Assessment of heavy metals accumulation in food crops irrigated with water of Boumerzoug river (Constantine, North East of Algeria)

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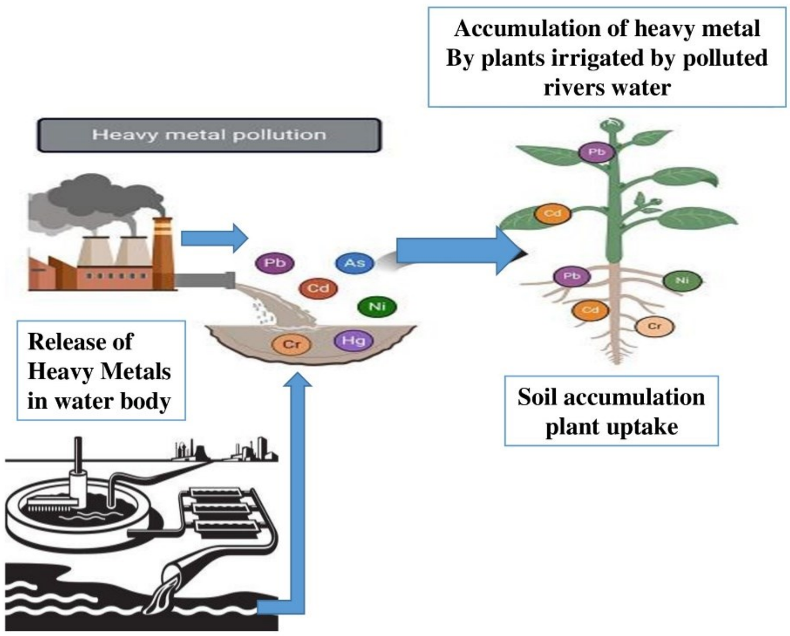
GRAPHICAL ABSTRACT



ABSTRACT

The progress of an economy is critically dependent on industrialization; however, this process can also negatively impact the ecosystem by releasing harmful elements, such as heavy metals, into surface aquifers. This study aimed to assess the contamination of edible plants (pepper, watermelon, and lettuce) grown on agricultural land surrounding the Boumerzoug River in Constantine Province, Algeria, which is irrigated with river water. The plants were subjected to total quantification of metals (Cu, Cd, Zn, Cr, Pb, and Fe) across four organs (root, stem, leaf, and fruit). While metal concentrations were generally within acceptable limits, the levels of Pb and Cd were found to be elevated, indicating an increased potential for health concerns with regular consumption. The type of plant organ significantly affected metal accumulation, with the root being the organ that accumulated the highest concentrations of metals, and lettuce exhibiting the greatest overall accumulation. The Transfer Factor (TF) for Cd and Cu was found to be greater than 1, suggesting a notable bioaccumulation of these metals.

Keywords: River contamination, Heavy metal, Plant, Wastewater, Metal accumulation, Irrigation water.

1. Introduction

Algeria is one of the countries where water availability is limited, while demand continues to rise. This increasing demand is attributed to population growth, industrial and agricultural development, as well as frequent drought episodes (Soltani, 2021). Desalination, recycling, and the reuse of wastewater are adaptation and mitigation strategies that can relieve pressure on traditional water resources, including surface water, fossil aquifers, and groundwater (Iglesias and Garrote, 2015). In many developing nations, the irrigation of crops with treated, insufficiently treated, diluted, or even raw wastewater is a common practice in urban and peri-urban areas (Gatto D'Andrea et al., 2015). These wastewaters represent a significant resource and an appealing alternative due to their fertilizing potential (Arborea et al., 2017). However, they also contain numerous pollutants, such as suspended solids, pathogenic microorganisms, and various chemicals, including heavy metals and pesticides, which can render these waters unsuitable for irrigation (Djegbe et al., 2018).

The use of untreated wastewater on agricultural lands leads to the accumulation of heavy metals in soils to levels that may pose long-term ecological and health-related concerns (Behbahaninia and Mirbagheri, 2008). Urban wastewater contains a wide range of heavy metals, with the most prevalent being iron, zinc, copper, and lead. Additionally, trace amounts of other metals, such as manganese, aluminum, chromium, arsenic, selenium, mercury, cadmium, molybdenum, and nickel, are also present. These metals originate from various sources, including consumer goods, material deterioration in water distribution networks, stormwater treatment in combined sewer systems, services (e.g., health and automotive), and potentially industrial waste. Nickel (Ni), cadmium (Cd), and lead (Pb) are identified as the three most hazardous elements (Sellami et al., 2022).

Heavy metal accumulation in plants is influenced by the source of wastewater, the nature of the crop, the specific plant species, and the uptake of metals, which can be evaluated through either the absorption by plants or by assessing metal transfer factors from soils (Rattan et al., 2005). Typically, the concentration of heavy metals present in plants is lower than that found in the wastewater or soil in which these crops are cultivated (Khan et al., 2008). Heavy metals impact living organisms in various ways. In contaminated areas, plants exhibit reduced growth, metabolic alterations (such as inhibited photosynthesis), decreased biomass production, and increased metal accumulation (Belabed et al., 2013). Furthermore, elevated levels of heavy metals in plant tissues can lead to toxicity issues for humans and other organisms that consume these plants (Masona et al., 2011).

The Boumerzoug River (BR) receives both the domestic wastewater from the city of El-Khroub and the effluents from the PMO complex (mechanical industry) via its confluence with the Hamimime River. According to Drici and Mouats (2018), the waters of the Boumerzoug River are contaminated. Given that surface water is the primary source of irrigation for agriculture in this region, utilizing this water may pose significant health and environmental risks. Therefore, the objective of this study is to assess the contamination by metal trace elements (MTE) or heavy metals in crops (Pepper, Watermelon, and Lettuce) irrigated with water from the Boumerzoug River.

2. Materials and methods

2.1. Study area:

The Boumerzoug sub-basin is located in the southeastern part of the Kébir-Rhumel basin. The Kébir River is formed by the confluence of two significant rivers: the Rhumel and Endja rivers. These rivers supply water to Algeria's largest dam, the Benni-Haroun, which commenced operations in 2003, underscoring the importance of the water quality in the tributaries feeding into it. The Boumerzoug River, a tributary of the Rhumel River, is situated to the south of Constantine, which is the third largest city in Algeria. It drains a watershed of 1,832 km², characterized by a fragmented landscape dominated by isolated and abrupt horst reliefs. The river is 50 km long and originates from the confluence of Oued El Kleb and Oued Meleh, approximately 25 km south of Constantine (Fig. 1). It traverses several settlements, including Ain Mlila, Ouled Rahmoun, El Guerrah, and El Khroub, before reaching the Rhumel River in the city of Constantine (Keddari et al., 2019).

The Boumerzoug River receives several tributaries along its course, with the most significant being the Hamimime River. The industrial units within the Boumerzoug River basin are organized into several areas, predominantly concentrated around major settlements and along the river. Notable industries in the region include gas production (NAFTAL), significant enterprises for the National Production of Agricultural Equipment (ENPMA), production of machine tools (ENPMO), the National Society for Tobacco and Cigarette Manufacturing (SNTA), various food processing industries, tanneries, and others (ABH, 2004).

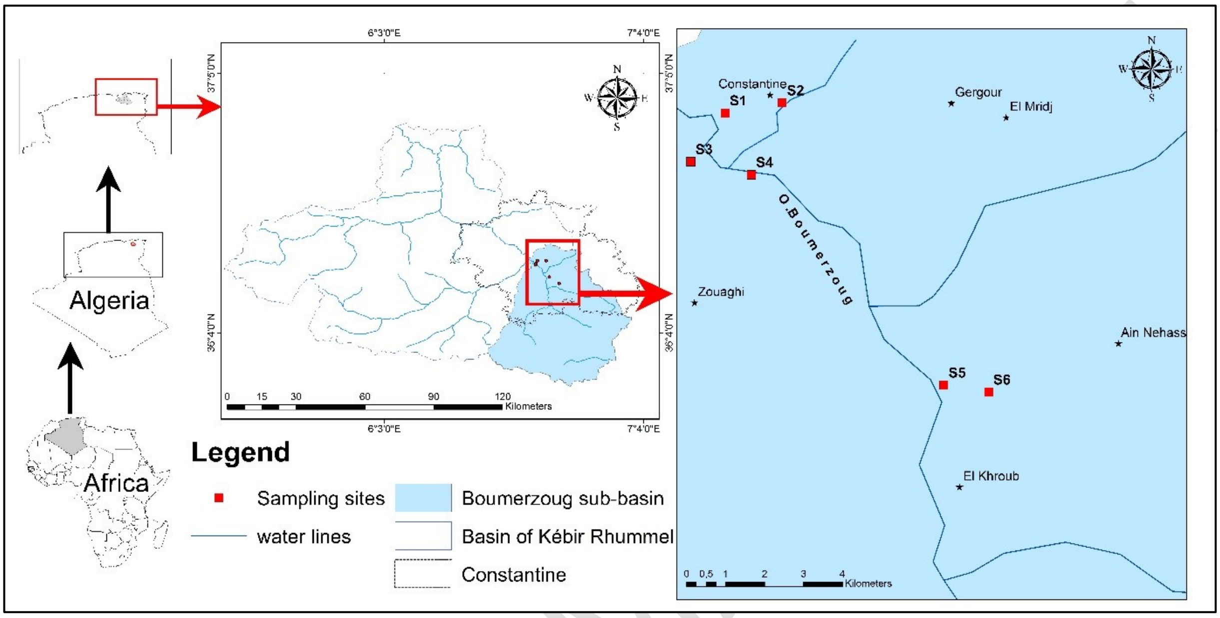


Figure 1. (A): situation of The Boumerzoug sub-basin in the southeastern part of basin Kébir-Rhumel in Constantine province (northeastern Algeria); **(B):** Locations of plants sampling sites at Boumerzoug river in the province of Constantine (northeastern Algeria).

2.2. Plants sampling and treatments:

Plant samples were taken in August 2020 from six sites (S1 to S6) (three plants from each site) irrigated at the edges of the BR. The tested plants were lettuce (*Lactuca sativa*), watermelon (*Citrullus lanatus*), and pepper (*Capsicum annuum*). Pepper and watermelon (stem, leaves, root, and fruit) and lettuce (root and leaves) were washed by rapid immersion in baths of demineralized water of high purity. Once washed, the plants were drained, dried at 40 °C, and then milled. The obtained powders were kept in hermetically sealed bags (Kadem, 2005).

2.3. Determination of heavy metal content:

According to the digestion-based procedure outlined by Hoening et al. (1979), plant extracts were created. Aqua regia (sulfonitric-hydrogen peroxide solution) was used for the recovery of heavy metals (Pb, Cd, Cr, Cu, Fe, and Zn). Briefly, a 1 g sample of plant powder was treated with 1 ml of sulfuric acid, 3 ml of nitric acid, and 3 ml of hydrogen peroxide at 30 volumes. In a 250 ml Erlenmeyer flask fitted to a condenser, the entire mixture was heated to boiling at 75 °C for 15 minutes. After cooling and rinsing the condenser with a few milliliters of demineralized water as required, the substance was filtered through filter paper of 0.45 µm into a 50 ml to 100 ml flask at a medium filtration speed.

The Bruker 820-MS ICP-MS Spectrometer was used to determine the heavy metal analyses on this test solution. The approved multi-element standard was used to create the calibration curve. The main stock solution was used to create an intermediate stock of 10 mg L-1, and standard stocks of 5, 10, 20, 50, 100, and 250 µg L-1 were used to build a calibration curve. Each of the three samples was tested three times using an ICPMS equipment. 1% HNO3 was used to prepare the blind sample (Temizer et al., 2018). Sengul (2016) found the standard slope, MTE (determination limit), and MTE (detection limit).

2.4. Transfer factor:

The metal trace elements (MTE) transfer factor from soil to plants was determined using the following equation (Cui *et al*., 2004):

$$TF = \frac{C_{Plant}}{C_{Soil}}$$

Where C_{Plante} and C_{Soil} represent the concentrations of MTE in the extract from plants and soil respectively.

2.5. Statistical analysis:

The studied parameters were subjected to a statistical test carried out by the analysis of variance (ANOVA). The software used was Excel Stat version 2014 software.

3. Results and Discussion

3.1. Heavy metals content in irrigated plants:

The results of MTE in plants (pepper, Watermelon, lettuce) in four organs (leaf (L), stem (S), root (R), fruits (Fr)), irrigated by the waters of the BR are presented in Table 1 and Figure 2.

Table 1. MTE (μg g^{-1}) contents of plants irrigated by water from the Boumerzoug river in Constantine, North-East of Algeria, Mean± S.D (standard deviation S.D), n=3.

MTE / Plants	Pb (μg g^{-1}) Mean±S.D	Cd(μg g^{-1}) Mean± S.D	Zn(μg g^{-1}) Mean± S.D	Fe(μg g^{-1}) Mean± S.D	Cr(μg g^{-1}) Mean± S.D	Cu(μg g^{-1}) Mean± S.D
Pepper L	0,51±0,02	0,14±0,02	6,64±1,01	12,58±1,35	0,32±0,06	0,48±0,03
Pepper S	1,32±0,37	0,36±0,21	12,24±2,05	19,86±2,37	0,99±0,01	0,16±0,02
Pepper R	2,68±0,27	0,14±0,14	1,91±0,23	61,37±4,27	1,59±0,16	0,54±0,06
Pepper Fr	1,22±0,19	0,19±0,07	6,65±1,23	34,47±2,17	1,17±0,39	0,52±0,19
Watermelon L	0,43±0,17	0,13±0,11	6,52±1,56	9,78±1,36	0,43±0,26	0,32±0,06
Watermelon S	1,35±0,12	0,25±0,07	8,94±1,32	14,53±1,43	0,93±0,01	0,14±0,01
Watermelon R	1,95±0,36	0,19±0,01	1,58±0,15	58,37±5,73	1,13±0,62	0,39±0,02
Watermelon Fr	0,86±0,58	0,19±0,14	5,99±1,13	36,49±2,08	1,27±0,02	0,52±0,32
Laittuce L	2,02±0,41	0,29±0,04	11,52±2,05	9,78±1,07	1,45±0,6	0,44±0,16
Laittuce R	1,58±0,13	0,20±0,03	4,58±1,01	58,37±5,52	1,06±0,03	0,32±0,04

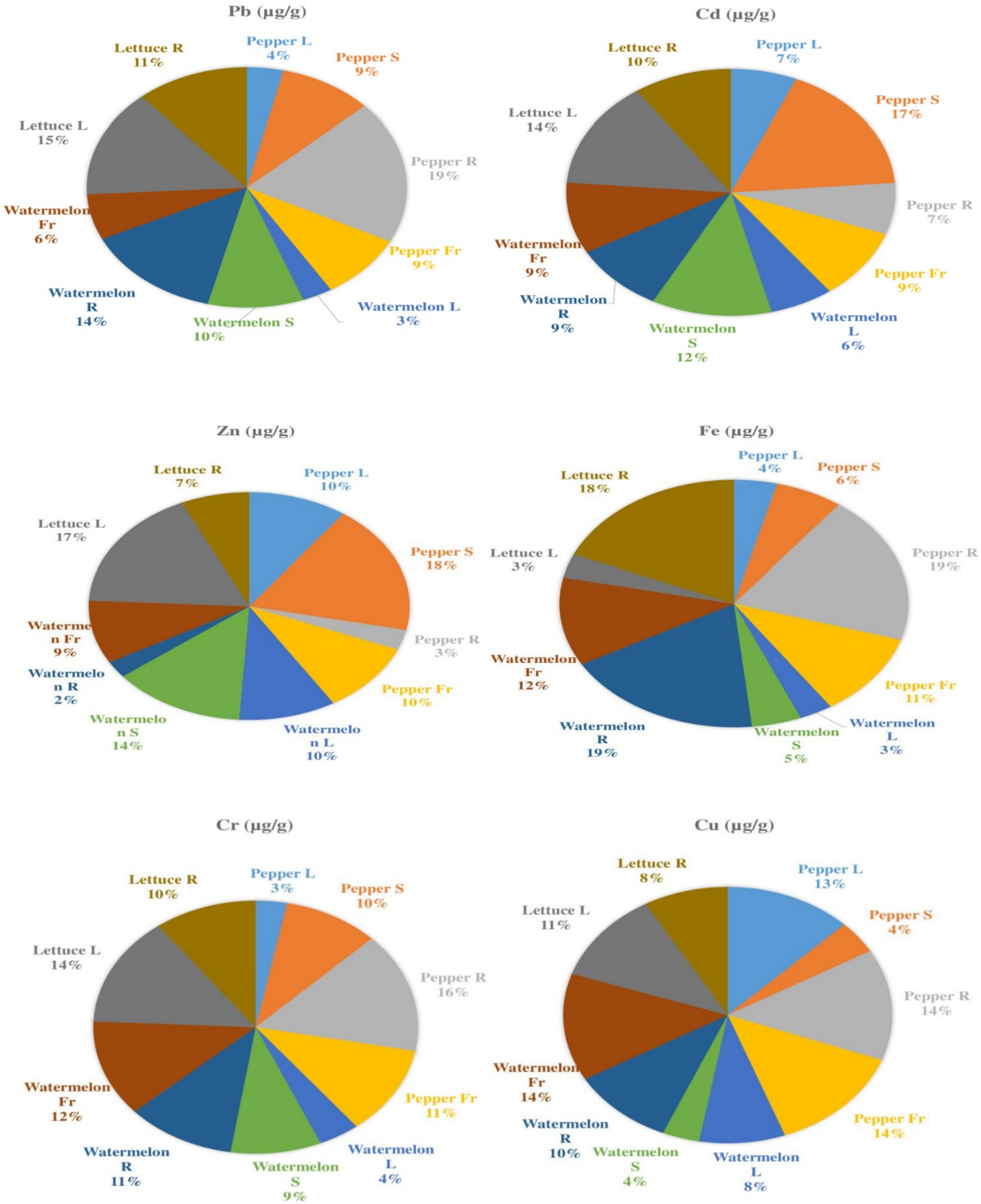


Figure 2. Rate of MTE in organs and plants irrigated by water from the Boumerzoug river (L: Leaf; S: Stem; R: Root; Fr: Fruits)

The organs that accumulated the most Pb were the roots of watermelon and pepper, and the leaves of lettuce. The average content of Pb in plants and organs can be classified in the following order: Lettuce > Pepper > Watermelon; Root > Stem > Fruit > Leaf.

The organs that accumulated the most Cd were the stems of pepper and watermelon, and the leaves of lettuce. The average content of Cd in plants and organs can be classified in the following order: Lettuce > Pepper > Watermelon; Stem > Fruit > Leaf > Root.

The organs that accumulated the most Zn were the stems of pepper and watermelon, and the leaves of lettuce. The average level of Zn in plants and organs can be classified in the following order: Lettuce > Pepper > Watermelon; Stem > Leaf > Fruit > Root.

For all plants, the roots were the most Fe-accumulating organs. The average Fe content in plants and organs can be classified in the following order: Lettuce > Pepper > Watermelon; Root > Fruit > Stem > Leaf.

The organs that accumulated the most Cr were the roots of pepper, the fruit of watermelon, and the leaves of lettuce. The average level of Cr in plants and organs can be classified in the following order: Lettuce > Pepper > Watermelon; Root > Fruit > Stem > Leaf.

The organs that accumulated the most Cu were the roots of pepper, the fruit of watermelon, and the leaves of lettuce. The average Cu content in plants and organs can be classified in the following order: Pepper > Lettuce > Watermelon; Fruit > Root > Leaf > Stem.

According to the total content, MTEs can be classified as follows: Fe > Zn > Cu > Pb > Cr > Cd.

The accumulation according to the plants is presented as: Lettuce > Pepper > Watermelon. The classification according to organ accumulation is: Root > Fruit > Stem > Leaf.

Heavy metals can be absorbed by plants grown in soil irrigated with municipal or industrial effluent in the form of mobile ions, through their roots, and bioaccumulate in different parts of the plant (Guerra et al., 2012). Analysis of heavy metals in plant organs can provide information on soil pollution where plants are grown (Siaka et al., 2014).

In the present study, plants were heavily loaded with Fe, while the Cd concentration was very low. The same result was reported in the study by Hassana et al. (2014). They investigated the accumulation of heavy metals in vegetables grown in the tropical savanna region of Nigeria and watered with untreated urban wastewater, where the accumulation pattern reveals a decreasing order of importance from Fe to Cd (Fe > Cu > Mn > Pb > Cd). In the study conducted in Dhaka (Bangladesh) by Shammi et al. (2016), a significant concentration of Fe was also noted in plant samples. High levels of Fe and Zn have previously been found in vegetables, according to a study by Abbasi et al. (2013) in the Lesser Himalayas (Pakistan), where the accumulation of heavy metals was higher in wastewater-irrigated vegetables than in control vegetables.

In the Arsukhna region of Jordan, vegetables are grown by irrigating with treated wastewater. The concentrations of Zn, Fe, and Pb in these vegetables were higher than those in the reference vegetables by 3423%, 155%, and 397% respectively for pepper. These results demonstrate how treated wastewater affects the buildup of heavy metals in vegetables and imply that some plant crops are more susceptible to heavy metal contamination than others (Al-Ansari et al., 2013).

Because of the high dietary consumption of these crops, peppers from the fields of Faisalabad (Pakistan) acquired more metals like Cd and Pb, which can be harmful to human health (Najam et al., 2015). High exposure to lead from eating vegetables can cause severe kidney and brain damage and can lead to death (Martin and Griswold, 2009), as well as particular alterations in the expression of genes and proteins and adverse effects at the cellular level (Gillis et al., 2012). Bioaccumulation of Pb in the human body causes toxic effects in multiple organs such as the liver, lungs, and spleen, resulting in various biochemical defects (Guerra et al., 2012).

Ingesting very high Cd concentrations severely irritates the stomach, resulting in vomiting and diarrhea. Long-term exposure leads to kidney accumulation and kidney disease, fragile bones, and lung damage (Khan et al., 2008).

The Fe, Zn, Cu, Pb, Cr, and Cd levels in the examined plants matched those found by Belaid et al. (2010) in El Hajeb-Sfax (Tunisia) and Abdu et al. (2011) in three West African cities. However, the levels were less than those reported by Benlkhoubi et al. (2016) in Kenitra (northwestern Morocco), Shammi et al. (2016) in Dhaka (Bangladesh), Alghobar et al. (2015) in Karnataka (India), Cary et al. (2015) in Chania (Crete), Raja et al. (2015) in Faisalabad (Pakistan), and Ahmad et al. (2014) in Punjab (Pakistan). Our results were higher than those found by Yami et al. (2016) in Awara Melka and Nura Erafarms (Ethiopia), Noor-Ul and Tauseef (2015) in Peshawar (Pakistan), and Laaziri et al. (2015) in Meknès-Tafilalet (Morocco) for Pb and Fe.

Numerous factors, such as soil characteristics (pH, organic matter, clay content, metal concentration, and salinity), can significantly impact the accumulation of heavy metals in plant tissues (Shammi et al., 2016). The composition of wastewater (Mojiri, 2011), plant type (Kapungwe, 2013), and environmental conditions (air and industrial pollution) (Twining et al., 2004) also play a role. Other studies have pointed to additional factors, including metal speciation, the intrinsic nature of the bioaccumulative organism, and biotic and physicochemical characteristics, that contribute to the bioaccumulation of hazardous metals by living organisms (Koumolou et al., 2013).

In actuality, a number of variables, including the pH and clay content of the soil, affect the bioavailability of heavy metals. Plants' ability to absorb heavy metals can be decreased by raising the pH and clay content of the soil. Heavy metals can be found as chelates in the presence of organic matter, which improves plants' capacity to absorb heavy metals. Soil irrigation using wastewater raises soil organic matter and decreases soil pH. Consequently, there is an increase in the uptake of heavy metals by plants (Mojiri et al., 2013).

In addition, the increase in the solubility of insoluble heavy metals in the soil brought on by the chelation or acidification action of the applied wastewater may result in an accumulation of MTE directly or indirectly depending on the wastewater's composition (Mojiri, 2011). Plants that are cultivated on soil that has been enhanced with metals also absorb metal ions to varying degrees. This uptake is dependent on the bioavailability of metals, which is in turn influenced by external (soil-associated) and internal (plant-associated) factors (Farahat and Linderholm, 2015). Moreover, plant species have different capacities to accumulate heavy metals (Kapungwe, 2013).

3.2. Effect of plant type and plant organ on MTE content:

The effect of plant type and plant organ on MTE content is shown in Table 2.

Table 2. ANOVA results of the effect of plant type and plant organ on MTE content in plants irrigated by water from BR.

Variable	Source	R^2	Sum of Squares	Mean of Squares	F	Pr>F
Pb	plant	0.59	7.11	0.64	1.6	0.15
Cd	type	0.63	0.08	0.007	1.55	0.17
Zn		0.46	107.91	9.81	3.65	0.003*
Fe		0.87	508.32	46.21	0.48	0.89
Cr		0.43	2.01	0.18	0.8	0.63
Cu		0.73	0.22	0.02	1.71	0.12
Pb	Organe	0.37	7.75	2.58	6.43	0.002**
Cd		0.42	0.12	0.04	8.52	0.0005**
Zn		0.81	403.96	134.65	50.23	<0.0001**
Fe		0.8	16217.58	5405.86	56.74	<0.0001**
Cr		0.11	2.31	0.77	3.38	0.03**
Cu		0.59	0.62	0.2	17.17	< 0.0001**

**Variation spatiale significative à p<0.05.*

*** Variation organes significative à p<0.05.*

This study showed that the "plant type" factor (pepper, watermelon, or lettuce) did not affect the accumulation of MTE, while the "organ type" did influence this accumulation. The same observation was reported in the study by Laaziri et al. (2015). We also noted that the root is the organ that accumulates the most MTE, and lettuce is the plant that accumulates the most. This result corroborates the findings of Mojiri et al. (2011) in Isfahan province (center of Iran), where the roots accumulated more heavy metals than the shoots did.

According to the study by Sbartai et al. (2012) in Annaba (Algeria), compared to the pepper leaves, the roots accumulate more Cd and Zn. With a percentage that ranges between 71% and 80% of the total Cd absorbed by the plant, the distribution of Cd that has collected inside the plant indicates that the majority of this element is trapped in the roots, while the percentage of Zn present in the leaves varies between 19% and 30% of the total Zn absorbed by the plant.

In Karnataka State (India), irrigation with wastewater has shown that plant roots accumulate significantly higher amounts of Cr, Ni, Pb, and Cd than in control irrigated land (Salakinkop and Hunshal, 2014).

In Romanian contaminated areas, leafy crops (lettuce) were cultivated with Pb levels that were 17 times the allowable limit (Siaka et al., 2014). According to Koumolou et al. (2013) and Youssef and Eissa (2015), leafy vegetables accumulated more MTE than non-leafy vegetables. Leafy vegetables accumulated more metals than fruit types because their translocation and transpiration rates are higher (Zhuang et al., 2009). Sinha et al. (2006) in Kanpur (India) reported that the accumulation of MTE was higher in the fruit and reserve organs than in the foliage, which is rich in chloroplasts. Therefore, it is not recommended to cultivate these kinds of plants on soil contaminated by MTE.

However, Al-Lahham et al. (2007) reported that it is possible to keep heavy metal contamination of vegetables below sanitary standards by diluting irrigation wastewater with potable water. The use of a mixture of wastewater and natural water is recommended to improve the yield of many crops without polluting effects (Salakinkop and Hunshal, 2014).

3.3. Enrichment factor:

The transfer factors of the different MTE are mentioned in Table 3.

Table 3. Average concentration of MTE (µg g^{-1}) in the 3 plants irrigated by the waters of the BR, compared to international limits.

	Cd	Cr	Cu	Pb	Zn	Fe
Pepper	0.52	1.01	4.23	1.43	6.86	32.07
Watermelon	0.48	0.94	3.42	1.14	5.76	29.79
Lettuce	0.62	1.25	3.77	1.80	8.05	34.08
Leaves	0.19	0.73	0.41	0.98	8.23	10.72
Stems	0.30	0.96	0.15	1.33	10.59	17.20
Roots	0.18	1.26	0.41	2.07	2.69	59.37
Fruits	0.19	1.22	0.52	1.04	6.32	35.48
Plants mean	0.21	1.07	0.38	1.46	6.89	31.98
MTE concentration in soil	0.18	1.62	0.35	3367	197.90	406.52
TF	1.17	0.66	1.09	0.04	0.03	0.08
PML	0.5	50	20	10	50	1000
MPLs	0.5	1	/	9	100	/
MPC	0.2	2.5	40	0.5	/	/
CC	5	3	20-100	12-300	100-400	1000
TN	0.05	1.5	10	1	50	150

PML: Permissible Maximum Limits (WHO standards) in (Masona, 2011).

MPLs : Maximum Permissible Limits in vegetable and fruits (Qishlaqi, 2008).

MPC : maximum permissible concentrations of heavy metals in vegetables, according to Codex alimentarius (OMS/FAO), Commission 2001 (Maleki et Zarasvand, 2008).

CC : critical contents of heavy metals in plantes (Baba Ahmed *et al*., 2010)

NC : normal content of heavy metals in plants (Baba Ahmed *et al*., 2010)

TF : transfer factor.

The levels of heavy metals in the samples of plants irrigated with BR's water were judged to be below the authorized level (normal levels, Table 3) except for Cd and Pb, which were higher than normal levels but did not reach the levels for intoxication. Remon et al. (2013) indicate that even if the plant does not show signs of phytotoxicity, humans and animals that feed on this plant can be affected, particularly if the MTE are of the kind that accumulates in the organism.

The transfer factors observed for the different heavy metals varied between 0.03 and 1.17. The highest were recorded for Cd (1.17), Cu (1.09), and Cr (0.66). This indicates that the contents of these metals were higher in the plant than in the soil. The ability of plants to accumulate metals is indicated by a TF > 1, providing information on the plant's status as an accumulator, excluder, or indicator (Galal and Shehata, 2013). The plant rejects the element from its tissues if the TF is less than 0.1. The probability that anthropogenic activities lead to metal contamination of vegetables increases when the transfer quotient value rises above 0.50. The larger TF of heavy metals suggests that the vegetable has accumulated that metal to a higher level (Khan et al., 2008).

The TF values observed in our study were lower than those found by Ahmad et al. (2014) in Punjab (Pakistan), which ranged from 4.15 to 5.93. Our findings show that Cd can transfer from soil to plants more easily than other metals. The struggle between Cd and Ca is the reason for this. It is easier for Ca to be replaced by Cd than by other metals since their ionic radius and valence are the same (Kim et al., 2002). Additionally, because Ca is an essential component of plants, it can enter plant tissues through active transport, but the majority of heavy metals, which are non-essential components, can only do so passively (via diffusion and penetration). Otherwise, Ca channels allow Cd to enter plant tissue (Chang et al., 2014). According to Lone et al. (2003), chronic use of food containing Cd causes renal issues by the accumulation of Cd in the kidneys.

Cadmium has been noted for its high mobility in soils (Fotiadis and Lolas, 2011), which can affect its retention in the soil, especially in the root zone of the crop. Therefore, the absorption of Cd in soil may be low. Soil Cd is mainly adsorbed, and its plant availability is closely related to the pH and organic matter content of the soil (Adah et al., 2013).

Conclusion:

The purpose of this study is to investigate whether plants (specifically lettuce, watermelon, and pepper) irrigated with BR's water are contaminated by heavy metals. The results indicate that these plants exhibit elevated levels of cadmium and lead; however, these concentrations do not reach critical thresholds for toxicity. The findings suggest that the "plant type" factor does not significantly influence the accumulation MTE, whereas the "organ type" does have an effect. Notably, roots are the primary organs for MTE accumulation, with lettuce exhibiting the highest levels among the studied plants. Additionally, our study reveals that the transfer factor is greater for Cd and Cu, suggesting that the concentrations of these metals in the plants exceed those in the soil, thereby contributing to the heavy metal contamination of vegetables. This study advocates for ongoing monitoring of irrigation water and crops to prevent the accumulation of heavy metals beyond permissible levels within the food chain. Consequently, in order to safeguard both human and animal health in the research area, it is imperative to implement preventive measures to limit heavy metal deposition resulting from wastewater irrigation.

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