

Reuse of treated wastewater from the Rabat slaughterhouse in agriculture: heavy metal accumulation and bioaccumulation

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Abstract. Slaughterhouses generate large volumes of wastewater that is rich in organic matter, nutrients, heavy metals, and microbial contaminants. In Rabat, as in many other cities, managing slaughterhouse wastewater is a significant environmental challenge. Direct discharge of untreated wastewater can contaminate soil, surface water, and groundwater, thereby threatening environmental quality and public health. This study evaluated soil quality by examining the accumulation and bioaccumulation of heavy metals, including chromium (Cr), lead (Pb), copper (Cu), zinc (Zn), cadmium (Cd), and iron (Fe), and their transfer from soil to bean plants (*Vicia faba*). Metal concentrations were determined in agricultural soils and different plant organs (roots, stems, and leaves) exposed to irrigation with untreated wastewater. The results showed that heavy metals mainly accumulated in the soil and roots, with lower concentrations detected in stems and leaves. Elevated concentrations of Pb and Zn were observed in the roots, indicating limited translocation of these metals to the plant's aerial parts. Additionally, soils irrigated with untreated wastewater exhibited higher metal concentrations, contributing to increased metal uptake by *V. faba*. These findings demonstrate that using untreated slaughterhouse wastewater for irrigation promotes the accumulation of heavy metals in soil and their transfer to crops. This highlights the importance of implementing effective wastewater treatment and regular monitoring programmes to prevent the contamination of agricultural products by heavy metals and to protect environmental and food safety.

Keywords: bean plant, environmental risk, metal translocation, soil contamination, wastewater irrigation.

Introduction. Water scarcity is one of the major challenges facing many regions of the world, particularly arid and semi-arid areas. Population growth, urbanisation, intensified agriculture, and climate change have put considerable pressure on conventional freshwater resources. Consequently, finding alternative water sources has become crucial for ensuring sustainable agricultural production and food security (Corcoran et al., 2010; Qadir et al., 2020).

Of the available alternative water resources, treated wastewater has attracted increasing attention due to its potential to supplement irrigation water supplies while reducing effluent discharge into the environment. In addition to providing water, treated wastewater can supply nutrients such as nitrogen and phosphorus, thereby reducing the need for fertilisers and promoting the circular use of resources. However, wastewater reuse may also introduce contaminants such as heavy metals, pathogens, and organic pollutants into agricultural systems (Wu et al., 2022; Zheng et al., 2022).

Heavy metals are of particular concern due to their persistence, non-biodegradable nature, and tendency to accumulate in soils over time. Long-term irrigation with wastewater can lead to the gradual enrichment of agricultural soils with metals such as chromium (Cr), lead (Pb), copper (Cu), zinc (Zn), cadmium (Cd), and iron (Fe). These elements can then be absorbed by plants and transferred through the food chain, which poses potential risks to environmental quality, crop productivity, and human health (Rattan et al., 2005; Sharma et al., 2006; Zhang et al., 2017).

Several studies have reported that the accumulation and bioaccumulation of heavy metals depend on the quality of the irrigation water used and the characteristics of the cultivated species. Plant roots often act as the primary site of metal retention, limiting the transfer of certain elements to aerial tissues. Nevertheless, prolonged exposure to heavy metals can lead to their accumulation in aerial tissues. Nevertheless, prolonged exposure to contaminated irrigation water can lead to increased metal concentrations in edible plant parts, posing a potential threat to food safety (Klay et al., 2010; Bouchouata et al., 2012). In Morocco, wastewater reuse has become an important strategy for addressing increasing water shortages. The city of Rabat produces large amounts of slaughterhouse wastewater with high organic content and the potential presence of metallic contaminants. Treating this wastewater for agricultural use could contribute to water conservation and environmental protection. However, the impact of this practice on soil quality and the transfer of heavy metals to crops remains insufficiently documented.

This study investigates the accumulation and bioaccumulation of heavy metals in soils irrigated with wastewater from the Rabat slaughterhouse, as well as in bean plants (*Vicia faba*) grown under various irrigation conditions. Particular attention is given to Cr, Pb, Cu, Zn, Cd, and Fe. The study aims to evaluate the distribution of these metals in soil and plant tissues, and assess the potential risks of their transfer into agricultural production systems.

Material and Method

Description of the study sites. The wastewater used in this study originated from the municipal slaughterhouse in the Yacoub Al Mansour district of Rabat. This traditional slaughterhouse covers an area of around 1,800 m² and serves one of the city's most densely populated areas (Figure 1). Slaughterhouse activities generate considerable volumes of wastewater containing organic matter, nutrients, and trace metals, which could affect the environment if discharged without adequate treatment.

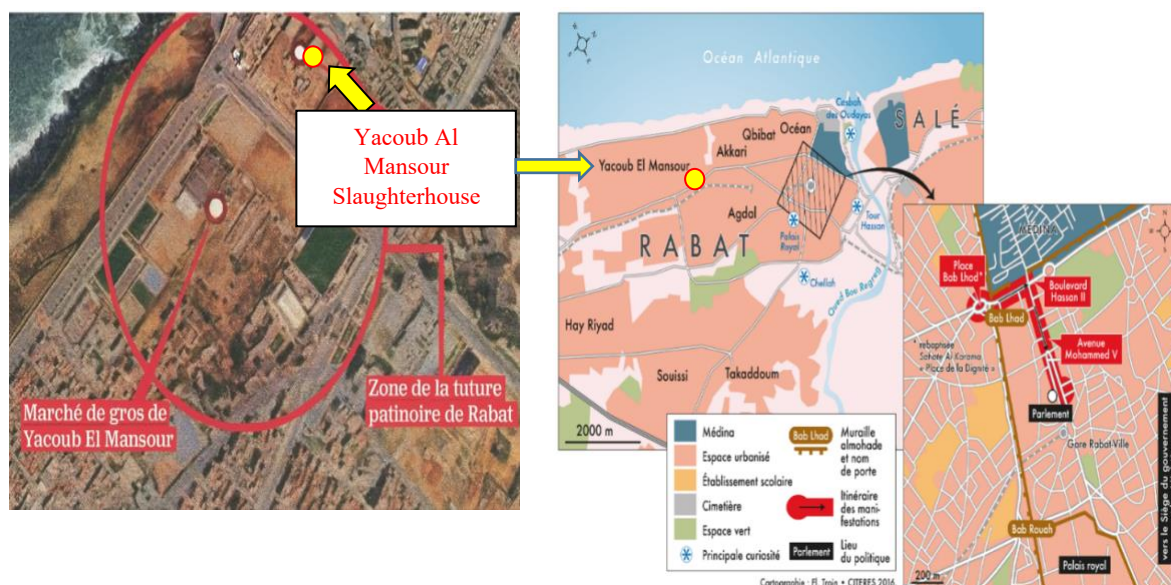


Figure 1. Synoptic diagram of the slaughterhouse of the city of Rabat.

Sampling and analytical procedures. Quantitative analyses of selected heavy metals (Cd, Cr, Cu, Pb, Zn, Fe, and P) were performed at the National Center for Scientific and Technical Research (CNRST), Rabat, within the Technical Support Unit for Scientific Research (UATRS). The experiment evaluated four irrigation water sources :

Table 1

Codes and corresponding irrigation water types

Code	Water type
RW	Raw wastewater
WW	Well water
BTW	Biologically treated wastewater
FW	Filtered treated wastewater

The concentrations of heavy metals were determined in the irrigation water, the agricultural soil, and the *V. faba*. Soil samples were collected before irrigation began and again after eight months of continuous irrigation. Plant samples were collected at harvest time and separated into roots, stems, and leaves for analysis.

V. faba were cultivated in four separate pots, each of which was irrigated with a different type of water. An irrigation volume of 80 ml was applied every two days for eight months (Figure 2).

For each treatment, samples were collected in triplicate. The water, soil, and plant samples were prepared according to standardised laboratory procedures. Prior to analysis, the soil and plant samples were dried, homogenised, and subjected to acid digestion in accordance with established protocols.

Metal concentrations were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) at the UATRS laboratory. Quality control procedures included replicate analyses and the use of certified reference materials to ensure the precision, accuracy, and reliability of the analytical results.

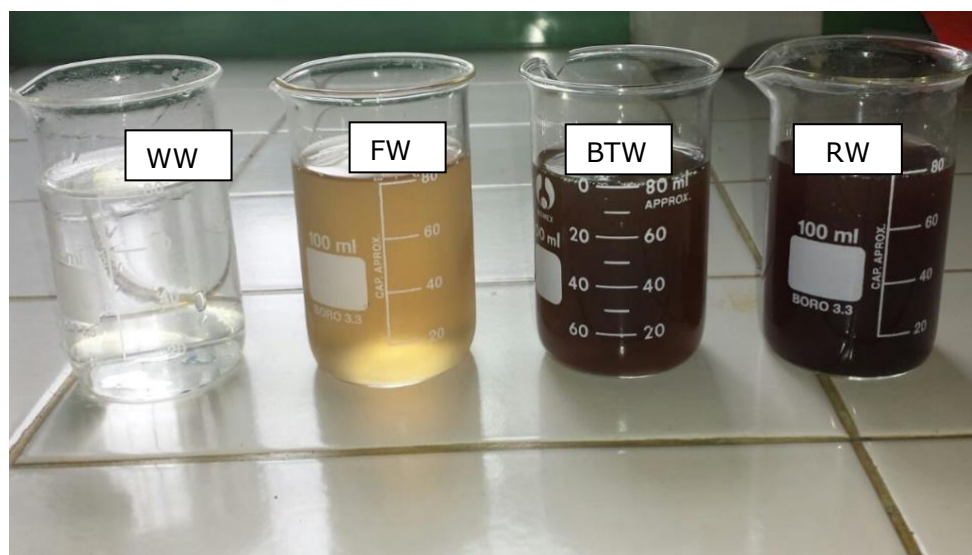


Figure 2. Types of water used for irrigation.

The results obtained were used to compare the influence of different sources of irrigation water on the accumulation of heavy metals in soils. They were also used to evaluate the transfer and bioaccumulation of these elements in *V. faba*, as well as to assess the potential environmental risks associated with the reuse of wastewater in agriculture.

Results and Discussion. Our study, which focused on the valorisation of treated wastewater from the Rabat slaughterhouse for agricultural use and on the accumulation and bioaccumulation of heavy metals, yielded several results.

Accumulation of trace metallic elements in irrigation water. Cd concentrations are fairly stable at 0.017 mg L⁻¹ for raw wastewater (RW), 0.014 mg L⁻¹ for well water (WW), 0.011 mg L⁻¹ for biologically treated wastewater (BTW), and 0.011 mg L⁻¹ for filtered treated wastewater (FW) (Table 2). The slight decrease observed after biological treatment and filtration may be related to adsorption processes onto suspended particles and organic matter removal during treatment. Previous studies have demonstrated that Cd is one of the most mobile trace metals in wastewater and can easily accumulate in agricultural soils when contaminated water is repeatedly used for irrigation (Alloway, 2013). Therefore, although the detected concentrations remain within irrigation guidelines, long-term monitoring is recommended to prevent gradual accumulation in soil and crops.

The chromium content is 0.067 mg L⁻¹ in RW, compared to 0.01 mg L⁻¹ in WW, BTW, and FW. These values comply with the general discharge limit standards stipulated in Article 12 of Decree No. 2-04-553 (Ehaouzi et al., 2015), as well as the required limit for irrigation water (1 mg L⁻¹) (National Academy of Sciences, 1972). These concentrations are lower than those recorded in treated wastewater from Marrakech (values ranging from 0.22 to 0.28 mg L⁻¹), which exceeds discharge standards (0.1 mg L⁻¹) (Ellouzi et al., 2015), and are also lower than values recorded in Fes wastewater (ranging from 0.1 to 8.24 mg L⁻¹), which far exceed the required limit for irrigation water (Khamar, 2002). The chromium concentration was higher in RW (0.067 mg L⁻¹) than in treated water samples, indicating the effectiveness of treatment in reducing chromium availability. Similar observations were reported by Ellouzi et al. (2015). However, chromium behavior depends strongly on its chemical form, as Cr(VI) is more toxic and mobile than Cr(III) (Kabata-Pendias, 2011).

Copper levels are 0.073 mg L⁻¹, 0.037 mg L⁻¹, 0.015 mg L⁻¹, and 0.033 mg L⁻¹ for RW, WW, BTW, and FW, respectively. These values do not exceed the maximum allowable concentration of 2 mg L⁻¹ for irrigation water, according to World Health Organization (WHO) standards (World Health Organization, 1989) and International Organization for Standardization (2023). Comparable results were reported by several authors studying wastewater reuse in agriculture, where copper generally remained below critical levels due to its strong affinity for organic matter and suspended solids (Mashauri et al., 2000). Nevertheless, continuous application of wastewater containing copper may lead to gradual accumulation in agricultural soils because copper is relatively persistent.

The iron content of the four types of water is approximately 1.73 mg L⁻¹, 0.417 mg L⁻¹, 0.663 mg L⁻¹, and 0.823 mg L⁻¹ for RW, WW, BTW, and FW, respectively. According to WHO standards (World Health Organization, 1989), these values do not exceed the maximum limit of 5 mg L⁻¹ for irrigation water. By contrast, wastewater from the city of Fès shows iron concentrations ranging from 0.93 to 24 mg L⁻¹, which surpass the recommended limits for irrigation water. This is mainly due to contributions from the old medina of the city, where discharges are enriched with iron (Khamar, 2002). This finding is consistent with previous studies indicating that iron is commonly present at higher concentrations in municipal and industrial wastewater due to corrosion of metallic infrastructures and the presence of iron-containing particles (Khamar, 2002; Kabata-Pendias, 2011). The lead concentrations are approximately 0.537 mg L⁻¹, 0.15 mg L⁻¹, 0.045 mg L⁻¹, and 0.043 mg L⁻¹ for RW, WW, BTW, and FW, respectively (Table 2). These values do not exceed the maximum allowable concentration (3.8 mg L⁻¹) for irrigation water according to WHO standards. This reduction may be explained by the association of lead with suspended solids, which are removed during biological treatment and filtration. Similar reductions in Pb concentrations after wastewater treatment have been reported by other researchers, confirming the tendency of lead to bind strongly to particulate matter and organic fractions (Alloway, 2013).

Table 2

Heavy metals in water intended for irrigation

Parameters (mg L ⁻¹)	RW	WW	BTW	FW	Indirect discharge limit values	Values for irrigation water
Cadmium	0.017	0.014	0.013	0.011	0.1	0.01
Chromium	0.067	0.010	0.010	0.010	0.1	1
Copper	0.073	0.037	0.015	0.033	0.1	2
Iron	1.73	0.417	0.663	0.823	-	5
Lead	0.537	0.15	0.045	0.043	0.2	3.8

Note: RW - raw wastewater; WW - wastewater; BTW - biologically treated wastewater; FW - filtered wastewater.

Analysis of trace metallic elements in the treated wastewater from the Rabat slaughterhouse indicates that the water is not heavily contaminated with heavy metals or other potentially toxic elements. All measured concentrations remained below the maximum permissible limits recommended for irrigation water, as set out in WHO guidelines (1989) and International Organization for Standardization (2023). Therefore, from a regulatory perspective, the treated wastewater meets the recommended quality standards for agricultural reuse.

While the concentrations detected in the RW were consistently higher than those measured in the BTW and the FW, they were still relatively low compared to those reported in previous studies on municipal wastewater in Morocco (Khamar, 2002; Ellouzi et al., 2015). The reduction observed after treatment is likely to be associated with the removal of suspended solids and organic matter, which are recognised as major carriers of trace metals in wastewater. Similar reductions in heavy metal concentrations following wastewater treatment have been reported in previous studies, where adsorption onto sludge particles, sedimentation, and precipitation were identified as the main mechanisms responsible for metal removal (Kabata-Pendias, 2011; Alloway, 2013).

Nevertheless, compliance with irrigation standards should not be interpreted as a complete absence of environmental risk. Trace metals are non-biodegradable and can build up in agricultural soils over time through repeated irrigation, especially under long-term reuse conditions. The accumulation and mobility of these metals depend on various factors, such as soil texture, pH, organic matter content, irrigation frequency, and the chemical form of each metal. Furthermore, some of the accumulated metals may become available for plant uptake, potentially entering the food chain and posing ecological and human health risks (Kabata-Pendias, 2011; Alloway, 2013).

Therefore, assessing the quality of treated wastewater should not rely solely on compliance with regulatory thresholds, but should also evaluate trace metal accumulation in soils and crops under long-term irrigation conditions. This integrated approach provides a more comprehensive evaluation of the environmental sustainability and safety of wastewater reuse in agriculture, justifying the investigation of heavy metal accumulation in soil and *V. faba* presented in the following section.

Accumulation of trace metallic elements in the soil. The Cd concentration remains below the detection limit (0.001 mg kg⁻¹) in all soils, regardless of the type of irrigation water used. This suggests that Cd is not being introduced or accumulated significantly from the different sources of irrigation water used (Table 3). The absence of detectable Cd accumulation in all soil samples indicates that the irrigation waters used in this study did not constitute an important source of Cd contamination. Similar observations have been reported in agricultural soils irrigated with treated wastewater, where Cd concentrations remained below detectable or critical levels due to the low Cd content of the irrigation water (Rattan et al., 2005). Since Cd is highly toxic even at low concentrations and can accumulate in edible crops over prolonged periods, its continued monitoring remains essential despite the favourable results obtained in the present study (Alloway, 2013).

In contrast, a significant increase in Cr is evident in soils irrigated with untreated wastewater (1.17 mg kg⁻¹) compared to the initial soil concentration (0.10 mg kg⁻¹). The other irrigation types (WW, FW, and BTW) show lower accumulation, but still exceed the initial soil concentration (0.10 mg kg⁻¹), indicating the relative effectiveness of the

treatments in reducing the Cr load (Table 3). The higher chromium concentration observed in soils irrigated with RW confirms that untreated effluents are an important source of chromium accumulation in agricultural soils. Similar results were reported by Zhang et al. (2017), who demonstrated that repeated irrigation with untreated wastewater significantly increased soil chromium concentrations. The lower concentrations measured in soils irrigated with BTW and FW indicate that the treatment processes effectively reduced chromium inputs before agricultural reuse. Cr is known to bind strongly to clay minerals and soil organic matter, which explains its progressive accumulation following repeated irrigation (Kabata-Pendias, 2011).

The initial soil concentration of Cu is 0.04 mg kg^{-1} . This decreases slightly in soils irrigated with RW (0.038 mg kg^{-1}), FW (0.025 mg kg^{-1}), and WW (0.026 mg kg^{-1}), except for soil irrigated with BTW, where notable accumulation is observed (0.23 mg kg^{-1}) (Table 3). This may indicate low accumulation or increased mobility of copper towards other compartments, such as water or plants. Copper exhibited different behaviour to the other trace metals. Its relatively low concentration in most irrigated soils can be explained by its mobility and uptake by plants. Similar observations have been reported by Sharma et al. (2006), who found that copper is often transferred from soil to plant tissues because it is an essential micronutrient involved in several physiological processes. However, excessive accumulation of copper can be toxic to both plants and soil microorganisms if wastewater irrigation is maintained over long periods (Alloway, 2013).

A significant increase in Fe is observed after irrigation. Soils irrigated with RW show the highest concentration of Fe (26.08 mg kg^{-1}), followed by soils irrigated with WW (20.44 mg kg^{-1}), BTW (20.40 mg kg^{-1}), and FW (19.24 mg kg^{-1}) (Table 3). This indicates a substantial input of iron from the irrigation water, particularly the RW. Iron was the predominant metal accumulated in all irrigated soils, particularly under RW irrigation. This observation agrees with the findings of Rattan et al. (2005), who reported that iron is generally the dominant metallic element in wastewater-irrigated soils because of its natural abundance and continuous input through irrigation water. Although iron is an essential nutrient for plants, excessive accumulation may modify soil chemical properties and influence the availability of other micronutrients (Kabata-Pendias, 2011).

For Pb, the initial soil concentration is 0.09 mg kg^{-1} and increases in all irrigated soils. Values range between 0.30 and 0.48 mg kg^{-1} , with soils irrigated with RW again showing the highest concentrations. Lead accumulation is therefore notable, although it remains lower than that of Fe or Cr (Table 3). The greater accumulation of lead in soils irrigated with RW reflects the strong affinity of Pb for soil particles and organic matter. Similar observations have been reported by Alloway (2013), who demonstrated that lead is one of the least mobile trace metals and therefore tends to accumulate progressively in agricultural soils following repeated wastewater irrigation. Although the concentrations measured in this study remain below critical levels, continuous monitoring is recommended because lead may become available for plant uptake under changing soil conditions.

Finally, the initial soil Zn concentration is 0.15 mg kg^{-1} . After irrigation, it increases moderately in soils irrigated with RW (0.45 mg kg^{-1}) and decreases slightly in soils irrigated with FW (0.26 mg kg^{-1}) and BTW (0.14 mg kg^{-1}) (Table 3). The higher zinc concentration measured in soils irrigated with RW is consistent with previous studies showing that zinc accumulation is directly influenced by the quality of irrigation water and the duration of wastewater application (Zhang et al., 2017). Although zinc is an essential micronutrient required for plant growth, excessive accumulation may disturb nutrient balance in soils and increase the transfer of this metal to crops (Kabata-Pendias, 2011).

Overall, the results suggest that using raw slaughterhouse wastewater for irrigation leads to greater accumulation of heavy metals in soil than using treated wastewater. While the concentrations measured in this study are relatively low, continuous application over long periods could lead to progressive soil enrichment with potentially toxic elements. This can alter soil quality and fertility, increase the availability of metals for plant uptake, and enhance their transfer into the food chain through bioaccumulation. While wastewater treatment significantly reduces metal concentrations and therefore limits these risks, it does not ensure complete removal. Therefore, the sustainable reuse of wastewater in agriculture requires adequate treatment and regular monitoring of heavy metal levels in

water, soil, and crops, in order to protect environmental quality, food safety, and public health.

Table 3

Concentration of metallic elements in soil irrigated with four types of water

<i>Soil type (mg kg⁻¹)</i>	<i>Cd</i>	<i>Cr</i>	<i>Cu</i>	<i>Fe</i>	<i>Pb</i>	<i>Zn</i>	<i>P</i>
Initial soil	< 0.001	0.10	0.04	0.069	0.09	0.15	-
Soil irrigated with RW	< 0.001	1.17	0.038	26.08	0.48	0.45	7.98
Soil irrigated with WW	< 0.001	0.79	0.026	20.44	0.31	0.23	4.27
Soil irrigated with FW	< 0.001	0.81	0.025	19.24	0.32	0.26	5.04
Soil irrigated with BTW	< 0.001	0.80	0.23	20.40	0.30	0.14	5.10

Note: Cd - cadmium; Cr - chromium; Cu - copper; Fe - iron; Pb - lead; Zn - zinc; P - phosphorus.

Overall, the results suggest that using raw slaughterhouse wastewater for irrigation leads to greater accumulation of heavy metals in soil than using treated wastewater. While the concentrations measured in this study are relatively low, continuous application over long periods could lead to progressive soil enrichment with potentially toxic elements. This can alter soil quality and fertility, increase the availability of metals for plant uptake, and enhance their transfer into the food chain through bioaccumulation. While wastewater treatment significantly reduces metal concentrations and therefore limits these risks, it does not ensure complete removal. Therefore, the sustainable reuse of wastewater in agriculture requires adequate treatment and regular monitoring of heavy metal levels in water, soil, and crops, in order to protect environmental quality, food safety, and public health.

From a practical perspective, the accumulation of heavy metals in agricultural soils can have significant implications for both crop production and the sustainability of ecosystems. Excessive concentrations of metals can interfere with plant physiological processes, such as nutrient absorption, photosynthesis, and growth. This can potentially reduce crop yield and quality. Furthermore, the gradual transfer of metals from soil to edible plant tissues may raise food safety concerns for consumers. Therefore, to prevent long-term environmental degradation and ensure the safe agricultural reuse of wastewater resources, it is essential to implement effective wastewater treatment systems and periodically monitor soils and crops.

Bioaccumulation of trace metal elements in the plant (Bean)

Chromium (Cr) bioaccumulation. The concentration of chromium is highest in the soil irrigated with untreated slaughterhouse RW, reaching 1.17 mg kg⁻¹, compared to soils irrigated with FW at 0.81 mg kg⁻¹, BTW at 0.80 mg kg⁻¹, and WW at 0.79 mg kg⁻¹ (Figure 3). Similarly, the concentration of chromium in the roots of beans irrigated with RW is 0.65 mg kg⁻¹, which is higher than in the roots of beans irrigated with FW, BTW, and WW. On the other hand, the leaves of beans show greater chromium accumulation with BTW (0.077 mg kg⁻¹), compared to those irrigated with RW, WW, and FW (Figure 3). In the stems, chromium concentrations vary according to the type of irrigation water: 0.004 mg kg⁻¹ for RW, 0.023 mg kg⁻¹ for FW, 0.008 mg kg⁻¹ for BTW, and 0.002 mg kg⁻¹ for WW (Figure 3).

These results are consistent with several previous studies that highlight the influence of irrigation water quality on the accumulation of heavy metals in soils and plants. For example, a study by Ziegler Rivera et al. (2023) also reported higher chromium accumulation in soils irrigated with RW compared to those irrigated with treated water. Chromium accumulated predominantly in the roots, whereas its concentrations in stems and leaves remained considerably lower. This distribution agrees with previous studies demonstrating that chromium is largely retained in root tissues because of its strong adsorption to cell walls and its limited translocation to aerial plant parts (Kabata-Pendias, 2011). The restricted movement of chromium from roots to shoots may represent a protective mechanism that minimizes its potential toxicity to photosynthetic tissues.

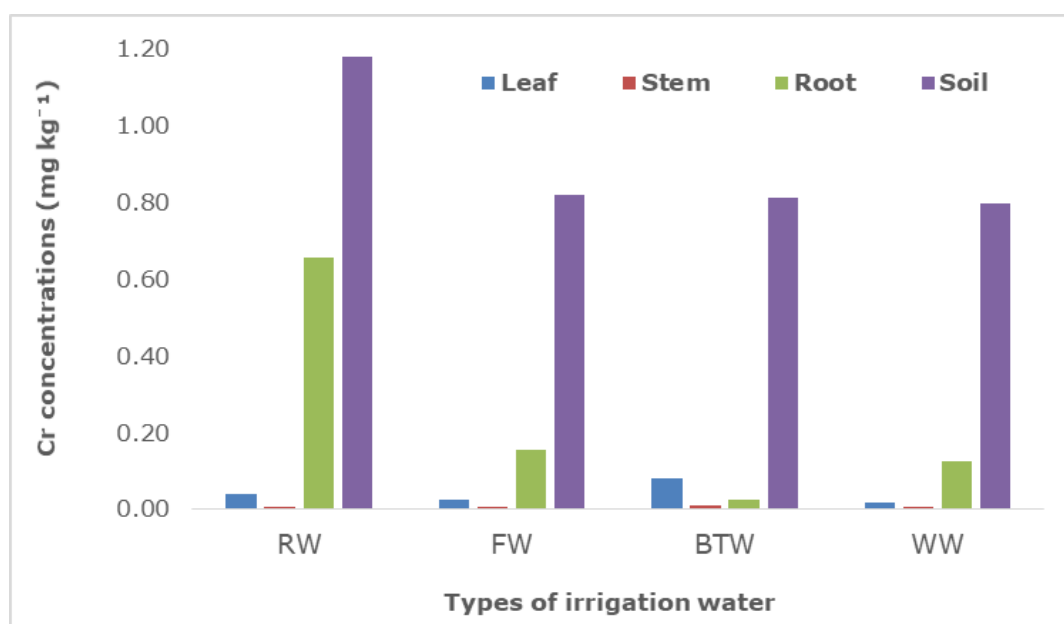


Figure 3. Bioaccumulation of chromium in the soil, stem, root, and leaf of *Vicia faba*.

ANOVA revealed a significant influence of plant organs on chromium distribution. The p-value indicates the probability that the observed differences occurred by chance, with values below 0.05 confirming a statistically significant effect. In the present study, chromium concentrations differed significantly between roots and stems, but no significant variation was detected in leaves ($p = 0.577$). This pattern suggests that chromium is preferentially retained in belowground tissues and stems, while its transfer to leaves is limited.

Copper (Cu) bioaccumulation. The highest copper concentration was observed in the roots of fava beans irrigated with RW, at a value of 0.18 mg kg^{-1} , followed by those irrigated with WW at 0.12 mg kg^{-1} , and FW at 0.032 mg kg^{-1} . In the leaves, the copper content was 0.076 mg kg^{-1} for plants irrigated with RW, which was higher than the values measured for FW (0.023 mg kg^{-1}), BTW (0.021 mg kg^{-1}), and WW (0.024 mg kg^{-1}). As for the stems, copper concentration is also higher in plants irrigated with RW (0.065 mg kg^{-1}) compared to those irrigated with FW (0.007 mg kg^{-1}) and BTW (0.016 mg kg^{-1}).

In the soil, copper content varies depending on the irrigation water type: soils irrigated with RW show a concentration of 0.038 mg kg^{-1} , while for the other water types (FW, BTW, WW), the respective values are 0.025 mg kg^{-1} , 0.023 mg kg^{-1} , and 0.026 mg kg^{-1} (Figure 4).

These results suggest that RW contributes more significantly to copper accumulation in the roots, leaves, and stems of fava beans, likely due to the metal load present in untreated water (Majdy et al., 2018). The differences in copper concentrations among various plant parts and in the soil also indicate a transfer of this element influenced by the quality of the irrigation water, with potential implications for metal accumulation and bioaccumulation in the food chain.

Copper showed a relatively balanced distribution among the different plant organs. As an essential micronutrient involved in photosynthesis, respiration, and enzyme activation, copper is readily transported within plants to satisfy metabolic requirements (Marschner, 2012). However, excessive copper accumulation may become phytotoxic by inducing oxidative stress and affecting plant growth.

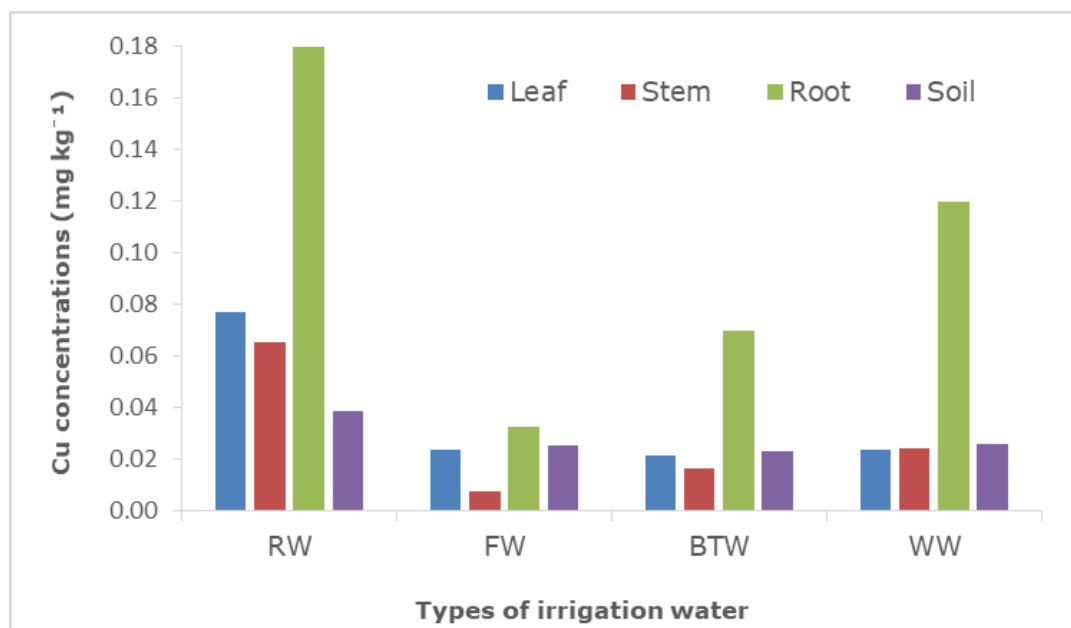


Figure 4. Bioaccumulation of copper in the soil, stem, root, and leaf of *Vicia faba*.

The statistical analysis showed that copper concentrations varied significantly between different plant organs. As all p-values were below the significance threshold of 0.05, it is unlikely that the observed differences are the result of random variation. The uneven distribution of copper may be related to its essential role in numerous physiological and enzymatic processes, resulting in differential accumulation within plant tissues.

Iron (Fe) bioaccumulation. The iron content of soil varies depending on the type of irrigation water used. The highest concentrations are found in soils irrigated with RW, at 26.08 mg kg⁻¹. Soils irrigated with other types of water show lower concentrations: 20.04 mg kg⁻¹ for FW, 19.24 mg kg⁻¹ for BTW, and 20.24 mg kg⁻¹ for WW. These results suggest that using RW for irrigation leads to greater iron accumulation in the soil than using other types of water. This can be attributed to the higher presence of particles or dissolved materials in RW, which often contains more unfiltered metallic elements (Klay et al., 2010). Iron accumulation in the soil may affect its availability to plants and ultimately influence crop quality and nutritional value. It may also pose an environmental risk if concentrations reach potentially toxic levels for local fauna or surrounding ecosystems (Rattan et al., 2005).

Iron concentration in the roots of fava beans also varies according to the irrigation water used. Fava beans irrigated with RW show the highest iron content at 4.6 mg kg⁻¹, followed by those irrigated with WW (2.3 mg kg⁻¹), FW (2.23 mg kg⁻¹), and finally BTW (1.7 mg kg⁻¹).

A similar trend is observed in the leaves: fava beans irrigated with RW contain 2.02 mg kg⁻¹ of iron, higher than those irrigated with FW (1.03 mg kg⁻¹), WW (1.07 mg kg⁻¹), and BTW (0.9 mg kg⁻¹). In the stems, iron concentration is also highest in plants irrigated with RW (2.25 mg kg⁻¹), compared to FW (1.16 mg kg⁻¹), BTW (1.5 mg kg⁻¹), and WW (0.74 mg kg⁻¹) (Figure 5).

These results show that fava beans irrigated with RW accumulate more iron throughout the plant. This can be attributed to the higher iron content of untreated wastewater, which has not been filtered or treated to remove metallic elements. While iron accumulation in plant tissues can be beneficial when levels are appropriate, as iron is essential for photosynthesis and growth (Wu et al., 2022), excessively high concentrations may pose toxicity risks to the plant and the food chain, especially if the beans are consumed directly. However, excessively high concentrations may pose a risk of toxicity to the plant and the food chain, particularly if the beans are consumed directly (Sharma et al., 2006).

Iron was the most abundant trace metal detected in the *V. faba*, particularly in the roots. Similar findings have been reported in wastewater-irrigated crops, where iron is preferentially retained in the root system because of its limited mobility within the xylem (Kabata-Pendias, 2011). Although iron is indispensable for chlorophyll synthesis and several enzymatic reactions, excessive accumulation may interfere with the uptake of other essential nutrients.

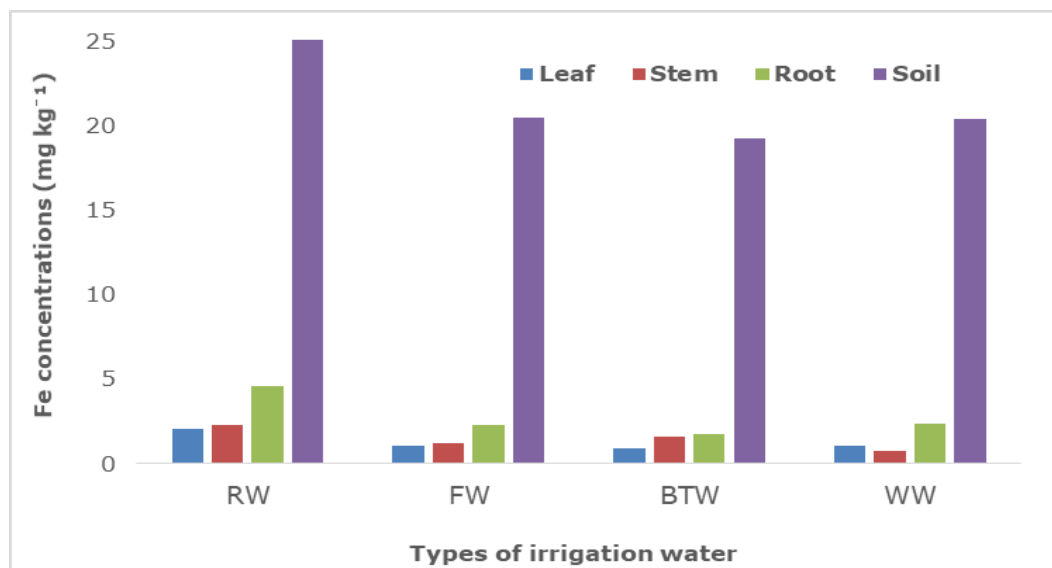


Figure 5. Bioaccumulation of iron in the soil, stem, root, and leaf of *Vicia faba*.

Analysis of variance (ANOVA) revealed a significant effect of plant organ on iron concentration. The low p-values obtained indicate that the variations recorded among the different organs are statistically significant ($p < 0.05$). These differences may reflect the specific physiological requirements of plant tissues, given that iron plays a crucial role in several metabolic functions, including photosynthesis, respiration, and various enzymatic reactions. Consequently, iron accumulation varies among plant organs according to their physiological and metabolic demands.

Lead (Pb) bioaccumulation. The lead content of the roots of fava beans irrigated with RW was high at 1.14 mg kg^{-1} , compared to roots irrigated with FW (0.036 mg kg^{-1}), BTW (0.016 mg kg^{-1}), and WW (0.15 mg kg^{-1}). In contrast, Pb concentrations in the leaves and stems of fava beans irrigated with different water types did not exceed 0.1 mg kg^{-1} .

The Pb content of soil irrigated with RW was 0.48 mg kg^{-1} , compared to 0.32 mg kg^{-1} for FW and 0.3 mg kg^{-1} for both BTW and WW. These results suggest that using RW for irrigation leads to higher lead accumulation in fava bean roots than using other water types. However, this accumulation was not observed in the leaves and stems, where Pb concentrations remained low. This suggests a limitation in the translocation of lead from the roots to the aerial parts (Figure 6).

Moreover, higher Pb concentrations in soil irrigated with RW may explain the greater absorption of this metal by the roots under these conditions (Zhang et al., 2017).

Lead accumulated mainly in the roots, whereas only limited amounts were translocated to stems and leaves. This behaviour is consistent with previous studies showing that lead exhibits very low mobility in plants because it is strongly bound to root cell walls and insoluble compounds (Alloway, 2013). The limited transfer of lead to aerial organs reduces its potential accumulation in edible tissues. However, prolonged wastewater irrigation may progressively increase plant exposure.

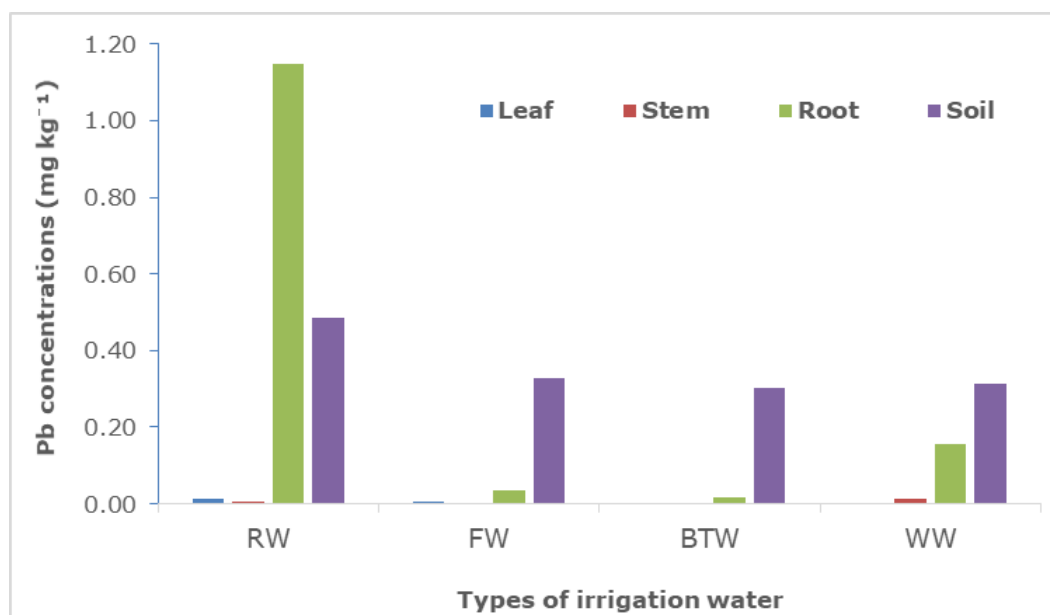


Figure 6. Bioaccumulation of lead in the soil, stem, root, and leaf of *Vicia faba*.

The results revealed a significant, organ-specific distribution of lead within the plant. A p-value lower than 0.05 confirms that the observed differences are statistically significant rather than being due to chance. The greater accumulation of lead in specific tissues can be explained by the restricted mobility of this non-essential element, as well as by plant mechanisms that limit its translocation to aerial organs.

Zinc (Zn) bioaccumulation. The highest zinc concentration was found in the leaves of fava beans irrigated with RW at 0.59 mg kg⁻¹, followed by those irrigated with WW at 0.18 mg kg⁻¹. In contrast, concentrations were much lower for FW (0.05 mg kg⁻¹) and BTW (0.025 mg kg⁻¹). This trend continued in the stems, where the Zn content was lower than in the leaves for all types of irrigation water. The highest concentration was again observed with RW (0.31 mg kg⁻¹), while BTW showed the lowest value (0.024 mg kg⁻¹).

In the roots, Zn concentration remains relatively high, particularly with RW irrigation (0.32 mg kg⁻¹). The other irrigation types show slightly lower, yet still significant concentrations (between 0.20 and 0.25 mg kg⁻¹). In the soil, irrigation with RW also leads to the highest Zn accumulation (0.45 mg kg⁻¹), which may promote greater uptake of this element by the plant. In contrast, the other water types show lower Zn concentrations in the soil, particularly BTW (0.14 mg kg⁻¹) (Figure 7).

These observations reveal a consistent pattern of zinc distribution: zinc levels are higher in all plant parts and the soil when RW is used for irrigation than when other sources are used.

The results show that heavy metals (Cr, Cu, Fe, Pb, and Zn) accumulate differently in the soil and in different parts of the fava plant, depending on the type of irrigation water used. Using RW for irrigation consistently results in higher metal concentrations in the soil and plants, particularly in the roots, than using other water sources (FW, BTW, WW). The increased bioaccumulation of heavy metals in fava beans associated with RW use highlights potential risks to food safety and the environment. Therefore, managing the quality of irrigation water is crucial in order to limit the transfer of heavy metals into agricultural ecosystems, and to protect soil and crop health.

Unlike chromium and lead, zinc showed greater translocation from roots to aerial plant organs. This behaviour reflects the essential physiological role of zinc in protein synthesis, enzyme activation, and plant growth (Marschner, 2012). Similar distribution patterns have been observed in legumes irrigated with treated wastewater, where zinc is more mobile than most other trace metals. Nevertheless, excessive zinc accumulation may disrupt nutrient balance and adversely affect plant metabolism.

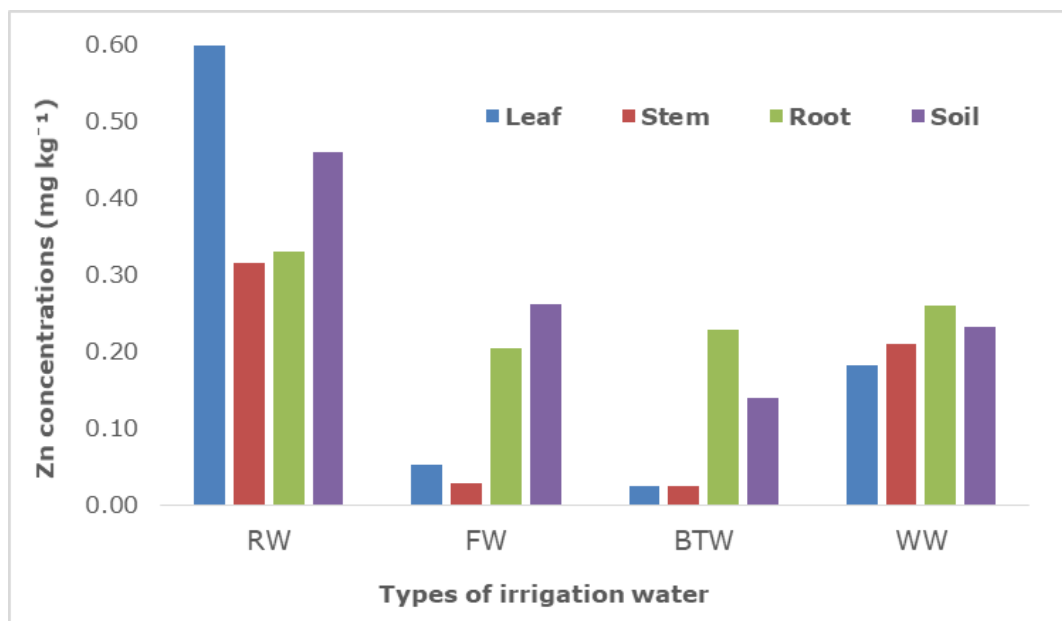


Figure 7. Bioaccumulation of zinc in the soil, stem, root, and leaf of *Vicia faba*.

ANOVA also identified significant differences in zinc concentrations between different parts of the plant. The statistical significance indicated by the p-values suggests that zinc is not distributed uniformly throughout the plant. This variability is consistent with the essential role of zinc in plant growth and metabolism, the influence of which can be seen in how it is taken up, transported, and stored in different tissues.

Conclusions. Heavy metals accumulated predominantly in soils and plant roots, indicating strong retention at the root level, particularly for Pb and Zn. Soils irrigated with RW exhibited elevated metal concentrations, confirming their role as a primary source of contamination within the soil-plant system.

Translocation to aerial parts was limited. Leaves showed moderate accumulation of certain elements, notably Zn, under EB irrigation, whereas stems generally contained the lowest concentrations.

Overall, metal distribution followed a clear downward trend (soil > roots > leaves > stems), highlighting restricted mobility within plant tissues. These findings emphasise the need for strict control of irrigation water quality to minimise the transfer of heavy metals into agricultural systems.

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