Pak. J. Bot., 57(1): 101-112, 2025. DOI: http://dx.doi.org/10.30848/PJB2025-1(3)

HAZARDOUS EFFECTS OF CHROMIUM IN VEGETABLES GROWN IN EXCESSIVE APPLICATION OF ORGANIC MANURE AND MINERAL FERTILIZERS WITH DIFFERENT WATER SOURCES

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Abstract

Food security is a significant focus for quantitative and qualitative global development. Chromium is essential to life but also toxic in specific frequencies and concentrations (including humans). While its existence in wastewater and fertilizers can benefit land when used in low quantities, it can also pose a prolonged risk to plants, water resources, animals, and humans. Consequently, a study was conducted to explore the potential ramifications of chromium in wastewater and soil treated with fertilizers, with a specific focus on the ability of Cr to migrate from contaminated soil into vegetables (*Daucus carota, Brassica oleracea, Spinacia oleracea, Raphanus sativus, Solanum tuberosum, Pisum sativum)*. The highest concentration of chromium (Cr) was observed in treatment T_8, which involved the combined use of poultry manure and wastewater with a higher pollution load index (2.24). *D. carota* had the most increased uptake of Cr (4.09) at T_8 during the second growing year out of six regularly used vegetables with 12 treatments. BCF < 1 means that less movement of metal from soil to vegetables. Enrichment factor values (1.082) exceeding 1 were observed, indicating that human activities have a discernible impact on the accumulation of heavy metals in the soil. The DIM for chromium was less than 1, indicating that consuming these vegetables will not cause higher level of toxicity for consumer. Long-term applications of wastewater and fertilizers can lead to increased concentrations of chromium (Cr) in the soil, vegetables, and finally in the individuals who consuming these vegetables. This means that, despite the many advantages of wastewater and fertilizers, heavy metals and metalloids in wastewater, fertilizers, soil, and vegetables should be strictly and regularly examined. Clear laws and regulatory control are required to reduce agricultural soil pollution from wastewater and fertilizer application.

Key words: Bio-concentration factor, Enrichment factor, Pollution load index, Poultry manure, Sugarcane bagasse, Wastewater.

Introduction

Long-term use of wastewater polluted with heavy metals for irrigation enhances heavy metal concentrations in soils over permissible levels (Khan *et al*., 2011). Sustained irrigation with wastewater results in the contamination of both soil and plants with heavy metals. However, the application of organic and inorganic fertilizers can mitigate this issue (Singh *et al*., 2010). Although vegetables are indeed beneficial for health, the use of chemicals such as fertilizers to boost production is harmful for the soil and human health (Mohamed *et al*., 2018). Due to the growing consumption of leafy vegetables and economic significance in tropical regions, it is prevalent for vegetable gardens and fields to be provided with too much nitrogen (N) and other chemical fertilizer to get a maximum yield (Baitilwake *et al*., 2011).

Fertilizers serve not only to supply essential nutrients but also contain adsorption sites that can aid in the removal of pollutants. Moreover, fertilizers have been utilized to mitigate the uptake of heavy metals by plants, thereby reducing potential contamination (Haytova, 2013). Due to the abundant organic content in compost, farmyard manure, and bio-solid compost, the presence of P and Fe makes it less likely for heavy metals to be detected in the soil (Ahmad *et al*., 2019). Inorganic variations, through the formation of binding sites, have been shown to limit the accessibility of metals to biota (Kiran *et al*., 2016). Introducing nitrogen and phosphorus through fertilizers can influence heavy metal removal by enhancing the natural metabolic activities of plants or by modifying the metal's chemical compositions (Adegoke *et al*., 2016).

Chromium (Cr), the second largest metal pollutant in groundwater, sediment, and soil, presents a significant environmental hazard for significant industrial applications. The two chemical elements of Cr that are more persistent are Cr (III) and Cr (VI) (Mishra *et al*., 2019). Due to its being more soluble, transportable, and dangerous than Cr (III), Cr (VI) state of chromium appears to be more harmful to both individuals and animals (Ukhurebor *et al*., 2021). As a non-essential element for plants, Cr lacks an absorption mechanism but it is assimilated along other essential components like sulphate through sulphate carriers. Humans require element Cr (III) related to the biosynthetic pathway, lipids, and carbohydrates (Zhao *et al*., 2020). On the other hand, elevated exposure to or consumption of Cr can be harmful to human organs (kidneys, lungs, skin and liver). Regarding inhalation and carcinogenic potential, Cr(VI) is a hundred times more poisonous than Cr(III) (EFSA Panel on Dietetic Products & Allergies, 2014). The mobility, uptake, and ecological toxicity in the soil-plant ecosystem are influenced by the chemical interactions of chromium (Ao *et al*., 2022). Recent study is showed that with a high level of soil organic matter (OM), potentially toxic elements (PTEs) were capable of being stabilized, and Cr was prevented from oxidation, reducing the chance of absorption of plants (Gattullo *et al*., 2020). Chromium prevents photosynthetic absorption by plants and impacts water absorption and minerals essential for crop growth (Das *et al*., 2021). Chromium toxicity produces reactive oxygen species (ROS), which influence various enzyme activities related to starch and nutrient uptake (Wakeel *et al*., 2020).

Species of plant may go extinct due to Cr as only a limited number of plant species can accumulate significant quantities of Cr without experiencing notable adverse effects. Phytoremediation involves hyper-accumulator and Cr-resistant plants (Chen *et al*., 2017). Humans require the mineral chromium in small amounts, but it must come from our food. Supplemental Cr can be beneficial for individuals with diabetes or reduced glucose metabolism (Vincent, 2018). Chromium enhances the body's fat metabolism, aiding in weight loss for individuals. Chromium supports mental skills, dissolves carbohydrates and fats and maintains sugar levels, which are all essential for health (Qafokua *et al*., 2022). Chromium (Cr) must enter biodiversity through climate transport, adversely affecting sprouting and biomass production, inducing structural abnormalities, metabolic and molecular modifications, and changes in metabolic activities (Hassanisaadi *et al*., 2022). Due to limited resources, farmers increasingly turn to wastewater as an alternative to supplement freshwater, causing adverse effects on crop production and humans (Sakiewicz *et al*., 2020). The present study aimed to evaluate the toxicity of chromium (Cr) in water, soil, and vegetables; assess the extent of soil contamination; and evaluate various indices of mobility and contamination, along with health risks. These assessments are vital for preventing the public from ingesting toxic levels of Cr through the consumption of contaminated vegetables. Researchers and farmers are likely learning about the effects of waste, canal water and fertilizers (organic manure + inorganic) on soil and vegetables from this research. This work provides a baseline for the farmer and researchers for appropriate applications of fertilizers and awareness of harmful effects of fertilizers and sewage water irrigation for vegetables

Material and Methods

Study area: The present research was conducted in Sargodha, an area located in the province of Punjab, Pakistan (Table 1). Plough thoroughly and ensured the ground was clear of weeds or large dirt clumps. The study contained 12 treatment combinations, which consisted of four types of organic manures (control without any manure, cow dung used at a rate of 20 tons per hectare, poultry droppings applied at 3 tons per hectare, leaf debris used at 50 tons per hectare, and sugarcane bagasse ash at 40 tons per hectare) along with inorganic fertilizer (nitrate) applied at 100 kg per hectare. The treatments also involved irrigation using canal water and wastewater. The winter vegetables, included Brassica Oleracea (cabbage), Daucus Carota (carrot), Pisum Sativum (pea), Solanum tuberosum (potato), Raphanus Sativus (radish), and Spinacia Oleracea (spinach), were cultivated in the period from October 2018 to 2019 (Table 2). Each seeds of vegetable were planted in its plot, thoroughly filled with compost. The vegetables received proper irrigation, using canal water and wastewater, at intervals ranging from 7 to 14 days. Following a growth period of 60 days' post-germination, the vegetables were harvested. From both irrigation sources, five replicate samples of water were taken.

To uphold the concentration of heavy metals in the irrigation water, one ml of HNO_3 was introduced per liter of the water sample. Vegetables were randomly harvested from each plot and watered with canal and wastewater at 5-day intervals for two (winter season) from 2018-2019 and 2019-2020. Only edible portions of the selected vegetables were collected. The study was conducted at a consistent location and maintained the same climate conditions to ensure uniformity. Metals in the vegetables were identified by analyzing the mature vegetable parts.

Table 1. Study region.

Region	Tehsil	Location	Source of irrigation	Geographic position
Sargodha	Sargodha	Lahore road Chak No 89 S.B	Wastewater and canal water	32.083°N 72.6719°E

Table 2. List of vegetables.

Scientific name	English name	Portion used for analysis
Daucus carota	Carrot	Root
Brassica oleracea	Cauliflower	Fruit
Spinacia oleracea	Spinach	Leaves
Raphanus sativus	Raddish	Root
Solanum tuberosum	Potato	Root
Pisum sativum	Pea	Fruit

Water, soil, vegetables sampling: Before manure addition, soil samples were collected at 0–30 cm depths for physical analysis. After manure addition, 72 samples of soil were collected from every plot of vegetables for metal analysis and physical analysis. A stainless steel auger was used to dig the soil for samples at a depth of 30 cm, partially containing all the layers. After that, the soil samples were air-dried in the air and placed in plastic bags for future research. Vegetables were irrigated with canal water and wastewater at 5-day intervals for two (winter season) from 2018-2019 and 2019-2020 and were collected randomly from each plot. Soil irrigated with wastewater (180) and soil irrigated with canal water (180). Five replicates of vegetable and soil samples were collected from each treatment plot. The irrigated water samples were collected from two irrigation sources (wastewater and canal water). Samples were washed with the water to remove dirt elements, and these samples were dried in air and kept for 48 hours in an oven set at 70 to 80°C. Using a mortar and pestle, dry samples were crushed. After that, the samples were air-dried and kept in sealed paper bags with labels, while water samples were collected in labeled plastic bottles. Experiment was conducted at a consistent location and maintained the same climate conditions to ensure uniformity. Metals in the vegetables were identified by analyzing the mature vegetable parts.

Heavy metal analysis: The following procedure was used to digest soil, vegetable, and water samples for heavy metal analysis.

Soil/ Vegetable sample digestion: Soil and vegetable samples were digested by FAO guidelines (1985). Then, 15 mL aqua regia was mixed to 0.5 g and dried in the air and a powder sample of soil and vegetables was placed in a

Pyrex beaker. It was left for overnight and then heated on a hot skillet until the brown smells ceased coming out—concentrated HClO4 (5 mL) with three parts of conc. Nitric acid (HNO_3) was added, and the solution was heated on low heat until nearly dry. The extracts were filtered, and a final volume of 25 mL was prepared in a clean volumetric flask with double-deionized water.

Water sampling and preparation: The samples were obtained in plastic bottles with a volume of 500 mL. To avoid contamination, the heavy metal sampling bottles in a 10% HCl solution for overnight and then washed thoroughly with distilled water. To avoid microbial heavy metal use, 1-2 drops of concentrated HNO_3 were mixed thoroughly, which was then kept at 4°C until evaluation.

Metal determination: By using an atomic absorption spectrophotometer (AAS) (Shimadzu Co., Ltd., Japan) which was calibrated before using it, the metals concentration in water, soil, and food crops was assessed. The samples were run by using the National Institute of Standard Technology. Standard Reference Material for all metals (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) confirmed the accuracy and precision of the analysis.

Following the filtration of the digestion mixture, distilled water was added to make the volume up to 50 ml. The assessment of chromium content in the digested samples was conducted at the University of Sargodha by employing an atomic absorption spectrophotometer (Shimadzu Co., Ltd., Japan). Calibration of the instrument was conducted prior to use. The analysis ensured accuracy and precision by utilizing the National Institute of Standards and Technology's Standard Reference Material (SRM 1570) for chromium metal (Qureshi *et al*., 2016). The operational parameters were as follows: acetylene flow rate at 2.8 L/min, Wavelength measured at 422.7 nm, Slit width of 0.7 nm, Lamp current at 10 mA, Burner height set to 9 mm (Gashaye, 2020).

Quality control: The study's results were checked against quality criteria. All of the quantitative compounds for the experiment were from Sigma Aldrich, Merck (Germany), and BDH (U.K.). Only Pyrex glassware was utilized in these tests. Prior to experiment the glassware was cleaned with Max liquid detergent and then oven-dried for 1 hour at 100°C, leaving it immaculate.

Indices for Pollution and Health Risks

Pollutant load index: The calculation of the pollutant load index was done using Equation 1 (Du *et al*., 2022).

$$\text{PLI} = \frac{(M)IS}{(M)RS} \quad \text{(1)}$$

The chromium levels were indicated as 9.07mg/kg in soil and 2.3mg/kg in food crops, as proposed by (Food & Organization, 2001).

Where M is the amount of metal present. IS was a metal (mg/kg) in the crop-growing soil that was under investigation. The metal in the soil's reference value was RS.

PLI 1 represents the absence of heavy metal contamination, while PLI > 1 or equal to 1 denotes the presence of heavy metal contamination and poor soil conditions (Qishlaqi *et al*., 2009).

BCF: Equation 2 was used to determine the Bio-concentration Factor (BCF), as described by (Akhtar *et al*., 2022).

$$\text{BCF} = \frac{\textit{Metal in vegetables}}{\textit{Metal in soil}} \quad \text{(2)}$$

M denotes the metal concentration (mg/kg). A BCF value greater than one indicates that toxic metals were found in large quantities in food crops (Kisku *et al*., 2000). BCF values of 0.01 indicated non-accumulator plants, 0.1-1 indicated modest accumulator plants, and 1-10 indicated hyperaccumulator plants (Netty *et al*., 2013)

Enrichment factor: Buat-Menard & Chesselet, 1979 was calculated using the formula:

$$\text{EF} = \frac{\text{Metal (vegetables/soil) sample}}{\text{(M)R Metal (vegetables/soil) Reference value}} \quad \text{(3)}$$

The enrichment factor (EF) ranges from 1 (no enrichment) to 1-2 (minor enrichment), 3–4 (moderate-to-severe enrichment), 5–9 (severe enrichment), 10–24 (severe), 25–49 (very severe), and 50 or more (very severe enrichment).

Daily intake of metal: Equation 4 was employed to ascertain the recommended daily intake of heavy metals (Akhtar *et al*., 2022).

$$\text{DIM} = \frac{\text{Crop metal x C factor D (Food intake)}}{\text{B (Average weight)}} \quad \text{(4)}$$

where D (food intake) is the daily consumption of a food crop (in grms per kg, or kg/person), Cf (conversion factor) has a value of 0.085, C (metal) is the amount of heavy metals present in the food crop (in grms per kg), and B is the average body weight (65 kg) Akhtar *et al*., 2022).

Health risk index: In order to determine the HRI, DIM was divided by the external reference dosage Eq. 5 by (Singh *et al*., 2018).

$$\text{HRI} = \frac{DIM}{RfD} \quad \text{(5)}$$

where RfD is the oral reference dosage for metals and DIM is the daily intake metal. A health risk score was calculated to estimate the risks related to consumption of food crops contaminated with heavy metals. If the HRI is less than 1, those kinds of crops are safe for people to eat (Cui *et al*., 2004). According to reports, the consumer was in danger if the HRI was higher than one (Khan *et al*., 2010).

Statistical research: The statistical significance of the data obtained from each parameter was determined by using the Microsoft Excel and Minitab 16. A three-factor factorial design was utilized to examine water, soil, and crop samples and identify potential variations in mean values. The threshold for statistical significance was set at a P-value below 0.05

Results

Presence of chromium in irrigation water: Both in terms of types and years, the quantity of Chromium present in the water varied between 0.01516 mg/L and 0.0683 mg/L. In 2nd year, the level of chromium increased in WW, however, the level of cadmium was less in FW and 1st year (Table 3). The analysis of variance indicated statistical significance ($p<0.05$) variation on water type and years but showed non-significance variation among Water Type* years (Table 4).

Table 3. Chromium in water.

Cr	Freshwater	Wastewater
Year-1	0.01516	0.05871
Year-2	0.02171	0.06839

Presence of chromium in agricultural soil: Analysis of variance revealed that significant ($p<0.05$) variation on treatment, vegetables, years, and their interaction among Treatments × vegetables, Treatments × Years, vegetables × Years, Treatments × vegetables × Years in chromium soil (Table 5). At the wastewater treated soil, there were more metals than recorded in the canal water treated soil. In this study, the average concentrations of chromium were ranged between 4.923-23.48 mg/kg (Table 7).

Presence of chromium in vegetables: Analysis of variance revealed that significant ($p<0.05$) variation on treatment, vegetables, years, and their interactions like Treatments × vegetables, Treatments × Years, vegetables × Years, Treatments × vegetables × Years in chromium soil (Table 6). In the wastewater treated vegetables, there were more metals than at the canal water treated vegetables. In this study, the average concentrations of chromium were ranged from 0.008-4.556 mg/kg (Table 8).

Pollution load index of chromium: Pollution load index of chromium ranged from 0.542 to 2.65. Higher value of PLI was found in 2nd year treatment T_8 in *Spineca oleracea* while lower value of PLI was found in *Brassica oleracea* 1st year treatment T_0 (Table 9).

Bio-concentration factor of chromium: The assessment of the bio-concentration factor included concentrations ranging from 0.00075 mg/kg to 0.356 mg/kg. Higher amount of BCF was found in *Dacus carota* 1st year treatment T_1 while minimum amount of BCF was found in *Brassica oleracea* 2nd year treatment T_6 (Table 10).

Table 4. Analysis of variance of chromium water.

Source	DF	Adj SS	Adj MS	F-value	P-value
Years	1	0.000329	0.000329	19.94	0.000
Water type	1	0.010176	0.010176	615.85	0.000
Error	17	0.000281	0.000017		
Lack-of-Fit	1	0.000012	0.000012	0.73	0.405
Pure error	16	0.000269	0.000017		
Total	19	0.010786			

Table 5. Analysis of variance of chromium soil versus treatments, vegetables, years.

Source	DF	Adj SS	Adj MS	F-value	P-value
Treatments	11	17782.9	1616.63	1349.24	0.000
Vegetables	5	45.6	9.12	7.61	0.000
Years	1	219.1	219.08	182.85	0.000
Treatments*vegetables	55	250.1	4.55	3.79	0.000
Treatments*Years	11	22.5	2.05	1.71	0.068
Vegetables*Years	5	49.9	9.98	8.33	0.000
Treatments*vegetables*Years	55	183.0	3.33	2.78	0.000
Error	576	690.2	1.20		
Total	719	19243.2			

Table 6. Analysis of variance of chromium in vegetables versus treatments, vegetables, years.

Source	DF	Adj SS	Adj MS	F-value	P-value
Treatments	11	63.776	5.798	26889.98	0.000
Vegetables	5	504.242	100.848	467729.74	0.000
Years	1	2.210	2.210	10248.61	0.000
Treatments*vegetables	55	94.894	1.725	8002.04	0.000
Treatments*Years	11	2.198	0.200	926.64	0.000
Vegetables*Years	5	1.520	0.304	1410.25	0.000
Treatments*vegetables*Years	55	10.250	0.186	864.36	0.000
Error	576	0.124	0.000		
Total	719	679.213			

Table 7. Effects of fertilizers and diverse water treatments on mean concentrations and standard errors of chromium in various soil samples.

Treatments	Year-i						
	D. carota	*B. oleracea*	*P. sativum*	*S. tuberosum*	*R. sativus*	*S. oleracea*	Mean
T0	5.593±0.123	4.923±0.244	5.635±0.125	5.2741±0.042	5.8104±0.0496	5.847±0.172	5.513±0.12606
T1	8.798±0.027	8.043±0.233	8.401±0.355	8.596±0.236	8.606±0.16	9.248±0.264	8.615±0.2125
T2	11.40±0.059	10.768±0.384	12.035±0.258	11.749±0.323	11.871±0.0626	11.66±0.0616	11.58±0.1913
T3	7.32±0.0255	6.704±0.35	10.26±3.26	7.5993±0.035	7.165±0.145	8.34±0.194	7.8987±0.6683
T4	9.56±0.0402	9.232±0.233	11.82±2.74	9.924±0.201	9.4149±0.0921	9.156±0.178	9.852±0.580
T5	10.60±0.034	10.689±0.0644	11.096±0.673	10.602±0.105	9.9955±0.0593	12.547±0.519	10.922±0.242
T6	13.41±0.024	13.628±0.0482	13.447±0.0813	12.493±0.324	12.661±0.0608	12.387±0.409	13.004±0.158
T7	20.36±0.187	18.601±0.426	18.337±0.347	19.616±0.226	20.275±0.145	14.368±0.263	18.59±0.26566
T8	20.1±0.32	17.667±0.384	19.682±0.226	19.006±0.29	18.384±0.24	19.509±0.0616	19.06±0.2542
T9	19.6±2.56	22.992±0.0255	23.436±0.12	23.487±0.231	22.39±0.345	23.297±0.228	22.53±0.5849
T10	12.8±0.49	14.769±0.351	14.823±0.35	14.639±0.235	15.412±0.13	16.478±0.225	14.82±0.2973
T11	12.52±0.4	13.768±0.0644	13.562±0.303	13.728±0.060	15.037±0.695	13.575±0.211	13.69±0.28905
Mean	12.6±0.35	12.64±0.2339	13.54±0.7365	13.05±0.192	13.08515±0.182	13.034±0.23218	
	Year-ii						
T0	6.725±0.0046	5.527±0.198	6.1353±0.0325	6.74±0.247	6.814±0.24	6.671±0.173	6.43±0.14918
T1	9.689±0.0698	8.77±0.211	8.376±0.32	9.962±0.24	10.31±0.304	10.272±0.305	9.56±0.24
T2	13.385±0.385	11.118±0.382	12.858±0.269	12.908±0.304	12.947±0.247	11.668±0.17	12.480±0.2928
T3	9.396±0.221	7.562±0.361	8.084±0.226	8.074±0.163	9.269±0.226	9.177±0.177	8.593±0.229
T4	10.652±0.032	9.853±0.226	9.551±0.386	11.133±0.226	11.203±0.233	10.113±0.205	10.4175±0.218
T5	12.104±0.017	11.208±0.24	11.79±0.106	12.16±0.12	11.315±0.226	13.329±0.462	11.984±0.1951
T6	14.76±0.0192	15.008±0.148	14.021±0.242	13.65±0.285	13.509±0.361	13.742±0.382	14.116±0.239
T7	19.24±0.444	19.169±0.32	19.665±0.187	21.132±0.387	20.787±0.257	21.094±0.304	20.181±0.3165
T8	23.362±0.428	19.831±0.382	20.255±0.262	21.466±0.226	21.217±0.158	20.197±0.297	20.888±0.2921
T9	19.878±0.324	20.449±0.382	18.048±0.226	19.656±0.29	20.931±0.234	20.061±0.24	20.83±0.282
T10	16.239±0.155	15.596±0.297	15.725±0.305	15.538±0.304	17.146±0.257	17.325±0.274	16.2615±0.2653
T11	15.243±0.148	13.549±0.346	13.925±0.276	14.208±0.221	15.092±0.226	15.523±0.269	14.59±0.2476
	14.473±0.1873	13.386±0.29108	13.702±0.23645	14.21±0.25108	14.461±0.2474	14.431±0.2715	

Table 8. Effects of fertilizers and diverse water treatments on mean concentrations and standard errors of chromium in vegetables samples.

Treatments	Year-i						
	D. carota	*B. oleracea*	*P. sativum*	*S. tuberosum*	*R. sativus*	*S. oleracea*	Mean
T0	1.155±0.003	0.026±0.002	0.1143±0.001	0.0081±0.002	0.0842±0.0031	0.167±0.015	0.259±0.0047
T1	3.135±0.001	0.47±0.0021	0.360±0.0030	0.159±0.0036	0.2528±0.0033	0.837±0.0036	0.869±0.0028
T2	3.591±0.002	0.48±0.0029	0.359±0.0022	0.186±0.0027	0.2853±0.0039	1.025±0.0026	0.988±0.0028
T3	1.912±0.002	0.40±0.0040	0.252±0.0036	0.076±0.0030	0.117±0.00144	0.454±0.0037	0.536±0.0031
T4	2.066±0.002	0.438±0.002	0.291±0.003	0.0946±0.002	0.1657±0.0024	0.521±0.0038	0.5964±0.003
T5	2.560±0.003	0.454±0.002	0.3466±0.003	0.1221±0.003	0.19595±0.003	0.558±0.0031	0.706±0.0032
T6	0.424±0.027	0.199±0.031	0.0878±0.002	0.1018±0.026	0.02454±0.006	0.163±0.0202	0.166±0.0192
T7	3.299±0.002	0.48±0.0038	0.382±0.0033	0.187±0.0025	0.2811±0.0033	0.972±0.0035	0.935±0.0031
T8	4.059±0.002	0.48±0.003	0.3966±0.003	0.1994±0.003	0.3062±0.0038	1.275±0.0024	1.120±0.0032
T9	1.970±0.002	0.504±0.004	0.286±0.0036	0.088±0.0033	0.1398±0.0032	0.481±0.0032	0.578±0.0034
T10	2.21±0.0033	0.27±0.0043	0.316±0.0053	0.1056±0.002	0.1884±0.0038	0.591±0.0024	0.615±0.0036
T11	2.79±0.0026	0.218±0.003	0.371±0.0027	0.135±0.0044	0.2226±0.0022	0.775±0.0022	0.752±0.003
Mean	2.43±0.0047	0.37±0.0057	0.297±0.0032	0.122±0.0051	0.1886±0.0034	0.651±0.0055	
	Year-ii						
T0	1.275±0.003	0.1009±0.003	0.192±0.0019	0.037±0.0028	0.0453±0.0001	0.2201±0.027	0.3119±0.006
T1	3.153±0.003	0.48±0.0020	0.398±0.0031	0.184±0.0030	0.3628±0.0031	0.8839±0.0029	0.9120±0.002
T2	3.769±0.002	0.605±0.002	0.408±0.0041	0.206±0.0030	0.3239±0.0029	2.0479±0.0029	1.2268±0.003
T3	1.929±0.002	0.59±0.0030	0.293±0.0041	0.098±0.0033	0.2588±0.0038	0.6146±0.0034	0.631±0.0033
T4	2.107±0.003	0.457±0.002	0.313±0.0036	0.112±0.0024	0.2039±0.0036	0.6438±0.0033	0.639±0.0031
T5	2.760±0.002	0.485±0.002	0.365±0.0030	0.147±0.0020	0.2206±0.0038	0.5917±0.0033	0.761±0.0029
T6	0.650±.0132	0.011±0.002	0.1878±0.003	0.0159±0.004	0.0751±0.0040	0.1312±0.0274	0.178±0.0091
T7	3.365±0.003	0.508±0.003	0.420±0.0030	0.205±0.0032	0.3217±0.0043	1.0013±0.0024	0.970±0.0034
T8	4.09±0.0033	0.520±0.001	0.423±0.0029	0.217±0.0033	0.3301±0.0041	2.2822±0.0029	1.3110±0.003
T9	1.99±0.0033	0.182±0.003	0.303±0.0036	0.107±0.0029	0.1688±0.0041	0.5838±0.0034	0.556±0.0034
T10	2.381±0.001	0.44±0.0033	0.342±0.0026	0.135±0.0031	0.9939±0.0033	0.7728±0.0030	0.84±0.00293
T11	4.556±0.003	0.480±0.002	0.394±0.004	0.1716±0.0029	0.2393±0.00382	0.8213±0.00276	1.11055±0.003
	2.669±0.0037	0.406±0.002	0.336±0.0033	0.1366±0.003	0.29±0.003444	0.882±0.00708	

Table 9. Effects of fertilizers and diverse water treatments on pollution load index of chromium metal in various vegetables for both years.

Treatments	Year-i						
	D. carota	***B. oleracea***	***P. sativum***	***S. tuberosum***	***R. sativus***	***S. oleracea***	**Mean**
T0	0.616648	0.542778	0.621279	0.581488	0.640617	0.644653	0.607911
T1	0.970033	0.88677	0.92624	0.94774	0.948842	1.019625	0.949875
T2	1.257883	1.187211	1.326902	1.295369	1.30882	1.285557	1.276957
T3	0.807486	0.73914	1.131202	0.83785	0.789967	0.919515	0.87086
T4	1.054928	1.017861	1.303197	1.094157	1.038026	1.009482	1.086275
T5	1.169019	1.178501	1.223374	1.168908	1.10204	1.383352	1.204199
T6	1.478501	1.502536	1.48258	1.377398	1.395921	1.365711	1.433774
T7	2.224873	2.050827	2.02172	2.162734	2.235391	1.584123	2.049945
T8	2.243705	1.94785	2.170011	2.09548	2.026902	2.150937	2.102481
T9	2.164278	2.53495	2.583903	2.589526	2.468578	2.568578	2.484969
T10	1.411577	1.628335	1.634289	1.614002	1.699228	1.816759	1.634032
T11	1.380375	1.517971	1.495259	1.513561	1.657883	1.496692	1.51029
Mean	1.398275	1.394561	1.49333	1.439851	1.442685	1.437082	
	Year-ii						
T0	0.741555	0.609372	0.676439	0.743109	0.751268	0.735502	0.709541
T1	1.068346	0.966924	0.923484	1.098346	1.136714	1.132525	1.05439
T2	1.475744	1.225799	1.417641	1.423153	1.427453	1.286439	1.376038
T3	1.035943	0.833738	0.89129	0.890187	1.02194	1.011797	0.947483
T4	1.174421	1.086329	1.053032	1.227453	1.235171	1.114994	1.148567
T5	1.334509	1.235722	1.29989	1.340684	1.247519	1.46957	1.321316
T6	1.628115	1.654686	1.545865	1.504961	1.489416	1.515105	1.556358
T7	2.121279	2.113451	2.168137	2.329879	2.291841	2.325689	2.225046
T8	2.665491	2.186439	2.233186	2.366703	2.33925	2.226792	2.302977
T9	2.432635	2.585336	2.651378	2.608159	2.638479	2.652811	2.628133
T10	1.790408	1.719515	1.733738	1.71312	1.890408	1.910143	1.792889
T11	1.680595	1.493826	1.535281	1.566483	1.663947	1.711466	1.6086
	1.595753	1.475928	1.51078	1.567687	1.594451	1.591069	

Table 10. Effects of fertilizers and diverse water treatments on bio-concentration factor of chromium metal in various soil in both years.

Treatments	Year-i						
	D. carota	***B. oleracea***	***P. sativum***	***S. tuberosum***	***R. sativus***	***S. oleracea***	**Mean**
T0	0.006176	0.005381	0.020284	0.005992	0.004223	0.019326	0.029507
T1	0.051465	0.058784	0.044007	0.018538	0.029375	0.090538	0.045553
T2	0.051302	0.044911	0.02988	0.015891	0.024033	0.087916	0.039111
T3	0.043318	0.060538	0.030508	0.010119	0.016366	0.054544	0.033707
T4	0.027769	0.047476	0.02973	0.009532	0.0176	0.056979	0.030531
T5	0.027912	0.042502	0.031244	0.011517	0.019604	0.044513	0.018898
T6	0.005533	0.014661	0.006533	0.008149	0.003491	0.011125	0.018834
T7	0.038363	0.026206	0.020838	0.009561	0.013864	0.067689	0.030146
T8	0.04493	0.027648	0.02015	0.010491	0.016656	0.065354	0.024269
T9	0.032593	0.021929	0.012235	0.003751	0.006244	0.029242	0.019963
T10	0.038147	0.018776	0.021326	0.007214	0.012224	0.035878	0.025053
T11	0.041741	0.016089	0.027427	0.009878	0.014803	0.057134	0.027865
Mean	0.034104	0.032075	0.024513	0.010053	0.014874	0.051687	
	Year-ii						
T0	0.006714	0.018256	0.031332	0.013031	0.01103	0.021001	0.034461
T1	0.074593	0.055792	0.045931	0.01849	0.031309	0.086049	0.048295
T2	0.050375	0.054452	0.031755	0.015967	0.025017	0.08981	0.043371
T3	0.038186	0.078656	0.029086	0.012249	0.027921	0.066972	0.037251
T4	0.02853	0.046392	0.027097	0.01006	0.0182	0.063661	0.0309
T5	0.026493	0.043308	0.031008	0.01215	0.019502	0.044392	0.018305
T6	0.006454	0.000756	0.0134	0.001171	0.007055	0.013972	0.017507
T7	0.044787	0.026535	0.021378	0.009709	0.0174	0.047468	0.030173
T8	0.041593	0.026269	0.020923	0.010142	0.015558	0.080309	0.024992
T9	0.036385	0.007766	0.012637	0.00454	0.007054	0.036732	0.022331
T10	0.036572	0.028264	0.021755	0.008688	0.022973	0.044606	0.029076
T11	0.041941	0.034919	0.028352	0.012078	0.015856	0.052909	0.03053
	0.036052	0.035114	0.026221	0.01069	0.01824	0.05399	

Table 11. Effects of fertilizers and diverse water treatments on enrichment factor of chromium metal in various vegetables for both years.

Treatments	Year-i						
	D. carota	*B. oleracea*	*P. sativum*	*S. tuberosum*	*R. sativus*	*S. oleracea*	Mean
T0	0.1091	0.1015	0.00312	0.0515	0.0027	0.0263	0.0490
T1	0.1414	0.2133	0.00718	0.1628	0.0114	0.4172	0.1589
T2	0.1620	0.2181	0.00842	0.1622	0.0128	0.5260	0.1816
T3	0.1727	0.1831	0.00346	0.1136	0.0520	0.3762	0.1502
T4	0.1323	0.1977	0.0042	0.1317	0.0747	0.4130	0.1589
T5	0.1155	0.0204	0.0055	0.1564	0.0884	0.5660	0.1587
T6	0.0521	0.09014	0.0045	0.0963	0.0210	0.0558	0.0533
T7	0.1488	0.2199	0.0846	0.7238	0.1268	1.0821	0.3977
T8	0.1831	0.2203	0.0899	0.9276	0.0138	1.0760	0.4184
T9	0.1788	0.2274	0.0397	0.63638	0.1307	0.95105	0.3607
T10	0.1000	0.1251	0.0476	0.50261	0.2499	0.7434	0.2947
T11	0.1258	0.1984	0.0611	0.47811	0.1004	1.0244	0.3314
Mean	0.1351	0.16798	0.0299	0.3452	0.0737	0.6048	
	Year-ii						
T0	0.0218	0.0304	0.0006	0.0206	0.0005	0.0079	0.0136
T1	0.0282	0.0639	0.0014	0.0651	0.0022	0.1251	0.0477
T2	0.0324	0.0654	0.0016	0.0648	0.0025	0.1578	0.0541
T3	0.0345	0.0549	0.0006	0.0454	0.0104	0.1128	0.0431
T4	0.0264	0.0593	0.0008	0.0526	0.0149	0.1239	0.0463
T5	0.0231	0.0061	0.0011	0.0625	0.0176	0.1698	0.0467
T6	0.0104	0.0270	0.0009	0.0385	0.0042	0.0167	0.0163
T7	0.0297	0.0659	0.0169	0.2895	0.0253	0.3246	0.1253
T8	0.0366	0.0661	0.0179	0.3710	0.0027	0.3228	0.1362
T9	0.0357	0.0682	0.0079	0.2545	0.0261	0.2853	0.1129
T10	0.0200	0.0375	0.0095	0.2010	0.0499	0.2230	0.0901
T11	0.0251	0.0595	0.0122	0.1912	0.0200	0.3073	0.10260
	0.02703	0.0503	0.0059	0.1381	0.0147	0.1814	

Table 12. Effects of fertilizers and diverse water treatments on daily intake of metal of chromium for both years.

Treatments	Year-i						
	D. carota	*B. oleracea*	*P. sativum*	*S. tuberosum*	*R. sativus*	*S. oleracea*	Mean
T0	0.00052	0.00001	0.00005	0.000003	0.00003	0.00007	0.00011
T1	0.00141	0.00021	0.00016	0.00007	0.00011	0.00037	0.00039
T2	0.00162	0.00021	0.00016	0.00008	0.00012	0.00046	0.00044
T3	0.00086	0.00018	0.00011	0.00003	0.00005	0.00020	0.00024
T4	0.00093	0.00019	0.00013	0.00004	0.00007	0.00023	0.00026
T5	0.00115	0.00020	0.00015	0.00005	0.00008	0.00025	0.00031
T6	0.00019	0.00009	0.00003	0.00004	0.00001	0.00007	0.00007
T7	0.00148	0.00021	0.00018	0.00008	0.00012	0.00043	0.00042
T8	0.00183	0.00022	0.00017	0.00008	0.00013	0.00057	0.00050
T9	0.00088	0.00022	0.00012	0.00003	0.00006	0.00021	0.00026
T10	0.00100	0.00012	0.00014	0.00004	0.00008	0.00026	0.00027
T11	0.00125	0.00009	0.00016	0.00006	0.00010	0.00034	0.00033
Mean	0.00109	0.00016	0.00013	0.00005	0.00008	0.00029	
	Year-ii						
T0	0.00057	0.00004	0.00008	0.00001	0.00002	0.00009	0.00014
T1	0.00142	0.00022	0.00017	0.00008	0.00016	0.00039	0.00041
T2	0.00170	0.00027	0.00018	0.00009	0.00014	0.00092	0.00055
T3	0.00087	0.00026	0.00013	0.00004	0.00011	0.00027	0.00028
T4	0.00095	0.00020	0.00014	0.00005	0.00009	0.00029	0.00028
T5	0.00124	0.00021	0.00016	0.00006	0.00009	0.00026	0.00034
T6	0.00029	0.000005	0.00008	0.000007	0.00003	0.00005	0.00008
T7	0.00151	0.00022	0.00018	0.000092	0.00014	0.00045	0.00043
T8	0.00184	0.00023	0.00019	0.000098	0.000148	0.00102	0.00059
T9	0.00090	0.00008	0.00013	0.000048	0.000076	0.00026	0.000251
T10	0.00107	0.00019	0.00015	0.00006	0.000448	0.00034	0.00038
T11	0.00205	0.00021	0.00017	0.00007	0.000107	0.00037	0.000501
	0.00120	0.00018	0.00015	0.000061	0.000133	0.00039	

Table 13. Effects of fertilizers and diverse water treatments on health risk index of metal of chromium for both years.

Treatments	*D. carota*	*B. oleracea*	*P. sativum*	*S. tuberosum*	*R. sativus*	*S. oleracea*	Mean
	Year-i						
T0	0.002117	0.003316	0.002354	0.004963	0.008894	0.028543	0.008364
T1	0.008737	0.030257	0.018768	0.046153	0.037183	0.073328	0.035738
T2	0.008993	0.03716	0.018618	0.059943	0.045642	0.006775	0.029522
T3	0.005489	0.017445	0.006722	0.036874	0.026197	0.060274	0.0255
T4	0.006015	0.020873	0.008782	0.04104	0.028573	0.065237	0.02842
T5	0.006948	0.02528	0.011294	0.045506	0.031127	0.071764	0.031986
T6	0.004596	0.013933	0.001978	0.012049	0.008106	0.022889	0.010592
T7	0.011144	0.036017	0.019971	0.054244	0.039115	0.006587	0.027846
T8	0.00973	0.040506	0.021746	0.066605	0.049296	0.007993	0.032646
T9	0.006482	0.019821	0.006918	0.038168	0.029558	0.06238	0.027221
T10	0.006076	0.02164	0.00976	0.044454	0.032054	0.066947	0.030155
T11	0.006752	0.030678	0.015339	0.052845	0.031912	0.073433	0.03516
Mean	0.002117	0.003316	0.002354	0.004963	0.008894	0.028543	
	Year-ii						
T0	0.001033	0.020753	0.001199	0.018037	0.01958	0.027626	0.014705
T1	0.012723	0.052454	0.024528	0.048732	0.0702	0.091374	0.050002
T2	0.009354	0.04313	0.023625	0.063703	0.071132	0.012948	0.037315
T3	0.008106	0.023219	0.010993	0.039491	0.032423	0.095554	0.034964
T4	0.008707	0.037807	0.011835	0.043341	0.041596	0.095479	0.039794
T5	0.010166	0.028122	0.014287	0.048965	0.041251	0.080606	0.037233
T6	0.003342	0.133385	0.004084	0.021776	0.040093	0.023565	0.037707
T7	0.009128	0.03892	0.024227	0.060725	0.074621	0.014001	0.036937
T8	0.012347	0.043882	0.02449	0.069012	0.052605	0.011474	0.035635
T9	0.009429	0.022753	0.010031	0.042017	0.032588	0.083674	0.033415
T10	0.009008	0.024994	0.012452	0.049868	0.009955	0.085073	0.031892
T11	0.010798	0.03322	0.018031	0.055823	0.037461	0.079328	0.03911
	0.008678	0.041887	0.014982	0.046791	0.043625	0.058392	

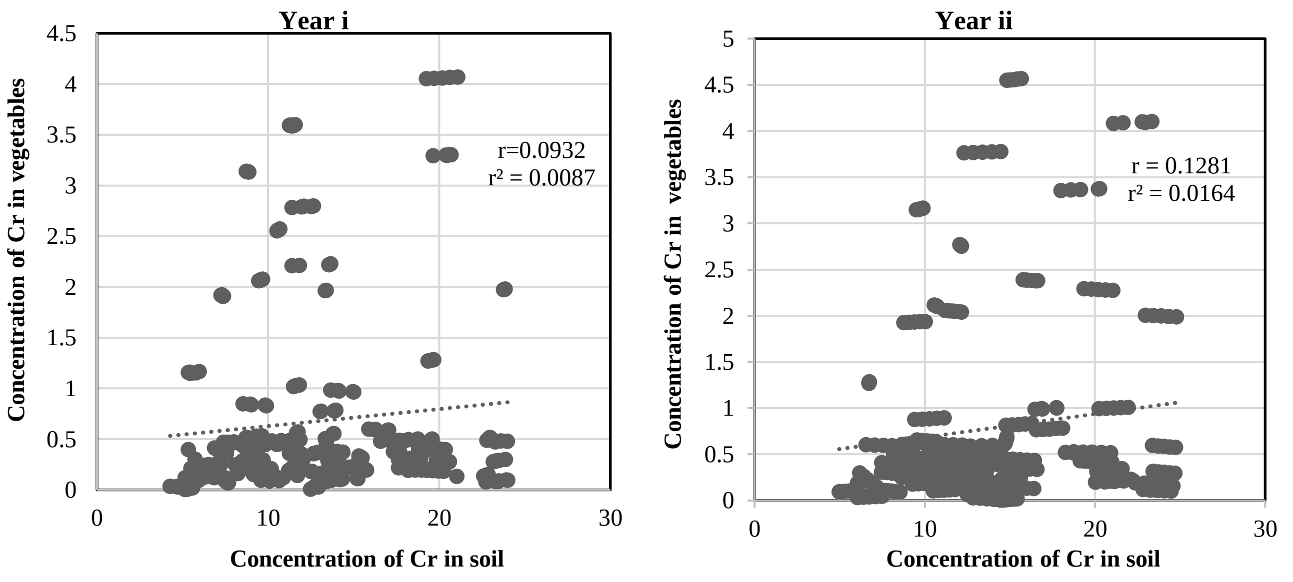

Fig. 1. Co-relation of soil and vegetables in chromium for both years.

Enrichment factor of chromium: The measurement of the enrichment factor included concentrations varying from 0.00298 mg/kg to 1.405 mg/kg. The highest quantity of EF was observed in *Dacus carota* 1st year treatment T_1 while Minimum amount of EF was found in *Brassica oleracea* during 2nd year in treatment T_6 (Table 11).

Daily intake of metal in chromium: Metal daily intake was assessed, with concentrations varying from 0.00000368 mg/kg to 0.00205 mg/kg. The greater quantity of DIM was recorded in *Dacus carota* 1st year treatment T_9 while minimum amount of DIM was found in *Solanum tuberosum* 1st year treatment T_0 (Table 12).

Health risk index of metal in chromium: The health risk index of the metal was evaluated, with concentrations varying from 0.00122 mg/kg to 0.133 mg/kg. Greater amount of HRI was identified in *Dacus carota* during 1st year treatment T_0 while minimum amount of HRI was found in *Brassica oleracea* during 2nd year in treatment T_6 (Table 13).

Chromium (Cr) contents analysis with a scatter plot: A scatter plot analysis compared chromium levels in soils and vegetables across all treatments, revealed a significant positive relationship. This association highlights the cumulative influence of Chromium (Cr) on the treatments administered to both the soil and the vegetables (Fig. 1).

Discussion

Presence of chromium in irrigation water: Agreed with present findings, Ugulu *et al*., (2021) who reported similar quantities of chromium. In comparison to the results (0.04 mg/L) of Alghobar & Suresha (2016), the current analysis found higher concentration of chromium in the water. The concentrations of chromium in the wastewater were lower than the permitted limits of heavy metals in irrigation water (FAO 1985; Pescod 1992). According to Sandeep *et al*., (2019) the concentration of chromium (0.067 mg/L) was similar as in the current finding. The concentration of chromium in each of the water samples was significantly lower than the WWF (2007) who recommended maximum allowable level of 0.1 mg/L. Chromium can infiltrate environmental watersheds through erosion of chromium sediments or through effluent application by industrial applications, soil leaching, and so on. Chromium may undergo conversion, oxidation, absorption, solubility, and deposition in the water habitats (Pradhan, 2012). Only small levels of contaminants could be found in water. Various industries can discharge the chromium and its metabolites into surface water. It is used in metal surface refinement and metallurgy, stainless steel industry (Krachler & Shotyk, 2009).

Presence of chromium in agricultural soil: The content of chromium in the soil in the present study was significantly lower than the values (24.06mg/kg) reported by (Sharma *et al*., 2007). Chopra & Pathak, (2012) reported extremely higher values of chromium (129.52mg/kg) in soil compared to the current experiment. Singh *et al*., (2010) found equivalent levels of chromium (22.00) in soil as in the current work. Ahmad *et al*., (2019) observed lower values (1.368mg/kg) of chromium content under various doses of water treatment as compared to current research. Concentration of chromium was higher in current study than the permissible limit (2.30mg/kg) of FAO/WHO (2011). The amendments of organic and inorganic fertilizers alone and in combination in the soil was done to reduce the availability of heavy metals in the wastewater irrigated soil and the consequent effects on heavy metal availability (Guo *et al*., 2020). Giannakis *et al*., (2014) found that at the range of 50 tons per acre compost treatment raised soil chromium value (0.18 mg/kg), similar results was found in present investigation. Chromium in the environment cause due to anthropogenic or natural sources, or even both. Anthropogenic and natural chromium has been linked to igneous rocks that seem to be naturally enriched in chromium and, in the existence of a reactive source, resulted in chromium occur in groundwater (Chrysochoou *et al*., 2016). The concentration of chromium in the current investigation was lower than the dangerous level specified by WHO (2000). Chromium poisoning can result in kidney and liver problems, gastrointestinal problems, diarrhea, hemolytic anemia, heart problems, and perhaps reproductive issues (Kim *et al*., 2015). The presence of chromium in the soils of farming land resulted in the long-term use of agrochemicals (inorganic fertilizers and synthetic insecticides).

Presence of chromium in vegetables: Muhammad *et al*., (2020) discovered level of chromium (9.81mg/kg) which was increased by the application of poultry manure and was higher value than current finding. Use of cattle manure enhanced the chromium content and showed similar results (3.36 mg/kg) as observed in the research (Zhao *et al*., 2014). Chromium concentration in the edible portions of most examined vegetables exceeded the maximum allowable amount (0.05 mg/kg). The highest concentration of chromium was found in *Dacus carota*. The ability of various crops in the accumulation of heavy metals affects their quantities in edible components (Remon *et al*., 2005). It is possible that the chromium found in the vegetables came from polluted soils. As a result, the agricultural soils in the research zone area were to be polluted. High levels of chromium are hazardous to all human and plants. Chromium influences germination of seeds, plant development, photosynthetic, and nutrient absorption (Shanker, 2005). The process of chromium absorption in plants is not well understood. Furthermore, because chromium is a non-essential component, it lacks a particular method for absorption and is similarly contingent on chromium speciation. Bioaccumulation of chromium is a gradual process that means the plant consumes no energy (Oliveira, 2012). The chromium concentration in the vegetables was higher than the WHO/FAO (Codex 2001). Acceptable guideline. Less chromium concentration was found in the vegetables because vegetables growing away from the roadway.

Bio-concentration factor, pollution load index and enrichment factor of chromium: In recent studies, BCF values for chromium are less than 1, indicating that less metal is being transferred from soil to vegetables. BCF value of chromium in soil investigated by Al-Jumaily *et al*., (2021) showed higher content compared to current study. Kulkarni *et al*., (2014) reported higher value of chromium through the application of fertilizers higher (0.93mg/kg) compared to present study. Asdeo, (2014) found lower value of chromium in vegetables (0.2890 mg/kg) as compared to current finding. Human exposure to toxic metals in food is largely determined by the BCF.

The pollution load index of chromium in the present study was observed to be higher contrasted to results of Asfaq *et al*., (2015). Plants store chromium mainly in their roots, and it rarely makes its way to the stems or leaves. In this study, chromium was observed at similar concentration levels in the edible components of leafy and root vegetables as observed by Ahmad *et al*., (2015). Vegetables samples were contaminated with chromium and had higher amount of chromium than the standard value suggested by FAO/WHO (2001). Pollution load index over 1.0 indicated that these metals were dangerous and should be avoided. Pollution load data can be used to determine the defilment soils status. Metals in water increase the concentrations of these metals in the soil.

Kumar & Thakur, (2018) reported higher value of EF for vegetables compared to present investigation. Pathak *et al*. (2011) found enrichment factor values for chromium insufficient enrichment types which were in agreement with the current research. Chary *et al*. (2008) reported that the rate of absorption of heavy metals depended on the amount of metals in the soil, their biochemical systems, and the ability of different plant species to uptake and grow.

Daily intake of metal, health risk index of chromium: Chopra & Pathak (2015) reported higher value (0.022 mg/kg/day) of DIM as shown in the current study. Singh *et al*., (2010) found that the amount of DIM through intake of polluted vegetables was too much higher (125.12 mg/kg/day) value compared to this study. Galal *et al*., (2021) observed higher value (0.0005 mg/kg/day) DIM compared to present investigation. It would, however, be dangerous for humans to eat the vegetables contaminated with metals because the HRI for heavy metals due to dietary intake was more than 1. The daily chromium consumption in this study seems to be much lesser than that of the levels (105 mg/kg/day) allowed by the (USEPA, 2010).

Shehata & Galal (2020) investigated the HRI of Cr vegetables ranging from 0.35-0.40, which had higher content than current research. Galal *et al*. (2021) indicated that the daily vegetable intake of Cr in non-polluted areas was 0.0005, while it was 0.01 in polluted areas. These values were higher than those observed in both years for all treatments in our study. The HRI was determined to be below one, suggesting no significant risk of Cr metal toxicity from consuming crops irrigated with wastewater.

Conclusion

The study aimed to evaluate the impact of various irrigation method and treatments (canal water, wastewater with organic manure, and inorganic fertilizers) on the chromium content in vegetables. The findings revealed that soil treated with wastewater and inorganic fertilizers accumulated higher levels of chromium compared to soil treated with freshwater, organic manure, and inorganic fertilizers. This accumulated chromium was then transferred to the edible parts of the vegetables. Treatment T_8 (wastewater + poultry manure) exhibited the highest chromium levels. Bio-concentration and enrichment factors indicated elevated chromium levels in soil from wastewater irrigation, posing risks to consumers. Analyses have shown that both soil and vegetables contain Chromium concentrations higher than the levels recommended by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Therefore, optimizing the use of wastewater and fertilizer application is essential to mitigate these risks in vegetable cultivation.

This paper is a minor part of thesis of a Ph.D. Scholar.

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(Received for publication 16 August 2023)