carrot preferentially accumulated Fe and Mn, while turnip favored Zn and Pb, reflecting differences in plant physiology, metal mobility, and soil-metal availability (Table 9).

Bioconcentration factor (BCF): BCF values further confirmed selective accumulation in crops. Carrot exhibited higher BCF for Zn, Fe, and Mn, while turnip showed elevated BCF for Pb and Zn. Site-specific differences were evident, with SW sites showing the highest BCF values due to elevated metal availability in soil (Table 10).

Estimated daily intake (EDI) and hazard quotient (HQ): Although both HQ and EDI values for metals in crops from sewage water sites remain below 1, depicting permissible exposure levels, the comparative risk between crops is important. Carrots show a higher accumulation of metals such as Zn, Pb, and Ni compared to turnips, indicating that carrots may pose a relatively higher risk for these specific metals. Despite this, the current HQ (Table 12) and EDI (Table 11) values for all metals remain below the threshold of concern, meaning that, under typical consumption patterns, the metal levels in these crops do not pose an immediate health risk.

Table 8. Contamination factor of metals in soil of *Brassica rapa* at different locations of site 1 & 2.

Sites	Cu	Fe	Mn	Zn	Cd	Cr	Ni	Pb
CW1	0.3198	0.2732	0.0923	0.2909	0.0060	0.0381	0.1817	0.1655
SW1	0.3462	0.3064	0.1044	0.3070	0.0097	0.0531	0.2491	0.2525
TW1	0.2604	0.2068	0.0786	0.253	0.0039	0.0157	0.1259	0.0896
CW2	0.2459	0.2012	0.0673	0.2472	0.0032	0.0185	0.1319	0.0854
SW2	0.3307	0.2677	0.0997	0.2614	0.0054	0.0361	0.1926	0.1450
TW2	0.1790	0.1536	0.0569	0.2232	0.0017	0.0058	0.1091	0.0744
Assessment of contamination factor of metals in *Daucus carrota* at different locations of site 1 & 2								
CW1	0.3359	0.2788	0.0831	0.2954	0.0064	0.0241	0.1940	0.1398
SW1	0.3813	0.3116	0.1198	0.3112	0.0094	0.0536	0.2582	0.2041
TW1	0.2695	0.2191	0.0716	0.2693	0.0038	0.0194	0.1361	0.1150
CW2	0.2759	0.2015	0.0725	0.2491	0.0035	0.0156	0.1438	0.0918
SW2	0.3465	0.2714	0.0946	0.2754	0.0054	0.0343	0.2045	0.1476
TW2	0.1829	0.1627	0.0615	0.2268	0.0019	0.0090	0.1122	0.0600

Table 9. Enrichment factor of metals at different locations of site 1 & 2.

Sites	Cu	Fe	Mn	Zn	Cd	Cr	Ni	Pb
CW1	0.0465	0.0105	0.0125	0.0586	0.4345	0.3183	0.0512	0.2882
SW1	0.0535	0.0121	0.0141	0.0743	0.4707	0.7173	0.0857	0.2481
TW1	0.0392	0.0080	0.0080	0.0462	0.5109	0.5285	0.0668	0.1119
CW2	0.0485	0.0074	0.0100	0.0531	0.7386	0.4598	0.0732	0.2062
SW2	0.0432	0.0115	0.0097	0.0775	0.6217	0.3995	0.0691	0.2328
TW2	0.0430	0.0062	0.0081	0.0320	0.4002	0.7383	0.0601	0.0394
Enrichment factor of *Daucus carrota* samples collected from different locations of site 1 & 2								
CW1	0.0501	0.0016	0.0033	0.0224	0.4975	0.6375	0.0772	0.9364
SW1	0.0567	0.0017	0.0028	0.0290	0.5442	0.8937	0.0768	0.8871
TW1	0.0430	0.0012	0.0021	0.0174	0.6631	0.4792	0.0754	0.7424
CW2	0.0457	0.0012	0.0022	0.0211	0.7272	0.6085	0.0657	0.8319
SW2	0.0453	0.0017	0.0024	0.0292	0.7668	0.7084	0.0679	0.9133
TW2	0.0427	0.0010	0.0018	0.0128	0.6497	0.5830	0.0528	0.9327

Table 10. Bioconcentration factor of metals at different locations of site 1 & 2.

Sites	Cu	Fe	Mn	Zn	Cd	Cr	Ni	Pb
CW1	0.1623	0.0105	0.1343	0.3664	0.1143	0.0807	0.0985	0.1061
SW1	0.1865	0.0121	0.1513	0.4646	0.1238	0.1819	0.1648	0.0913
TW1	0.1368	0.0080	0.0865	0.2890	0.1344	0.1340	0.1284	0.0412
CW2	0.1691	0.0074	0.1073	0.3324	0.1943	0.1166	0.1408	0.0759
SW2	0.1507	0.0115	0.1047	0.4847	0.1636	0.1013	0.1329	0.0857
Estimation of bioconcentration factor of metals in *Daucus carrota* collected from different locations of site 1 & 2								
CW1	0.1754	0.0922	0.1548	0.3595	0.1309	0.1616	0.2007	0.3446
SW1	0.1985	0.0981	0.1331	0.4647	0.1432	0.2266	0.1997	0.3265
TW1	0.1505	0.0668	0.1016	0.2789	0.1745	0.1215	0.1962	0.2732
CW2	0.1601	0.0665	0.1061	0.3379	0.1913	0.1543	0.1708	0.3062
SW2	0.1588	0.0947	0.1153	0.4674	0.2018	0.1796	0.1766	0.3361
TW2	0.1496	0.0561	0.0879	0.2057	0.1709	0.1478	0.1373	0.3433

Table 11. Estimated daily intake of metals by human through *Brassica rapa* at different sampling sites.

Sites	Cu	Fe	Mn	Zn	Cd	Cr	Ni	Pb
CW1	0.0256	0.0483	0.1011	0.2038	0.0018	0.0047	0.0068	0.0248
SW1	0.0318	0.0627	0.1287	0.2728	0.0031	0.0147	0.0149	0.0326
TW1	0.0175	0.0280	0.0554	0.1398	0.0013	0.0032	0.0048	0.0052
CW2	0.0205	0.0253	0.0589	0.1572	0.0016	0.0033	0.0067	0.0091
SW2	0.0246	0.0521	0.0851	0.2423	0.0023	0.0056	0.0093	0.0175
TW2	0.0132	0.0160	0.0402	0.0854	0.0004	0.0016	0.0045	0.0015
Estimated daily intake by human through contaminated *Daucus carrota* from multiple sampling sites								
CW1	0.0290	0.0559	0.1049	0.2076	0.0022	0.0059	0.0141	0.0299
SW1	0.0373	0.0665	0.1300	0.2766	0.0035	0.0186	0.0187	0.0364
TW1	0.0200	0.0318	0.0592	0.1436	0.0017	0.0036	0.0096	0.0062
CW2	0.0218	0.0291	0.0627	0.1610	0.0017	0.0036	0.0089	0.0130
SW2	0.0271	0.0559	0.0889	0.2462	0.0028	0.0094	0.0131	0.0214
TW2	0.0135	0.0198	0.0441	0.0892	0.0008	0.0020	0.0056	0.0024

Table 12. Hazard quotient of metals in *Brassica rapa* grown at different irrigation sites.

Sites	Cu	Fe	Mn	Zn	Cd	Cr	Ni	Pb
CW1	0.6406	0.6903	0.7221	0.6795	0.1836	0.0031	0.3442	0.6215
SW1	0.7968	0.8961	0.9198	0.9095	0.3174	0.0098	0.7458	0.816
TW1	0.4398	0.4007	0.3961	0.4662	0.1389	0.0021	0.2422	0.1306
CW2	0.5131	0.3624	0.4207	0.5240	0.1657	0.0022	0.3378	0.2295
SW2	0.6151	0.7449	0.6083	0.8079	0.2333	0.0037	0.4653	0.4398
TW2	0.3315	0.2295	0.2877	0.2847	0.0484	0.0011	0.2295	0.0382
Hazard quotient of metals in *Daucus carrota* collected from diverse irrigation regimes								
CW1	0.0290	0.7996	0.7495	0.6923	0.2218	0.0039	0.7076	0.7490
SW1	0.9339	0.9507	0.9289	0.9222	0.3557	0.0124	0.9371	0.9116
TW1	0.5004	0.4553	0.4234	0.4789	0.1772	0.00240	0.4845	0.1561
CW2	0.0218	0.4171	0.4480	0.5367	0.1797	0.00246	0.4462	0.3251
SW2	0.0271	0.7996	0.6356	0.8206	0.2894	0.0062	0.6566	0.5355
TW2	0.0135	0.2841	0.3151	0.2975	0.0867	0.0013	0.2805	0.0605

Discussion

The comparative assessment of heavy metal accumulation in carrot (*Daucus carota*) and turnip (*Brassica rapa*) revealed clear species-specific differences in metal uptake under identical irrigation conditions. In carrot tissues, copper concentrations ranged from 0.35 to 0.97 mg kg^{-1}, with the highest values observed at sewage-water-irrigated sites (SW1), while tube-well-irrigated sites (TW2) consistently exhibited the lowest concentrations. Turnip showed comparatively similar but slightly lower copper accumulation, ranging from 0.34 to 0.83 mg kg^{-1}. Iron accumulation was higher in carrot (0.52–1.74 mg kg^{-1}) than in turnip (0.42–1.64 mg kg^{-1}), indicating a greater affinity of carrot roots for Fe uptake. Manganese followed a similar pattern, with carrot tissues containing 1.15–3.40 mg kg^{-1} compared to 1.05–3.36 mg kg^{-1} in turnip. Zinc showed the highest overall accumulation among the studied metals in both crops, ranging from 2.33 to 7.23 mg kg^{-1} in carrot and 2.23 to 7.13 mg kg^{-1} in turnip, again peaking at sewage-water-irrigated sites.

Cadmium concentrations remained low in both vegetables but showed a clear irrigation-dependent trend, with carrot accumulating 0.02–0.09 mg kg^{-1} and turnip 0.01–0.08 mg kg^{-1}. Chromium concentrations in carrot ranged from 0.05 to 0.48 mg kg^{-1}, which were slightly higher than those recorded in turnip (0.04–0.38 mg kg^{-1}), suggesting differential translocation efficiency between the two species. Nickel levels were higher in carrot (0.14–0.49 mg kg^{-1}) than in turnip (0.12–0.39 mg kg^{-1}), while lead concentrations followed the same trend, with carrot accumulating 0.06–0.95 mg kg^{-1} and turnip 0.04–0.85 mg kg^{-1}. Across all metals, the highest concentrations were consistently recorded at sewage-water sites, whereas tube well sites showed minimal accumulation, reflecting the influence of irrigation source on metal transfer to edible tissues.

Although sewage water irrigation enhanced metal availability in soils, the concentrations recorded in both carrot and turnip tissues remained within internationally accepted permissible limits for food crops (Ghafar *et al*., 2021: Kaur *et al*., 2025). The slightly higher accumulation of Fe, Mn, Ni and Pb in carrot suggests that carrot may serve as a more sensitive bioindicator of soil contamination compared to turnip, while turnip demonstrated relatively efficient accumulation of Zn under similar conditions (Ghafar *et al*., 2022; Novak *et al*., 2023). These differences can be attributed to variations in root architecture, surface area, and metal binding affinity of each species (Pandey *et al*., 2022). Importantly, despite observable differences in uptake efficiency, both crops exhibited controlled accumulation patterns, indicating limited immediate risk to human consumers (Pirasteh-Anosheh *et al*., 2023; Luz *et al*., 2025).

The integrated comparison confirms that wastewater irrigation increases metal accumulation in root vegetables but does not result in excessive contamination under the current conditions. However, the consistently higher concentrations at sewage-water sites highlight the potential for cumulative buildup over prolonged cultivation periods. The combined evaluation of carrot and turnip strengthens the generalizability of the findings to root vegetables grown under wastewater irrigation and underscores the need for regular monitoring to safeguard long-term food safety and public health.

Conclusion

The findings of the current study showed that wastewater irrigation significantly affects heavy metal accumulation in root vegetables, with carrot samples showing higher metal uptake than turnip. Although essential metals like Cu and Zn are present in beneficial levels, elevated concentrations of toxic metals like Cd and Pb raise concerns about long-term exposure. The findings underscore the need for careful management and monitoring of irrigation sources, soils and crops to minimize risks to food safety.

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