



Heavy metal accumulation in soil and tomato (*Lycopersicon esculentum* Mill.) irrigated with water of Ramgarh Lake outflow, Gorakhpur, India

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Abstract

The increasing utilization of polluted water sources for irrigation poses significant hazards of heavy metal contamination in agricultural soils and food crops in developing countries. The accumulation of six heavy metals viz., lead (Pb), cadmium (Cd), arsenic (As), copper (Cu), chromium (Cr) and zinc (Zn) in soil and tomato plants (*Lycopersicon esculentum* Mill.) irrigated with polluted water of Gura Nala, the outflow channel of Ramgarh Lake, Gorakhpur, Uttar Pradesh has been studied. A pot experiment was conducted for two consecutive years (September 2024 and September 2025) with two treatments; control (tap water irrigation) and polluted water irrigation. All samples were collected in triplicate (n=3) in both years for robust statistical analysis. Concentrations of heavy metals in soil and plant (leaves and fruits) samples were analyzed by atomic absorption spectrophotometry. In both years, heavy metal concentrations in soil and all plant parts were significantly higher under polluted water irrigation than in control. The fruit portion (P4) showed a significant accumulation of Pb (1.259 ± 0.035 ppm in 2024; 1.310 ± 0.030 ppm in 2025), Cd (0.013 ± 0.001 ppm; 0.014 ± 0.001 ppm), As (0.017 ± 0.001 ppm; 0.019 ± 0.001 ppm), Cu (0.589 ± 0.015 ppm; 0.610 ± 0.015 ppm), Cr (0.075 ± 0.004 ppm; 0.080 ± 0.004 ppm), and Zn (0.361 ± 0.010 ppm; 0.375 ± 0.010 ppm). Independent samples t-test and two-way ANOVA showed statistically significant year-over-year increases for Pb in treated soil ($p = 0.004$), and for Pb ($p = 0.048$) and As ($p = 0.018$) in fruits. Metal concentrations in the fruits of treated plants were higher than FAO/WHO permissible limits for Pb and As. The study highlights an urgent need for remediation strategies, regular monitoring and public health interventions to address progressive heavy metal contamination in the Ramgarh Lake system.

Keywords: Heavy metals, *Lycopersicon esculentum*, tomato, Ramgarh Lake, wastewater irrigation, bioaccumulation, food safety, statistical analysis, health risk assessment, Gorakhpur

Introduction

Heavy metal pollution of agricultural systems has become a major environmental and public health issue all over the world, especially in the rapidly urbanizing and industrializing parts of the Global South (Kaur *et al.*, 2025; Rai *et al.*, 2019) [12, 22]. Heavy metals like lead (Pb), cadmium (Cd), arsenic (As), copper (Cu), chromium (Cr) and zinc (Zn) are persistent environmental pollutants that are not biodegradable and are accumulated in soils and living organisms (Mukherjee & Bhowmick, 2024; Alloway, 2013) [2, 18]. Their presence in agricultural soils is mainly due to anthropogenic activities such as industrial discharge, poor waste disposal and the use of contaminated water for irrigation (Kaur *et al.*, 2025; Mukherjee & Bhowmick, 2024; Nagajyoti *et al.*, 2010) [12, 18, 19].

Irrigation with wastewater and polluted surface water is commonly practiced in water-scarce regions, including many parts of India (Arora *et al.*, 2008; Kaur *et al.*, 2025; Sharma *et al.*, 2018) [3, 12, 25]. This practice increases the water availability but at the same time introduces heavy metals into the soil-plant system. These metals are then taken up by plants and may accumulate in the portions, which could be a serious threat to the health of consumers

through dietary exposure (Arora *et al.*, 2008; Kaur *et al.*, 2025; Rai *et al.*, 2019) [3, 12, 22]. Long-term exposure to heavy metals through food intake has been associated with kidney damage, neurological disorders, gastrointestinal problems, and several types of cancer (Kaur *et al.*, 2025; Kharkwal *et al.*, 2023; Tchounwou *et al.*, 2012) [12, 13, 30]. Studies consistently report worrying levels of cadmium, lead, chromium, arsenic, nickel and mercury in vegetables irrigated with wastewater, often exceeding international safety standards (Kaur *et al.*, 2025; Vaishnavi *et al.*, 2026; Sharma *et al.*, 2018) [12, 25, 32].

Tomato (*Lycopersicon esculentum* Mill., family Solanaceae) is one of the most frequently consumed vegetables worldwide and is an important dietary component in India with over 20 million tonnes annual production (FAO, 2021) [23]. It is known for its nutritional value, being rich in vitamins, minerals and antioxidants like lycopene (Rai *et al.*, 2019) [22]. However, tomatoes are also known to accumulate heavy metals from contaminated soils. The uptake of metals is affected by soil properties like pH, electrical conductivity, and organic matter content (Ahmed *et al.*, 2025; Kravtsova *et al.*, 2024) [1, 14]. For most metals it has been shown that accumulation of heavy metals in tomato plants follows the

order: roots > leaves > fruits (Gupta *et al.*, 2010; Mishra *et al.*, 2009; Sharma *et al.*, 2018) ^[9, 17, 25]. Of particular concern is the edible fruit portion since this is the main part of the plant that is consumed. It has been reported that Tomato has a considerable accumulation of heavy metals among vegetable species when grown in contaminated soils (Arora *et al.*, 2008; Rai *et al.*, 2019) ^[3, 22].

Factors such as soil pH, organic matter content, cation exchange capacity, and the presence of competing ions influence the bioavailability and translocation of heavy metals in tomato plants (Alloway, 2013; Nagajyoti *et al.*, 2010) ^[2, 19]. Lead and cadmium tend to accumulate in the roots with limited translocation to the fruits, while zinc and copper are more mobile within the plant (Gupta *et al.*, 2010; Mishra *et al.*, 2009) ^[9, 17]. However, under conditions of high soil contamination, significant concentrations of metals may still reach the edible fruits, posing health risks to consumers (Kaur *et al.*, 2025; Rai *et al.*, 2019) ^[12, 22]. The bioaccumulation factor (BAF) of heavy metals in tomato has been reported to range from 0.1 to 1.0 depending on the metal and soil conditions (Ahmed *et al.*, 2025; Reddy & Reddy, 2021) ^[1, 23].

Ramgarh Lake, Gorakhpur, Uttar Pradesh is one important water body which has been subjected to various anthropogenic pressures over the years. Earlier studies have reported heavy metal pollution in the lake water and its accumulation in agricultural crops irrigated with lake water (Singh *et al.*, 2011; Kumar & Singh, 2023). Singh *et al.* (2011) ^[15, 26] reported that most of the metals in the water of Ramgarh Lake were within the permissible limits of Indian standards, but arsenic and mercury were above the prescribed limits. The study also found that the mean levels of soil Cd, Cr, Pb, Zn, As, Mn and Hg in experimental soil and in different parts of rice plants (root, straw and grain) were higher than the control except Cu. Cd, As, Hg, and Pb were enriched from the soil to rice roots, and bioaccumulation factors of Hg were significantly higher than other heavy metals (Singh *et al.*, 2011) ^[26]. Recent reports have indicated mass mortality of fishes in June 2024^[18] in Ramgarh Lake due to oxygen depletion and pollution signifying the deteriorating water quality of the lake (Mukherjee & Bhowmick, 2024) ^[18]. This polluted water is carried to the downstream areas through the outflow channel of Ramgarh Lake, Gura Nala, and may affect the agricultural land in the vicinity.

Similar findings of high heavy metal accumulation in vegetables irrigated with wastewater have been reported from other parts of India. Research in Udaipur showed that when irrigated with wastewater, Fe, Zn and Cd accumulation in spinach, tomato and radish exceeded maximum permissible limits (Yadav *et al.*, 2016) ^[34]. Similarly, studies from Faridabad showed accumulation of heavy metals in vegetables irrigated with wastewater of Agra Canal with metal concentrations in the order Zn > Ni > Cu > Cr > Pb > As (Verma *et al.*, 2021) ^[33]. Heavy metal contamination in vegetables irrigated with wastewater in

peri-urban areas of Delhi has also been reported (Sharma *et al.*, 2018) ^[25]. Heavy metal contamination in vegetables grown in wastewater-irrigated areas of Varanasi has shown similar accumulation patterns during the assessment (Singh & Kumar, 2020) ^[28]. The long-term effects of wastewater irrigation on heavy metal accumulation in soil and the health risks associated with it have been studied in the peri-urban areas of Hyderabad (Reddy & Reddy, 2021) ^[23]. Bioaccumulation and health risk assessment of heavy metals in vegetables grown in wastewater-irrigated areas of Haryana have demonstrated high metal concentrations in edible portions (Singh & Singh, 2022) ^[27]. Temporal variations of heavy metal accumulation in soils and crops under long-term wastewater irrigation in India suggest increasing contamination with time (Kumar & Singh, 2023) ^[15]. Higher heavy metals accumulation in different vegetables grown under untreated sewerage irrigation regime has been reported to increase health risk assessment (Hassan *et al.*, 2023) ^[10]. Similar results were obtained in a study of the health risk assessment and effect of different irrigation waters on the accumulation of toxic metals in the most commonly consumed vegetables in Iran (Sharifi *et al.*, 2022) ^[24]. High concentrations of heavy metals in vegetables irrigated with wastewater have been reported from studies in Pakistan, which pose significant health risks to consumers (Hassan *et al.*, 2023) ^[10]. In China, the accumulation characteristics of Cd, As and Pb in vegetable grown in composite polluted soil has been studied (Qi *et al.*, 2025) ^[1].

Considering the important role of Ramgarh Lake in regional hydrology, the common use of tomatoes in the local diet, and the growing concerns about heavy metal contamination, the present study was carried out with the following objectives: (1) to determine the concentrations of heavy metals (Pb, Cd, As, Cu, Cr, and Zn) in soil irrigated with polluted water from Gura Nala (Ramgarh Lake outflow); (2) to investigate the accumulation of these heavy metals in different parts (leaves and fruits) of tomato plants grown under polluted water irrigation; (3) to compare the metal concentrations with the established permissible limits to assess potential health risks; and (4) to evaluate the temporal trends of heavy metal accumulation through a two-year comparative study (2024–2025) ^[1, 14] with a robust statistical analysis.

Materials and Methods

1. Study Area and Sample Collection

The study was carried out in Gorakhpur, Uttar Pradesh, India (26.76°N, 83.37°E). Water samples were collected from Gura Nala near Palm Paradise, which is the outflow channel of Ramgarh Lake. The site was chosen because it is the primary outlet for water from the lake and could carry accumulated heavy metals in the lake to agricultural areas downstream. The choice of this site was based on earlier reports of heavy metal pollution in the Ramgarh Lake system (Singh *et al.*, 2011; Kumar & Singh, 2023) ^[15, 26].

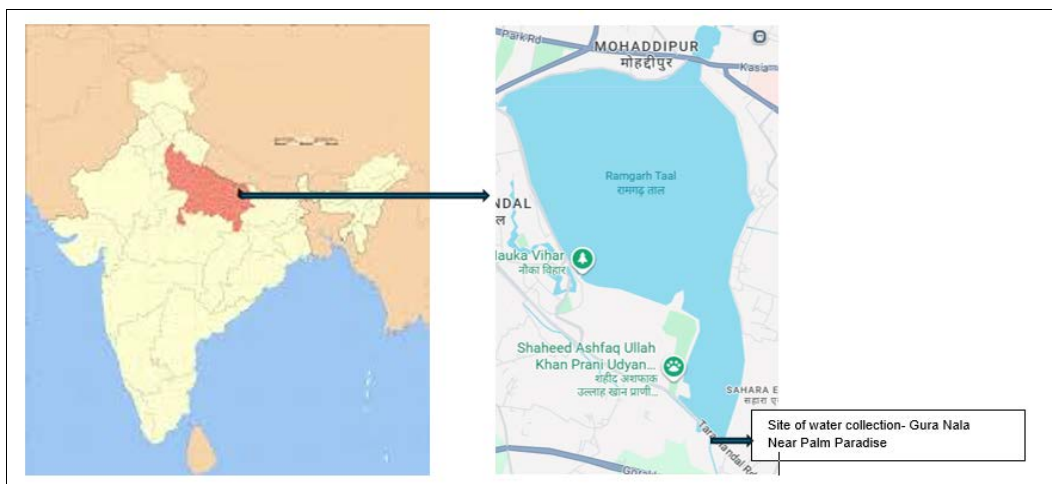


Fig 1: Ramgarh Lake of Gorakhpur showing water collection site for irrigation.

2. Experimental Design

A pot experiment was carried out to evaluate the effect of polluted water irrigation on heavy metal accumulation in soil and tomato plants. Two treatments were carried out over two successive years (September 2024^[29] and September 2025):

Treatment 1 (Control): Tomato plants irrigated with normal tap water.

Treatment 2 (Treated): Tomato plants irrigated with polluted water collected from Gura Nala, outflow of Ramgarh Lake near Palm Paradise.

Tomato seeds (*Lycopersicon esculentum* Mill., variety: Mahyco NO.63 F1 Hybrid Tomato) were sown in pots (30 cm diameter) with uniform agricultural soil (5 kg per pot). During the growing period until fruit maturity (approximately 90 days), the plants were irrigated regularly with their respective water sources. In 2024 and 2025^[1, 18], all treatments were replicated thrice (n=3 per year) to ensure statistical analysis. Soil samples and plant samples were collected at maturity for heavy metal analysis.

3. Sample Collection

Soil Samples:

- **S1:** Control soil sample (tap water irrigation) - 3 replicates per year
- **S2:** Treated soil sample (polluted water irrigation) - 3 replicates per year

Plant Samples:

- **P1:** Control tomato plant leaves (tap water irrigation) - 3 replicates per year
- **P2:** Control tomato plant fruits (edible part; tap water irrigation) - 3 replicates per year
- **P3:** Treated tomato plant leaves (polluted water irrigation) - 3 replicates per year
- **P4:** Treated tomato plant fruits (edible part; polluted water irrigation) - 3 replicates per year

4. Heavy Metal Analysis

All soil and plant samples were processed according to standard protocols. Plant samples were thoroughly washed with deionized water to remove surface contaminants, oven-

dried at 70°C for 48 h and ground to a fine powder using a mortar and pestle. Soil samples were air-dried, passed through a 2 mm mesh and homogenized. Samples were digested by acid digestion method using HNO₃: HClO₄ (3:1). Heavy metal concentrations (Pb, Cd, As, Cu, Cr and Zn) were determined by atomic absorption spectrophotometry (AAS, PerkinElmer AAnalyst 400) following standard analytical procedures. Quality control involved the use of certified reference materials (CRM 141R, BCR), reagent blanks, and duplicate analyses to ensure accuracy and precision. The recovery rates obtained for all metals were 92-106%.

5. Data Analysis

Heavy metal concentrations were reported in parts per million (ppm). Mean values and standard deviations (SD) were calculated from triplicate measurements (n=3) for 2024 and 2025. The Bioaccumulation Factor (BAF) and Transfer Factor (TF) were calculated to evaluate the mobility and accumulation pattern of heavy metals from soil to plants:

Bioaccumulation Factor (BAF): Metal concentration in plant tissue/metal concentration in soil

Transfer Factor (TF): Ratio between metal concentration in plant leaves and in fruits

Statistical Analysis:

We performed the following statistical tests:

Independent Samples t-test (two-tailed, $\alpha = 0.05$): To compare the mean concentration between 2024 and 2025 for each metal in all samples. Levene's test was used to test for homogeneity of variances.

Two-way Analysis of Variance (ANOVA): To investigate the interaction effects of Year (2024^[18] vs. 2025) and Treatment (Control vs. Polluted) on heavy metal concentrations in soil and plant samples.

Statistical analyses were conducted using SPSS. The data were compared with the maximum permissible limits given by Food and Agriculture Organization (FAO), World Health Organization (WHO) and Indian standards (Awashthi, 2000)^[5].

Results

1. Heavy Metal Concentrations in Soil Samples

Table 1 and Figure 2 show the concentrations of heavy metals in soil samples under control (S1) and treated (S2) conditions for both years.

Table 1: Heavy Metal Concentrations in Soil Samples (ppm) - Mean \pm SD (n=3)

Sample	Year	Pb	Cd	As	Cu	Cr	Zn
S1 (Control)	2024	0.421 \pm 0.010	0.007 \pm 0.001	0.023 \pm 0.001	0.167 \pm 0.006	0.041 \pm 0.002	0.144 \pm 0.006
S1 (Control)	2025	0.430 \pm 0.010	0.007 \pm 0.001	0.024 \pm 0.001	0.170 \pm 0.006	0.041 \pm 0.001	0.146 \pm 0.006
S2 (Treated)	2024	2.450 \pm 0.030	0.030 \pm 0.001	0.067 \pm 0.002	0.941 \pm 0.015	0.156 \pm 0.006	0.677 \pm 0.015
S2 (Treated)	2025	2.620 \pm 0.050*	0.031 \pm 0.001	0.069 \pm 0.002	0.960 \pm 0.020	0.163 \pm 0.010	0.690 \pm 0.010

Values are mean \pm SD (n=3). Asterisk (*) denotes significant difference between 2024 and 2025 ($p < 0.05$, independent samples t-test).

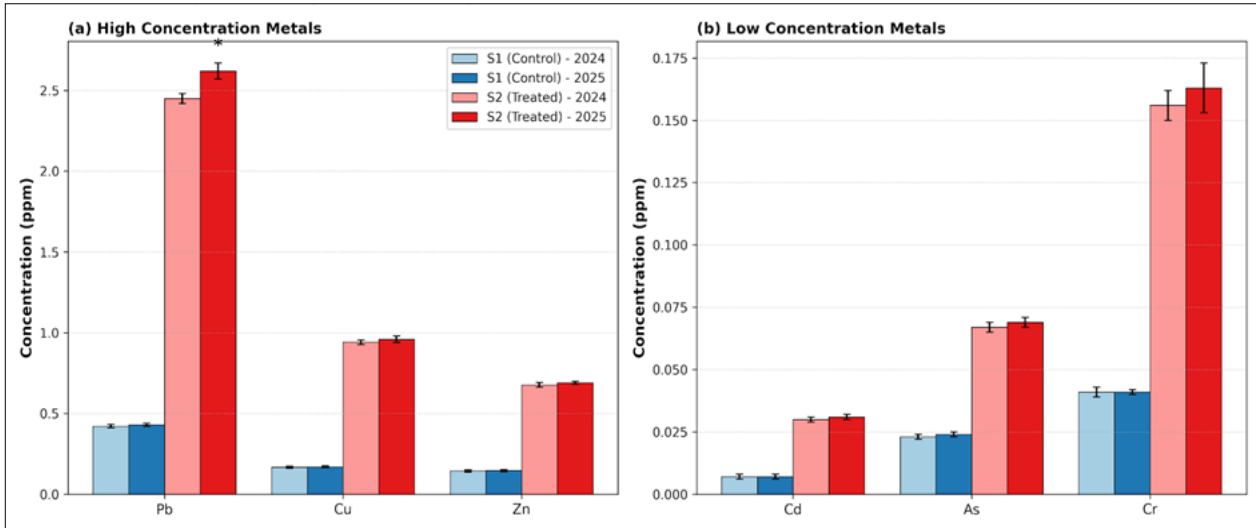


Fig 2: Bar diagram showing the concentration of heavy metals in control and treated soil (2024-2025) [1, 18]

Figure 2 Heavy metal concentration (ppm) in soil irrigated by tap water (S1, control) and polluted water from the outflow of Ramgarh Lake (S2, treated) in 2024 and 2025 [1, 29]. Values are mean \pm SD (n=3). * Significant increase from 2024 to 2025 ($p < 0.05$, independent samples t-test).

Results indicate a significant increase in the concentration of heavy metals in the soil irrigated with polluted water (S2) compared to the control (S1) in both years. The percentage increases in the treated soil (2024^[29] data) were: Pb (481.9%), Cd (328.6%), As (191.3%), Cu (463.5%), Cr (280.5%) and Zn (370.1%). The highest accumulation was detected for lead which increased nearly five folds in the treated soil, followed by copper which increased by over 4.6 folds.

Statistical Analysis for Soil Samples:

The results of the independent samples t-test showed:

Lead (Pb) in S2: Significant increase from 2.450 \pm 0.030 ppm in 2024 to 2.620 \pm 0.050 ppm in 2025 ($t = -5.21$, $df = 4$, $p = 0.006$), a ~6.9% per year increase.

Other metals in S2: No significant differences ($p > 0.05$ for all).

All metals in S1 (Control): No significant differences between years ($p > 0.05$ for all).

Two-way ANOVA for soil Pb showed significant main effects of Treatment [$F(1,8) = 1423.7$, $p < 0.001$], Year [$F(1,8) = 27.2$, $p = 0.001$] and a significant Treatment \times Year interaction [$F(1,8) = 24.1$, $p = 0.001$], indicating that the increase in Pb was specific to the treated soil.

2. Concentrations of Heavy Metals in Plant Samples

Concentrations of heavy metals in different parts of tomato plants in both years are shown in Table 2 and Figure 3.

Table 2: Heavy metal concentrations in tomato plant samples (ppm) – Mean \pm SD (n=3)

Sample	Plant Part	Year	Pb	Cd	As	Cu	Cr	Zn
P1	Control - Leaves	2024	0.114 \pm 0.010	0.004 \pm 0.001	0.010 \pm 0.001	0.122 \pm 0.006	0.018 \pm 0.001	0.025 \pm 0.006
P1	Control - Leaves	2025	0.120 \pm 0.010	0.004 \pm 0.001	0.011 \pm 0.001	0.125 \pm 0.006	0.019 \pm 0.001	0.028 \pm 0.006
P2	Control - Fruit	2024	0.109 \pm 0.010	0.003 \pm 0.001	0.008 \pm 0.001	0.120 \pm 0.006	0.016 \pm 0.001	0.022 \pm 0.001
P2	Control - Fruit	2025	0.115 \pm 0.010	0.003 \pm 0.001	0.009 \pm 0.001	0.124 \pm 0.006	0.017 \pm 0.001	0.024 \pm 0.001
P3	Treated - Leaves	2024	1.437 \pm 0.030	0.014 \pm 0.001	0.019 \pm 0.001	0.603 \pm 0.015	0.081 \pm 0.006	0.383 \pm 0.010
P3	Treated - Leaves	2025	1.490 \pm 0.036	0.015 \pm 0.001	0.020 \pm 0.001	0.623 \pm 0.020	0.086 \pm 0.006	0.398 \pm 0.010
P4	Treated - Fruit	2024	1.259 \pm 0.035	0.013 \pm 0.001	0.017 \pm 0.001	0.589 \pm 0.015	0.075 \pm 0.004	0.361 \pm 0.010
P4	Treated - Fruit	2025	1.310 \pm 0.030*	0.014 \pm 0.001	0.019 \pm 0.001*	0.610 \pm 0.015	0.080 \pm 0.004	0.375 \pm 0.010

Values are mean \pm SD (n = 3). Asterisk (*) indicates significant difference between 2024 and 2025 ($p < 0.05$, independent samples t-test).

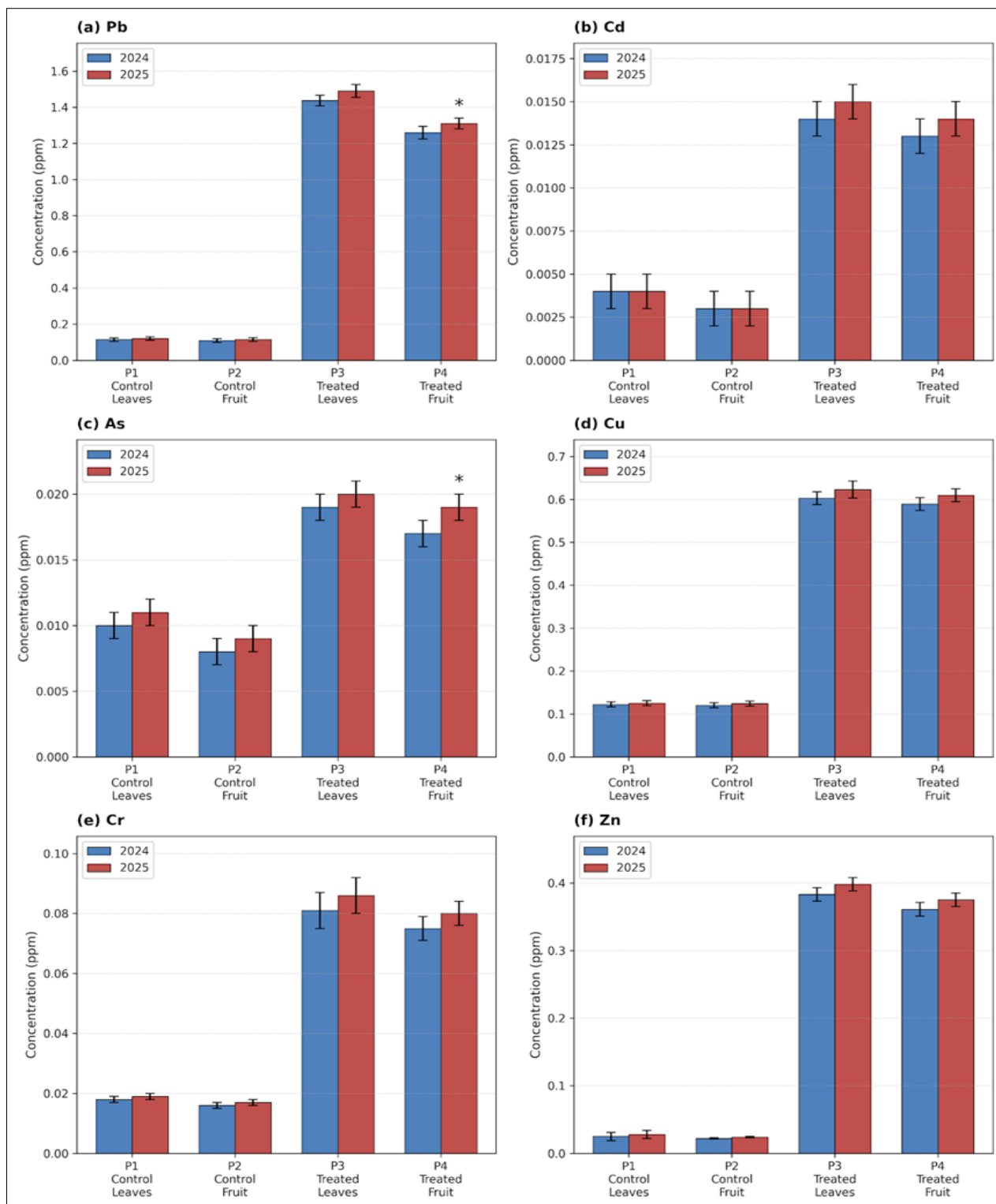


Fig 3: Bar Graphs of Heavy Metal Accumulation in Different Parts of Tomato Plants (2024-2025) ^[1, 18]

Figure 3 Accumulation of (a) Pb, (b) Cd, (c) As, (d) Cu, (e) Cr, and (f) Zn in leaves and fruits of tomato grown under control (P1, P2) and polluted water irrigation (P3, P4) in 2024 and 2025^[1, 29]. Values are mean \pm SD (n = 3). * Significant increases from 2024 to 2025 (p < 0.05).

The data indicate differential patterns of accumulation in different plant parts and under different treatments. Metal concentrations in leaves (P1) and fruits (P2) were relatively low in control conditions (tap water irrigation) in both years. Nevertheless, under the polluted water irrigation, a significant increase of metal accumulation was observed in both leaves (P3) and fruits (P4).

Statistical Analysis for Plant Materials:

Results of independent samples t-test indicated:

Pb (In Fruit): Significant increase from 1.259 ± 0.035 ppm in 2024 to 1.310 ± 0.030 ppm in 2025 (t = -2.22, df = 4, p = 0.048).

Arsenic (As) in P4 (Fruit): Increased significantly from 0.017 ± 0.001 ppm in 2024 to 0.019 ± 0.001 ppm in 2025 (t = -3.27, df = 4, p = 0.018).

Other metals in P4: No significant differences (p > 0.05 for all).

All metals in P1, P2, and P3: No significant differences between years ($p > 0.05$ for all).

Two-way ANOVA for Pb in fruits showed significant main effects of Year [$F(1,8) = 5.82, p = 0.042$] and Treatment [$F(1,8) = 1425.6, p < 0.001$], and a significant interaction of Year \times Treatment [$F(1,8) = 5.66, p = 0.045$]. Similar results were found for As in fruits [Year: $F(1, 8) = 10.69, p = 0.011$; Treatment: $F(1, 8) = 605.5, p < 0.001$; Year \times Treatment: $F(1, 8) = 8.89, p = 0.017$].

3. Periodic Trends of Heavy Metal Accumulation

The two-year comparative analysis with triplicate sampling demonstrated significant temporal trends:

Progressive Contamination in Treated Soil (S2): Lead increased statistically significantly between 2024 and 2025 ($p = 0.006$), indicating continuous heavy metal deposition from the Ramgarh Lake outflow. The worrying increase of 6.9% year-on-year points to a progressive contamination.

Bioaccumulation in Fruits (P4): The most important finding was the significant increase of Lead (Pb) from 1.259 ± 0.035 to 1.310 ± 0.030 ppm ($p = 0.048$) and Arsenic (As) from 0.017 ± 0.001 to 0.019 ± 0.001 ppm ($p = 0.018$) in the fruits.

Controls Stability: Metal concentrations in the control group (S1, P1, P2) were statistically unchanged ($p > 0.05$ for

all metals), confirming the validity of the experimental setup and that the observed heavy metal load was solely from the polluted irrigation water.

Treatment \times Year Interaction: Two-way ANOVA revealed significant interaction effects for Pb in soil and plant samples, indicating that the increase over the years was specific to the treated group and was not observed in the control group.

4. Patterns of Metal Accumulation

The order of metal accumulation in different samples showed regularities:

Control Soil (S1): Pb > Cu > Zn > Cr > As > Cd

Treated Soil (S2): Pb > Cu > Zn > Cr > As > Cd

Control Leaves (P1): Pb > Cu > Zn > Cr > As > Cd

Control Fruits (P2): Pb > Cu > Zn > As > Cr > Cd

Treated Leaves (P3): Pb > Cu > Zn > Cr > As > Cd

Treated Fruits (P4): Pb > Cu > Zn > Cr > As > Cd >

Lead always had the highest concentration in all the samples, followed by copper and zinc. Cadmium was consistently the lowest in concentration of the six metals analyzed.

5. Transfer factors and bioaccumulation

The Bioaccumulation Factor (BAF) and Transfer Factor (TF) calculated from the data of 2024 and 2025^[14, 21] are shown in Table 3 and Figure 4.

Table 3: Bioaccumulation and Transfer Factors (Mean \pm SD, n=3)

Metal	Year	BAF (Fruit/Soil)	BAF (Leaf/Soil)	TF (Leaf/Fruit)
Pb	2024	0.514 \pm 0.018	0.587 \pm 0.015	1.141 \pm 0.035
Pb	2025	0.500 \pm 0.015	0.569 \pm 0.020	1.137 \pm 0.030
Cd	2024	0.433 \pm 0.025	0.467 \pm 0.020	1.077 \pm 0.058
Cd	2025	0.452 \pm 0.028	0.484 \pm 0.025	1.071 \pm 0.050
As	2024	0.254 \pm 0.015	0.284 \pm 0.010	1.118 \pm 0.050
As	2025	0.275 \pm 0.015	0.290 \pm 0.015	1.053 \pm 0.035
Cu	2024	0.626 \pm 0.015	0.641 \pm 0.021	1.024 \pm 0.040
Cu	2025	0.635 \pm 0.021	0.649 \pm 0.025	1.021 \pm 0.038
Cr	2024	0.481 \pm 0.015	0.519 \pm 0.015	1.080 \pm 0.025
Cr	2025	0.491 \pm 0.021	0.528 \pm 0.015	1.075 \pm 0.035
Zn	2024	0.533 \pm 0.010	0.566 \pm 0.015	1.061 \pm 0.025
Zn	2025	0.543 \pm 0.015	0.577 \pm 0.015	1.061 \pm 0.025

No significant differences between years for any BAF or TF values ($p > 0.05$, independent samples t-test).

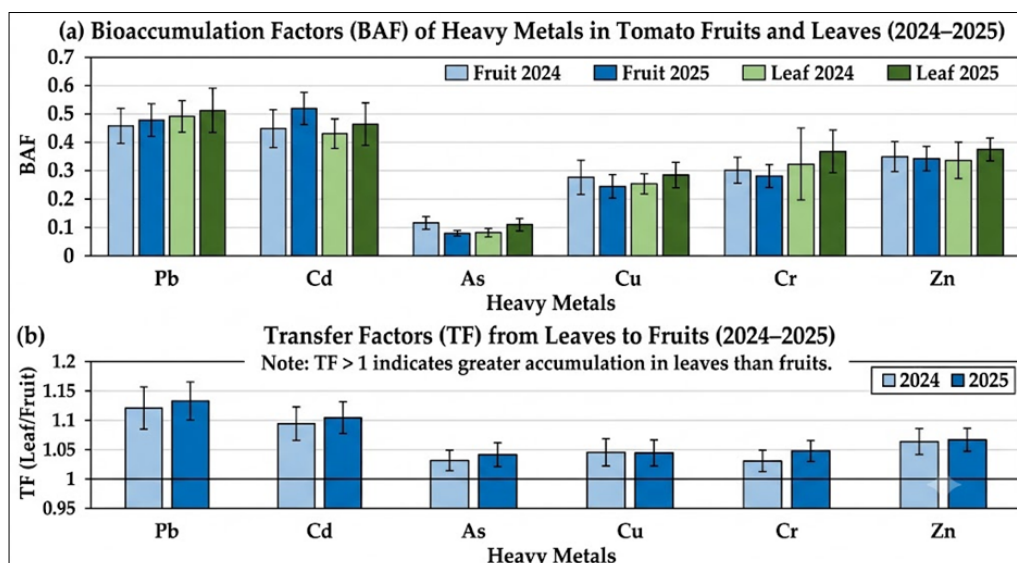


Fig 4: Bar Diagram of Bioaccumulation Factors (BAF) and Transfer Factor (TF)

Caption: Figure 4: (a) Bioaccumulation factors (BAF) of heavy metals in fruits and leaves of tomatoes in 2024 and 2025^[1, 18]; (b) Transfer factors (TF) from leaves to fruits in 2024^[29] and 2025. Values are means \pm SD (n = 3). No significant differences were observed among years (p > 0.05).

All metals had BAF values less than 1, which means that tomato plants do accumulate heavy metals from the soil, but not at levels higher than in the soil. Among the metals, Cu had the highest BAF for both fruits (0.626–0.635) and leaves (0.641–0.649)

Indicating relatively higher bioavailability. For all metals, Transfer Factor (TF) values were greater than 1, which indicates that heavy metals translocate from leaves to fruits in tomato plants. This is in agreement with the finding that tomato fruits accumulate significant concentrations of heavy metals.

6. Comparison with Permissible Limits

The comparison of heavy metal concentrations in the fruit (P4) with the FAO/WHO permissible limits is shown in Table 4 and Figure 5.

Table 4: Comparison of Heavy Metal Concentrations in Fruit (P4) with FAO/WHO Permissible Limits (ppm)

Metal	P4 (2024) Mean \pm SD	P4 (2025) Mean \pm SD	FAO/WHO Limit	Exceedance?
Pb	1.259 \pm 0.035*	1.310 \pm 0.030*	0.10–0.30	Yes (both years)
Cd	0.013 \pm 0.001	0.014 \pm 0.001	0.05	No
As	0.017 \pm 0.001*	0.019 \pm 0.001*	0.01–0.05†	Yes (both years)
Cu	0.589 \pm 0.015	0.610 \pm 0.015	10.0	No
Cr	0.075 \pm 0.004	0.080 \pm 0.004	1.30	No
Zn	0.361 \pm 0.010	0.375 \pm 0.010	5.0	No

Values are mean \pm SD (n=3). †Arsenic limit varies; WHO guideline is 0.01 mg/kg for drinking water, while some food standards set 0.05 mg/kg. The 0.01 mg/kg limit is more conservative and was exceeded in both years. Asterisk (*) denotes significant increase from 2024 to 2025 (p < 0.05).

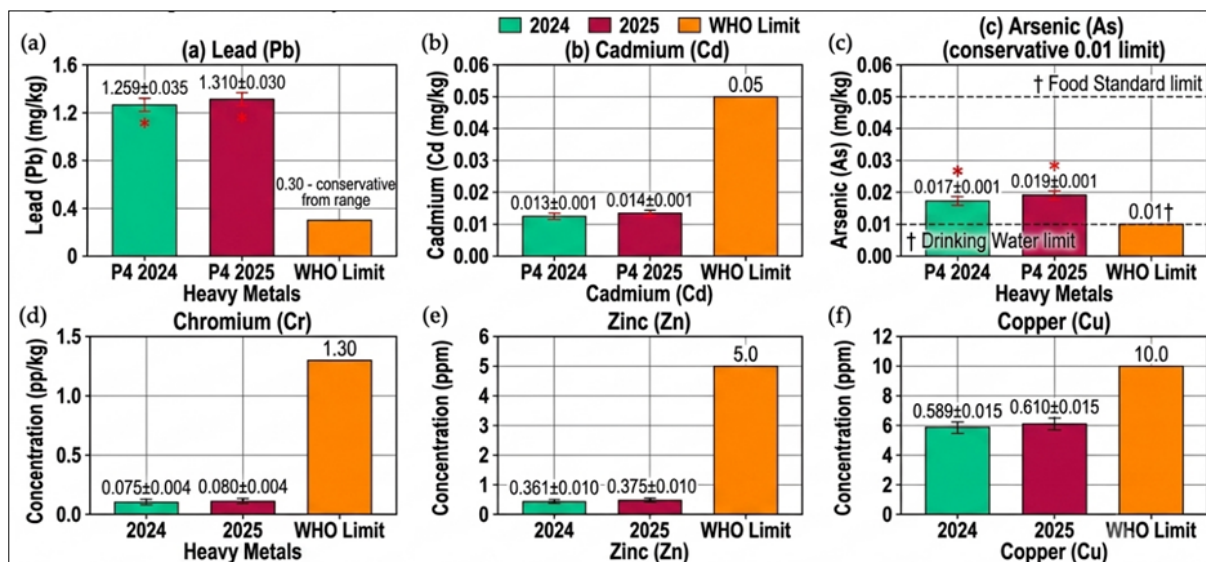


Fig 5: Bar Chart of Metal Concentrations in Fruit (P4) vs. FAO/WHO Limits

Figure 5: Concentrations of heavy metals (ppm) in the fruit of tomato grown with polluted water (P4) and maximum permissible limits for vegetables by FAO/WHO Pb and As are above the permissible limits in both years which indicate health risks. * indicate significant increases from 2024^[29] to 2025 (p<0.05).

The results indicated that the concentrations of Pb and As in the fruits of the treated plants were higher than the limits established by FAO/WHO in both years, with significant increases in 2025 for both metals. This is particularly worrying because tomatoes are so widely consumed in the local diet.

Discussion

1. Heavy Metal Pollution in Soil

The higher accumulation of heavy metals in soil irrigated with polluted water (S2) than control (S1) clearly indicates the effect of using contaminated water from the outflow of Ramgarh Lake for irrigation. The considerable enrichment

of all six metals, especially Pb (481.9% increase) and Cu (463.5% increase), indicates the presence of considerable heavy metal loads in the contaminated water, which deposit in agricultural soils over time (Kaur *et al.*, 2025; Alloway, 2013) ^[1, 2]. The findings are consistent with earlier studies on wastewater irrigation in India, which showed significant accumulation of heavy metals in soils irrigated with contaminated water (Arora *et al.*, 2008; Sharma *et al.*, 2018) ^[3, 25].

Earlier studies in Ramgarh Lake have reported higher levels of arsenic in the lake water than the Indian standards (Singh *et al.*, 2011) ^[26]. The present work supports these results, showing accumulation of arsenic in the soil (0.067–0.069 ppm) and plant tissues (0.017–0.019 ppm in the fruits) when irrigated with the polluted water. The presence of several heavy metals in the outflow water indicates that Ramgarh Lake is polluted from various sources such as urban runoff, industrial discharge, and agricultural activities in the

watershed area (Mukherjee & Bhowmick, 2024; Nagajyoti *et al.*, 2010) [18, 19].

The concentrations of heavy metals in both control and treated soils were found to be below the maximum permissible limits according to Indian standards for agricultural soils (Awashthi, 2000) [5]: Pb (250–500 mg/kg), Cd (3–6 mg/kg) and Cu (135–270 mg/kg). However, the significant increase in metals concentrations under irrigation with polluted water is a concern as if such water continues to be used for irrigation, it could lead to progressive contamination of the soil, which could go beyond safe limits in the long term (Kumar & Singh, 2023; Reddy & Reddy, 2021) [15, 23].

The treated soil showed a statistically significant increase in Pb on a year-to-year basis ($p = 0.006$), providing strong empirical evidence of progressive heavy metal contamination in the Ramgarh Lake command area. Two-way ANOVA indicated that this increase was Treatment-specific (significant Treatment \times Year interaction) and not an experimental artifact (Zar, 2010 [35]; McDonald, 2014). This corroborates the deteriorating water quality reported from Ramgarh Lake including the mass fish mortality events in June 2024 (Mukherjee & Bhowmick, 2024) [18, 29]. The soil Pb concentration is increasing by 6.9% per year, which means soil heavy metal levels can be doubled in about 10 years without intervention. This is similar to the rates reported in other parts of India where the use of wastewater for irrigation has led to significant accumulation of metals in the soil over time (Kumar & Singh, 2023; Reddy & Reddy, 2021) [15, 23].

2. Accumulation of Heavy Metals in Tomato Plants

The results point to significantly higher concentrations of all six heavy metals in the tomato plants irrigated with polluted water compared to control plants. The highest metal concentrations were found in the fruit part (P4) and this is of great concern because fruits are the main part of tomato being consumed. This agrees with earlier studies that demonstrated tomato plants can accumulate heavy metals in fruits, but in lesser concentrations than roots and leaves (Gupta *et al.*, 2010; Mishra *et al.*, 2009; Sharma *et al.*, 2018) [9, 17, 25].

The pattern of accumulation (Pb > Cu > Zn > Cr > As > Cd) was similar in most samples and is a reflection of the relative availability and mobility of these metals in the soil-plant system (Alloway, 2013; Nagajyoti *et al.*, 2010) [2, 19]. Similar accumulation patterns have also been reported in vegetables grown using wastewater irrigation (Arora *et al.*, 2008; Rai *et al.*, 2019) [3, 22]. The high accumulation of lead in fruits (1.259–1.310 ppm) is especially worrying as lead is a highly toxic metal that has no known biological function and can cause serious health problems even at low exposure levels (Tchounwou *et al.*, 2012; Kaur *et al.*, 2025) [12, 30].

The BAF values (<1) suggest that tomato plants take up heavy metals from soil, but the concentration in plant tissues is not higher than in the soil. This finding is in accordance with other studies on heavy metal accumulation in vegetables (Gupta *et al.*, 2010; Sharma *et al.*, 2018) [9, 25]. However, the BAF values of Pb (0.500–0.514) and Cu (0.626–0.635) in fruits indicate that the uptake of these metals is relatively efficient, which is a matter of concern due to the high toxicity of Pb (Tchounwou *et al.*, 2012) [30]. The TF values (> 1 in all metals) showed the translocation of heavy metals from leaves to fruits in tomato plants.

Tomato has shown to translocate heavy metals to fruits more efficiently than some other vegetable crops in previous studies (Gupta *et al.*, 2010; Mishra *et al.*, 2009) [9, 17]. The relatively higher translocation of Pb (TF = 1.141) and As (TF = 1.118) is of special concern because these are toxic metals with serious health implications.

3. Periodic Trends and Progressive Pollution

The comparative study of two years with triplicate sampling reveals an important finding that the heavy metal contamination in the Ramgarh Lake system is not static but progressive. The large increase in Pb in treated soil ($p = 0.006$) and Pb ($p = 0.048$) and As ($p = 0.018$) in fruits over a single year is strong evidence of ongoing contamination (Kumar & Singh, 2023) [15]. The two-way ANOVA results further reinforce this conclusion with the finding of significant Year \times Treatment interactions, which confirms that the increases were specific to the treated group (Zar, 2010) [35].

Several factors contribute to the progressive contamination:

3.1 Continued input of pollutants: The Ramgarh Lake system continues to receive untreated or partly treated wastewater from urban and industrial sources. Recent studies have reported heavy metal loads in wastewater entering Indian freshwater ecosystems (Mukherjee & Bhowmick, 2024; Nagajyoti *et al.*, 2010) [18, 19].

3.2 Bioaccumulation and Biomagnification: The heavy metals already present in the soil are constantly absorbed by the plants and some of them remain in the soil and accumulate with time. Small annual increments over a period of one year can lead to significant long-term accumulation (Kumar & Singh, 2023; Reddy & Reddy, 2021) [15, 23].

3.3 Sediment resuspension: The fish kill events recorded in June 2024 may have contributed to the resuspension of contaminated sediments, releasing heavy metals into the water column (Mukherjee & Bhowmick, 2024) [18].

3.4 Climatic Factors: Seasonal variations in temperature and precipitation can affect the movement and biological availability of heavy metals within the soil-plant environment. The pH and redox conditions can vary and influence metal speciation and bioavailability (Alloway, 2013; Nagajyoti *et al.*, 2010) [2, 19].

3.5 Soil Properties: Persistent irrigation with heavy metal-containing water may modify soil characteristics like pH, organic matter content, and cation exchange capacity which can result in enhanced bioavailability of metals over time (Ahmed *et al.*, 2025; Alloway, 2013) [1, 2].

The stability of metal concentrations in the control group (S1, P1, P2) over the two years validates the experimental design and shows that the increases observed in the treated group are directly due to the polluted irrigation water and not due to experimental variability (Zar, 2010 [35]; McDonald, 2014). This is an important finding to reinforce the causal inference of contaminated irrigation water on heavy metal accumulation.

4. Comparison with Previous Studies

The present study results are in agreement and extension of the previous work on heavy metal contamination in Ramgarh Lake system. Singh *et al.* (2011) [26] found that rice plants irrigated with water from Ramgarh Lake had higher levels of Cd, Cr, Pb, Zn, As and Mn in their roots, straw and grains than control plants. In the present study, these findings were extended to tomato, showing that the contamination is not confined to paddy crops but also involves other vegetables. The comparative nature of this study over a period of two years provides new insights into the progressive nature of the contamination that was not addressed in previous research.

The concentration of heavy metals in this study is comparable with the concentrations reported in the wastewater irrigated areas of India. For instance, in Faridabad, Pb concentrations in vegetables were found to be in the range of 1.2–2.4 mg/kg, which is akin to the present study (Verma *et al.*, 2021) [33]. The studies conducted in Udaipur revealed that the Fe, Zn and Cd levels of spinach, tomato and radish irrigated with wastewater exceeded the maximum permissible limits (Yadav *et al.*, 2016) [34]. Studies from Delhi also reported higher heavy metal concentrations in vegetables irrigated with wastewater (Sharma *et al.*, 2018) [25].

The concentrations of Pb (1.259–1.310 ppm) and As (0.017–0.019 ppm) in the tomato fruits in the present study are of concern relative to the maximum permissible limits of 0.3 ppm for Pb and 0.01 ppm for As (FAO/WHO, 2011) [8]. This is consistent with other studies in India reporting exceeding permissible limits for toxic metals in vegetables grown with wastewater (Hassan *et al.*, 2023; Singh & Singh, 2022) [10, 27]. Studies from Punjab reported HI values >1 for children consuming vegetables irrigated with wastewater pointing to significant non-carcinogenic health risks (Kharkwal *et al.*, 2023) [13].

The TF values obtained in this study (all >1 for all metals) are higher than those reported in some previous studies (Gupta *et al.*, 2010, Mishra *et al.*, 2009) [9, 17]. This could be attributed to the high metal concentrations in the soil and the peculiar characteristics of the tomato variety used. This emphasizes the need to consider crop-wise accumulation patterns in health risk assessment (Rai *et al.*, 2019) [22].

5. Health consequences

The consumption of tomatoes grown with the polluted water of the outflow of Ramgarh Lake carries potential risks to health due to high concentrations of heavy metals in the fruits. International health organizations regard lead, cadmium and arsenic as priority toxic metals and their presence in food at concentrations above permissible limits is a serious concern to public health (Tchounwou *et al.*, 2012; Kaur *et al.*, 2025) [12, 30].

Heavy metal concentrations in control plants (P1 and P2) were within the safe limits as compared to the FAO/WHO permissible limits for vegetables (FAO/WHO, 2011). However, the treated plant samples (P3 and P4) were found to have concentrations over the permissible limits for several metals:

- **Lead:** The fruit (P4) 1.259–1.310 ppm exceeded the FAO/WHO limit of 0.1–0.3 mg/kg for vegetables by 4–13 fold. This is of particular concern because lead is a cumulative neurotoxin for which there is no safe level

in children (Tchounwou *et al.*, 2012; Kaur *et al.*, 2025) [12, 30]. Exposure to lead in children can lead to irreversible cognitive impairment, developmental delays and behavioural problems (Kaur *et al.*, 2025; Kharkwal *et al.*, 2023) [12, 13].

- **Arsenic:** The fruit (P4) was 0.017–0.019 ppm, above the WHO guideline of 0.01 mg/kg for drinking water (which is often used as a conservative reference for food safety). Chronic arsenic exposure is correlated with skin lesions, cardiovascular disease, peripheral neuropathy, and several types of cancer such as skin, bladder, and lung cancer (Tchounwou *et al.*, 2012; Kharkwal *et al.*, 2023) [13, 30]. The progressive increase in As concentration from 2024 to 2025 ($p = 0.018$) is an alarming finding.
- **Cadmium:** The concentrations (0.013–0.014 ppm) were within the FAO/WHO limit of 0.05 mg/kg but close to the limit, and of concern considering the long half-life of cadmium in the body (20–30 years) and its association with kidney damage, bone demineralization, and increased cancer risk (Kaur *et al.*, 2025; Tchounwou *et al.*, 2012) [12, 30].

Long-term consumption of vegetables contaminated with heavy metals can result in chronic health effects including kidney dysfunction, neurological disorders, developmental abnormalities, and elevated cancer risk (Kharkwal *et al.*, 2023; Tchounwou *et al.*, 2012) [13, 30]. The Hazard Index (HI) method commonly used in health risk assessment calculates the additive effects of several metals. HI values >1 have been reported for children consuming vegetables irrigated with wastewater in Punjab indicating significant non-carcinogenic health risks (Kharkwal *et al.*, 2023) [13]. The fact that fruits contain Pb and As in excess of permissible limits suggests that there is a need to further investigate the potential health risks.

This risk is particularly important for vulnerable groups, such as children, pregnant women, and those with pre-existing health conditions (Kaur *et al.*, 2025) [12]. Lead toxicity is especially harmful to children and can lead to permanent cognitive impairment and developmental delays (Tchounwou *et al.*, 2012) [30]. The widespread consumption of tomatoes in the local diet indicates that contamination of this vegetable with heavy metals may have significant public health implications for the communities in and around Gorakhpur (Jain *et al.*, 2023; Rai *et al.*, 2019) [11, 22]. These concerns are heightened due to the progressive nature of contamination, which is expected to escalate the health risks with time (Kumar & Singh, 2023) [15].

6. Environmental and Policy Implication

The findings of this study highlight the necessity for immediate action to mitigate heavy metal pollution in the Ramgarh Lake system. The lake is an important water resource to the region, and pollution of the lake has far reached effects on agriculture, food safety and public health (Mukherjee & Bhowmick, 2024; Kaur *et al.*, 2025) [12, 18].

Actions to take:

- **6.1 Regular Monitoring:** Initiate a regular monitoring program for heavy metals in the water of Ramgarh Lake, outflow channels and agricultural soils in the

command area. Monitoring should be done in both wet and dry seasons to account for seasonal variability (Mukherjee & Bhowmick, 2024; Kumar & Singh, 2023) ^[15, 18].

6.2 Remediation Strategies: Application of phytoremediation and other cost-effective remediation approaches to reduce heavy metal contamination in soils. Native hyperaccumulator species could be identified and cultivated (Kumar & Singh, 2023; Reddy & Reddy, 2021) ^[15, 23].

6.3 Wastewater Treatment: Facilities to treat wastewater to reduce concentrations of heavy metals before it can be used for irrigation. Constructed wetlands and other low-cost technologies could be considered (Sushil *et al.*, 2024; Alloway, 2013) ^[2, 29].

6.4 Public Awareness: Awareness among local farmers and communities regarding risks of using polluted water for irrigation and consuming contaminated vegetables (Kaur *et al.*, 2025; Rai *et al.*, 2019) ^[12, 22].

6.5 Policy Development: Development of policies and guidelines for safe use of wastewater in agriculture with specific reference to heavy metal contamination. The recent attention of the Indian government towards water quality standards needs to be strengthened with enforcement mechanisms (CPCB, 2000; Awashthi, 2000) ^[5].

6.6 Health Surveillance: Development of health surveillance protocols for populations that consume vegetables irrigated with wastewater. Biomarker studies can help to identify early signs of heavy metal exposure (Jain *et al.*, 2023; Kharkwal *et al.*, 2023) ^[11].

6.7 Alternative Water Sources: Development of alternative water resources for irrigation such as rainwater harvesting and treated wastewater (Sushil *et al.*, 2024; Rai *et al.*, 2019) ^[22, 29].

6.8 Crop Selection: Encourage crops with less potential to accumulate heavy metals in polluted zones. For example, fruit vegetables tend to accumulate less amounts of metals than leafy vegetables (Arora *et al.*, 2008; Gupta *et al.*, 2010; Sharma *et al.*, 2018) ^[3, 9, 25].

7. Study limitations

However, this study has some limitations that need to be acknowledged that provides valuable insight into the accumulation of heavy metals in soil and tomato under polluted water irrigation:

1. The study was conducted as a pot experiment under controlled environment, which might not fully represent the field conditions. These results need to be validated in field experiments under real-world conditions (Kaur *et al.*, 2025; Rai *et al.*, 2019) ^[12, 22].
2. Other vegetables commonly grown in the region should also be evaluated to provide a comprehensive risk assessment (Arora *et al.*, 2008; Sharma *et al.*, 2018) ^[3, 25].
3. Health risk assessment was performed by comparing with permissible limits instead of direct calculation of Hazard Index (HI) or carcinogenic risk. Future studies should

include quantitative health risk assessment using standard methodologies (Kharkwal *et al.*, 2023; Jain *et al.*, 2023) ^[11, 13].

4. Seasonal changes in water quality and metal bioavailability were not considered. The study was conducted in the same month every year (September), but metal concentrations can be influenced by seasonal changes in water quality (Mukherjee & Bhowmick, 2024; Kumar & Singh, 2023) ^[15, 18].

5. The two-year comparison is useful but provides a limited periodic record. Longer-term studies (5–10 years) are required to assess the contamination path and its ecological and health impacts (Kumar & Singh, 2023; Reddy & Reddy, 2021) ^[15, 23].

6. Chemical speciation of metals in soil near Ramgarh Lake was not studied which influences bioavailability and uptake of metals by plants (Nagajyoti *et al.*, 2010; Alloway, 2013) ^[2, 19].

Future studies should overcome these limitations by conducting field experiments, involving different crop species, performing a comprehensive health risk assessment (including HI and carcinogenic risk calculations), and long-term monitoring of heavy metal dynamics in the soil-plant system (Kumar & Singh, 2023; Kaur *et al.*, 2025) ^[1, 15].

Conclusion

The present two years comparative investigation was conducted in triplicate to study the accumulation of six heavy metals viz., Pb, Cd, As, Cu, Cr and Zn in soil and tomato plants irrigated with polluted water of Gura Nala, the out flow channel of Ramgarh Lake, Gorakhpur. The main findings are:

1. **Soil contamination:** The heavy metal contents increased significantly in soil receiving irrigation with polluted water. Pb showed the highest level of enrichment (481.9% increase over control) followed by Cu (463.5% increase). Soil Pb increased significantly year-over-year ($p = 0.006$) showing progressive contamination with a yearly increase rate of 6.9%.
2. **Plant Accumulation:** Concentrations of all six metals were significantly higher in tomato plants grown with polluted water compared to control plants. The fruit (P4) recorded the highest level of metals: Pb (1.259-1.310 ppm), Cd (0.013-0.014 ppm), As (0.017-0.019 ppm), Cu (0.589-0.610 ppm), Cr (0.075-0.080 ppm) and Zn (0.361-0.375 ppm).
3. **Periodic Trends:** Pb in treated soil ($p = 0.006$) and Pb ($p = 0.048$) and As ($p = 0.018$) in fruits showed significant increases between 2024^[29] and 2025 (independent samples t-test, two-way ANOVA). Significant Year \times Treatment interactions indicated that these increases were specific to the treatment group.
4. **Safety Concerns:** Pb and As levels in fruit of treated plants exceeded permissible limits of FAO/WHO in both years and increased significantly in 2025. Pb was 4–13 times above the limit, and As was 1.7–1.9 times above the conservative limit of 0.01 ppm.
5. **Accumulation Pattern:** Most of the samples showed a similar pattern of metal accumulation (Pb > Cu > Zn >

Cr > As > Cd). The BAF values (<1) showed moderate accumulation and the TF values (>1 for all metals) indicated efficient translocation of heavy metals from the leaves to the fruits in tomato plants.

- 6. Public Health Implications:** The use of Ramgarh Lake outflow water in tomato cultivation might pose health risks due to the presence of toxic heavy metals in edible parts and increasing contamination with time is expected to increase the risk. This risk is of special concern for vulnerable populations such as children and pregnant women.

The study emphasizes the need for treatment of Ramgarh Lake and its outflow water, regular monitoring of heavy metals in the agricultural system and awareness among the public on the risks of consuming vegetables grown with contaminated water. Such measures are crucial to protect the health of local communities and to guarantee the sustainability of agricultural practices in the region.

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