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Risk Assessment of Heavy Metals from Algerian Urban Wastewater in Agricultural Soils and Fava Beans

Mahdi S[1,2*], Djeziri M[2,3], Berrabah D[4], Louiza Ouksel[5], Mohamed Zine Msssessaoud Boureghda[2], Krim Louhab[2]

[(1)] Research Unit Materials Proceeding Environment; Faculty of Technology, M'hamed Bougara University of Boumerdes, Frantz fanon city, 35000, Boumerdes, Algeria.
[(2)] Research laboratory of food Technology, Faculty of Technology, M'hamed Bougara University of Boumerdes, Frantz fanon city 35000, Boumerdes, Algeria.
[(3)] Center for scientific and technical research in physico-chemical analysis (CRAPC) BP 384 Bouismail, Tipaza, Algeria.
[(4)] Scientific and Technical Research Center in Arid Regions
[(5)] Materials Electrochemistry Laboratory (LEM), Department of Process Engineering, Faculty of Technology, Ferhat Abbas University, Sétif-1, 19000, Algeria.
***Correspondance** : s.mahdi@univ-boumerdes.dz

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Abstract:
Description of the subject. This work involves assessing the degree of Cadmium, Chromium and Iron bioaccumulation in agricultural soils in the M'sila area and in fava beans grown and irrigated with purified wastewater.
Objectives. The objective was to assess the risk level incurred by the population consuming this vegetable, and evaluate cancer factors.
Method. In our study, we opted for fava beans, which were planted in a plastic greenhouse, and irrigated at the same times and in the same quantities with purified wastewater from the wastewater treatment station. The study was conducted over a period of 12 weeks. The experiment was carried out in three stages (time) during plant growth so as monitor heavy metals behaviour of (Cr, Cd and Fe) from the soil towards the various plant organs. Heavy metals levels are evaluated through ICP-OES.
Results. The results revealed levels of heavy metals in the soil, values extend from 0 to 400 mg/kg. On the other hand, values vary between 0 and 9.75 mg/kg were obtained from the various organs of the bean. This suggests that sandy soils allow greater transfer of trace elements to plants than heavier (more clayey) soils. As far as the carcinogenic risk quotient (CRQ) is concerned, the results obtained in broad fava beans (Fe: 5.15- 6.22× 10^{-6}) are higher than the admissible limit set by the United States Environmental Protection Agency (USEPA, 2022), which is 10^{-6}, representing a risque for public health.
Conclusions. Special consideration should be given to the accumulation of heavy metals in various food products, as they present dangers for the population and must be reduced to acceptable levels.

Keywords: Heavy metals, agricultural activity, irrigation, plants contamination, cleanliness, public health.

1. Introduction

Rapid population growth requires large drinking water quantities to be available. This liquid commodity, which has a significant impact on human health, hygiene and food production, has prompted us to consider the reuse of urban wastewater as a more appropriate solution. It provides additional quantities of water, ensures the stability of the natural water cycle, environmental protection and the real implementation of a sustainable circular economy policy (Augustsson et al., 2017).
The Food and Agriculture Organization of the United Nations (FAO) points out that agriculture is the largest water consumer, accounting for 69% of global water withdrawals (FAO, 2021). Urban wastewater represents a significant source of water that could be exploited (OECD, 2016).
Reusing wastewater can provide a significant amount of fresh water to irrigate arable land in semi-arid area. This operation makes it possible to recycle this water type, which is a source of health problems and requires effective treatment. This fluid is used in agriculture because of its composition, which is rich in organic and mineral matter. The reuse of wastewater in agriculture can have negative consequences for human health and the environment, the problem being toxicity as a result of elevated concentrations of certain heavy metals (Chekroun et al., 2010). Studies from the past have confirmed that electroplating processes contribute to soil contamination exhibited the features of polymetallic contamination, with Cr contamination being the most notable (Xiao *et al.,* 2019). Electroplating processes can lead to soil contamination through various pathways, including leakage of electroplating solution, discharge of wastewater and piling of electroplating sludge. In addition, contaminated soils generally have high concentrations of Cr (VI), associate with elevated carcinogenic risks (Luo et al., 2022). Cadmium exerts detrimental effects on plants, resulting in complex genetic, biochemical and physiological alterations that result in delayed growth (Bhaben et al., 2019), water and nutrient absorption, as well as lipid peroxidation and protein degradation.

In plants, iron Fe (II) is also one of the essential micronutrients and is involved in many physiological processes. including RNA synthesis, chlorophyll biosynthesis, several enzyme activities, respiration and redox (Feifei et al., 2023).
High heavy metals concentrations in plant cells lead to molecular damage, mainly via the production of reactive oxygen spices including superoxide radicals, hydroxyl radicals and hydrogen peroxide (Steliga et al., 2020).
In the present work, we want to assess the transfer of Cd, Cr and Fe in three different soils irrigated in arid zones (M'sila zone with treated urban wastewater) to the roots and aerated parts of the fava bean (leaf, flower and fruit) of plants. This experiment will be carried out in pots under glass. Not all the metals carried into the soil by treated wastewater are in a form that can be assimilated or bioavailable.

2. MATERIAL AND METHODS

2.1. Study area and site characteristics

This is the first study in Algeria in which three agricultural soils from different regions of M'sila region were selected for experiments in order to simulate what happens in reality. The research was carried in semi-arid agricultural areas in the M'sila region (Algeria), where wastewater is regularly used without treatment, there is a potential risk to agricultural products, which can consequently have adverse effects on human health. The soils in question are irrigated with wastewater to irrigate vegetables. Consequently, consumption of vegetables from these regions may be dangerous and have adverse given the potential effects on the health of the local population, we hypothesized that wastewater irrigation may lead to heavy metal contamination and accumulation in vegetables, ultimately reducing their nutritional quality and posing risks to human health. Therefore, This study seeks to assess the concentration of certain heavy metals (Cd, Cr and Fe) in agricultural soil and the edible parts of broad fava beans, to assess the soil's potential to translocate heavy metals to these vegetables as a transfer factor, to study The potential harmful health impacts associated with dietary exposure due to broad fava bean consumption by determining the daily consumption of metals (EDI), the Non-carcinogenic health risk quotient (NCRQ) and the cancer risk (CRQ), and to identify the estimated origins of metals in soils areas using statistical analysis. This study's results will play a role in future agricultural planning and policy formulation aimed at improving human health by Decreasing the danger of heavy metal contamination in agricultural soils. (**Figure 1**). ~~Three soils type differing in their physical and chemical properties were sampled in three different areas of the M'sila region (**Figure 1**).~~ Soil samples were collected at a depth of 30 Cm and the geographical coordinates are as follows: Site S1 is 35º68' North in latitude and 4º78' E in longitude. site S2 is 35º81'N in latitude and 4º55' E in longitude. and site S3 is 35º3'N in latitude and 4º27' E in longitude.

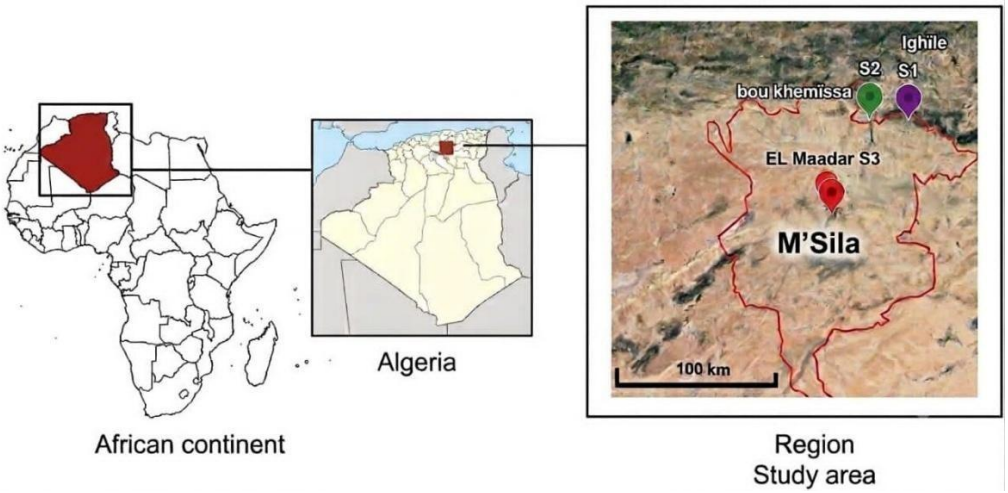


Figure 1. Locations of the three soils S1, S2 and S3 in the M'sila area.

2.2. Biological material

In our study, we opted for fava beans, which were planted in a plastic greenhouse, witch the plants placed in plastic pots and irrigated at the same times and in the same quantities Using purified effluent from the wastewater treatment station in Boussaâda in the M'Sila city. The study was conducted over a period of 12 weeks. The experiment was carried out in three stages (time) during plant growth for the purpose of observing how heavy metals (Cr, Cd, and Fe) behave from the soil towards the various plant organs.

2.3. Soil analysis

After drying in ambient air, the soil samples were ground and passed through a 2 mm sieve. The resulting sieve was subjected to a series of physico-chemical analyses; The pH of the water was measured in a 1:2.5 (w/v) water-to-soil suspension using a pH meter (HM-50V, TOA DKK, Tokyo, Japan). Total nitrogen (TN) content was determined by the Kjeldahl method according to (Nelson & Sommers, 1996). The total $CaCO_3$ concentration was quantified in an acidic medium using a Bernard calcimeter. Electrical conductivity (EC), which represents the quantity of soluble salts in the soil, was measured on the basis of a 1/5 suspension using a brand-name conductivity meter (Aubert, 1978).
The Ca^{2+}, K^+ and Na^+ exchanged by the soil were measured by x-ray fluorescence, Rigaku type ZSX primus II. Organic matter (OM) was estimated using the Anne method described by Aubert, (1978). This method involves the use of a powerful oxidant. Phosphorus was determined using the Olsen method. Complexation of phosphorus by ammonium heptamolybdate and reduction of the complex by $SnCl_2$ (blue color), Absorbance was recorded at

660 nm with a Shimadzu UV-1800 UV-Vis spectrophotometer. Granulometric analysis was carried out using the international Robinson pipette method on air-dried samples of fine soil (less than 2 mm) (Aubert, 1978). The total soil porosity is equal to the volume of voids expressed as a % of the total volume. It is used to assess the permeability and soil aeration (Aubert, 1978).

2.4. Analysis of heavy metal concentrations in samples by ICP-OES

The analysis is carried out using 0.5g of sample and placing it in a digestion vessel, a volume of 1.5mL nitric acid (69%), 0.8mL H_2O_2 and 4mL water are added along with 0.2mL stock standard solution (10 ppm), the mixture is heated, stirred and Placed on a hot plate and heated at 120°C for 2 hours on a hot plate at 120°C for 2 hours. Once digestion is complete, the mixture was transferred into a 10 mL volumetric flask and brought up to volume with distilled water. The sample is centrifuged and the supernatant used for analysis. The blank is prepared under the same conditions (Phadke & Gaitonde, 2016). The spectrometer used is Agilent 5900 ICP-OES. For Cd, Fe, and Cr, the device's detection limits (LOD) were 0.049, 0.160, and 0.564 parts per billion (ppb), respectively. Additionally, the recovery rates for these metals were 98.5 ± 6.66, 97.6 ± 3.3, and 95.4 ± 3.6, respectively (Kimars et al., 2019).

Quality control and assurance

Blank reagents, Duplicate samples and a standard reference material (SRM 1753a, tomato leaves) from the National Institute of Standards and Technology (NIST) were incorporated into the strict quality control protocol to validate the results (Gaithersburg, MD).

$$\boldsymbol{RR}\ (\%) = \frac{\boldsymbol{C_{found}} - \boldsymbol{C_{real}}}{\boldsymbol{C_{added}}}$$

The equation above was applied to determine the percentage recovery of the analyte in the spiked samples: Where C_{found} is the total concentration of the metal after adding a known amount of standard to the real sample, C_{real} is the original concentration of the metal in a real sample, and C_{added} is the concentration of the known amount of standard that was added to the real sample (Mohammad et al., 2022).

To convert the concentration from mg/L to mg/Kg, we use the formula below (Uwah et al., 2012).

$$[\boldsymbol{C}]\ (\frac{\boldsymbol{mg}}{\boldsymbol{kg}}) = \frac{[\boldsymbol{C}]\ (\frac{\boldsymbol{mg}}{\boldsymbol{L}}) * \boldsymbol{V}}{\boldsymbol{P}}$$

Here [C] is the concentration in (mg/L).

V is the total volume of the solution in L.

P is the mass of the sample to be analysed in (Kg).

2.5. Estimation of risks to human health

In this research work, Risk evaluation was performed based on the estimated daily intake (EDI), the non-carcinogenic risk quotient (NCRQ), and the carcinogenic risk quotient (CRQ) will be determined in order to evaluate the potential health hazards arising from the intake of fava beans (Muhib *et al.,* 2016).

Estimated daily intake (EDI)

The Estimated Daily Intake (EDI) quantifies the daily exposure to a given element or substance. It is determined using the following equation

$$\boldsymbol{EDI(mg/kg/day)} = \frac{\boldsymbol{Cm * Ci}}{\boldsymbol{70}}$$

Here Cm (mg/Kg) = metal concentrations in the fava bean (mg/Kg), *Ci* (mg/day) represents the average daily fava bean consumption rate in Algeria, while 70 kg is considered the average body weight of an adult. (Belete et al., 2014).

Non-carcinogenic risk quotient

The EDI is used in the calculation of the hazard quotient (NCRQ). This valor is compared to a reference dose (RfD), which represents an estimate of a daily level of exposure (in milligrams per substance), which is not expected to pose any significant health risk over a lifetime. The following formula is used to calculate the non-carcinogenic risk quotient (NCRQ)

$$\boldsymbol{NCRQ} = \frac{\boldsymbol{EDI}}{\boldsymbol{RfD}} * \boldsymbol{10^{-3}}$$

Where EDI is the estimated daily intake of metal (mg/Kg/day), RfD is the oral reference dose (mg/kg/day), in this case, the RfD is 0.001 for Cd, 0.003 for Cr and 0.7 for Fe (Akele et al., 2017, USEPA, 2022).

If the NCRQ is found to be greater than 1, this indicates that the EDI exceeds the RfD and implies a possible risk of non-carcinogenic health effects (Ezemonye et al., 2019).

The carcinogenic risk factor

The cancer risk (CRQ) is the average probable carcinogenic risk related to a lifetime individual exposure to trace element, in the case of this study, via their ingestion. The lifetime cancer risk factor is expressed as follows

$$\boldsymbol{CRQ} = \frac{(\boldsymbol{FEr * DE * EDI * CSF0}) * \boldsymbol{10^{-3}}}{\boldsymbol{AT}}$$

Where FEr represents the frequency of exposure (350 days per year), DE denotes the duration of exposure (30 years), AT stands for the average lifetime exposure period used for cancer risk assessment (365 days/year × 65 years, based on life expectancy in Algeria), and CSFo refers to the oral cancer slope factor obtained from the Integrated Risk Information System (IRIS) database, which is 8.5×10^{-3} (mg/kg/day) for lead (Pb). (Akele et al., 2017).

2.5. Statistical analysis

The statistical analysis of the study data was performed using XLSTAT 2019 software. A one-way analysis of variance (ANOVA) followed by Fisher's test was conducted, with a significance level set at $p < 0.05$. In addition,

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were employed to reveal group patterns associated with the physico-chemical parameters

3. RESULTS

3.1. Physicochemical characteristics of the soils

The physicochemical properties of the soil samples are presented in (**figure 2**).

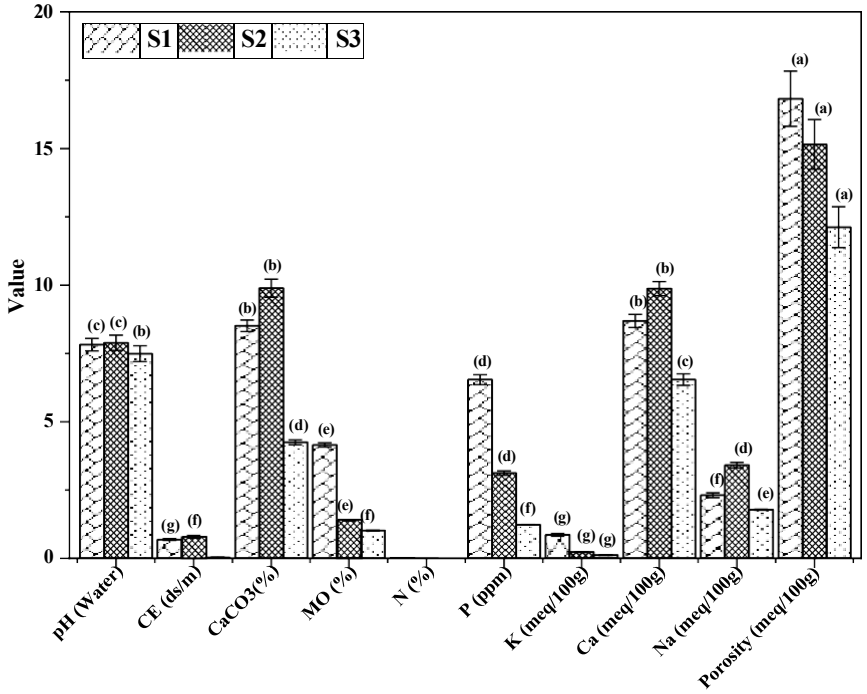


Figure 2. Soils physicochemical parameters

The Soils pH studied varies between 7.49 and 7.89. The pH of soils S1 and S2 are slightly alkaline and that of soil S3 is neutral (a neutral to basic pH will tend to immobilise heavy metals and therefore greatly reduce their uptake by the roots (Jung & Thornton, 1996; Harter, 2007). The electrical conductivity values of the 1:5 extract indicate very low salinity in soil S3 (unsalted) and low salinity in soils S1 and S2. The electrical conductivity of the soil, which is a major indicator of soil salinity, was found to be low at 0.220 - 0.602 mS/Cm. For total $CaCO_3$ content, the low value was attributed to S3, with relatively similar values for S1 and S2 Limestone provides plants with the calcium they need. Among other things, limestone blocks certain elements that are essential for plants, such as iron and various trace elements (particularly manganese and copper), causing deficiencies (manifested, for example, by chlorosis in plants) (Rattan et al., 2005). According to the results obtained, the organic matter content is low for soils S2 and S3 (soil poor in organic matter), In contrast, soil S1 is rich in organic matter, which serves as a reservoir of nutrients essential for plant growth and mineral recycling (Rattan et al., 2005) found the organic matter in their work to be low (2.73 to 4.17%), This may be due to the predominance of sandy texture in the soil. From the figure.2, exchangeable cations vary significantly between soil types and are ranked in order of importance Ca^{2+}, Na^{+} and K^{+}. Nitrogen content is very low in S1 and S2 and zero in S3. The lowest porosity (12.12%) was found in soil S3, while soils S1 and S2 showed higher porosity 15.15 and 16.82% respectively. According to the ANOVA statistical analysis supplemented by the Fisher test, there is a there a marked divergence between the soil parameters.

The soils textural distribution are shown in (**figure 3**).

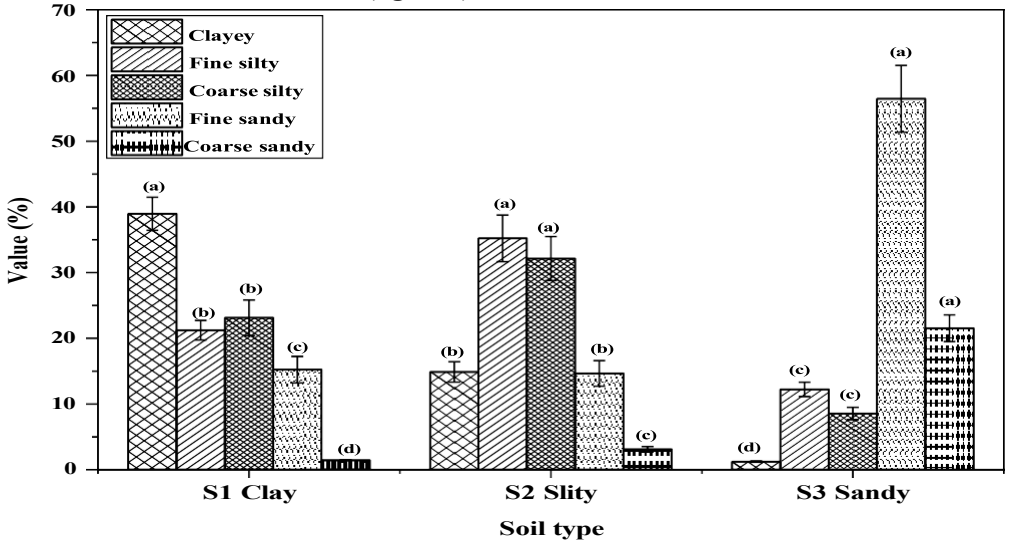


Figure 3. Soils type.

Soil texture, which represents the relative mass of sand, silt, and clay, which defines key soil characteristics affecting plant growth. Sandy soils have large pores, which improve aeration and the flow of water through these pores. Since sandy soils do not capture nutrients and do not become fertile, this type of soil is generally well-watered. Figure 3 present the textural distribution of the three soils selected in the study area (**Figure 1**). The clay-loam texture is dominant in soil S1, while silt dominates soil S2, while sand is found in the fine silt soil S3. Soils S1, S2 and S3 can therefore be classified respectively according to their textural classes (Figure 3) as clayey, silty and sandy. The statistical approach clearly shows that there is a significant distinction between the different soil categories.

3.1. Determination of heavy metals in selected soils

The values for total heavy metals (Cd, Cr and Fe) in the selected soils are given in **Table 1**.

Table 1. Levels of heavy metals detected in the three different soil types.

Heavy metal	Time	S1	S2	S3
Cd (mg/Kg)	T0	$0\pm0^{(c)}$	$0.008\pm0^{(b.c)}$	$0.007\pm0^{(b.c)}$
	T1	$0.039\pm0^{(b.c)}$	$0.049\pm0^{(a.b.c)}$	$0.037\pm0^{(b.c)}$
	T2	$0.081\pm0^{(a.b.c)}$	$0.051\pm0^{(a.c)}$	$0.083\pm0^{(a.b.c)}$
	T3	$0.253\pm0.01^{(a.c)}$	$0.091\pm0.01^{(a.c)}$	$0.219\pm0.01^{(a.c)}$
Cr (mg/Kg)	T0	$4.446\pm0.51^{(b.c)}$	$13.874\pm1.43^{(b)}$	$5.194\pm0.39^{(b)}$
	T1	$10.189\pm1.42^{(b.c)}$	$14.057\pm1.91^{(a.b)}$	$6.247\pm0.53^{(b)}$
	T2	$16.935\pm2.21^{(a.b)}$	$15.208\pm2.27^{(a.b)}$	$6.887\pm0.71^{(a.b)}$
	T3	$22.096\pm5.33^{(a.b)}$	$16.934\pm3.03^{(a.b)}$	$18.366\pm3.02^{(a.b)}$
Fe (mg/Kg)	T0	$370.654\pm7.65^{(a.c)}$	$350.783\pm6.66^{(a.b)}$	$390.081\pm8.88^{(a.b)}$
	T1	$380.852\pm8.22^{(a.b.c)}$	$370.101\pm7.44^{(a.b)}$	$390.336\pm9.23^{(a.b)}$
	T2	$390.281\pm9.01^{(a.b)}$	$380.841\pm8.07^{(a)}$	$400.611\pm9.69^{(a.b)}$
	T3	$390.706\pm9.11^{(a)}$	$390.498\pm9.09^{(a)}$	$400.794\pm9.72^{(a)}$

The total content of heavy metals varied from 0 to 400 mg/kg. It should be noted that the Cd and Cr contents recorded in soil S1 are high compared with those found in soils S2 and S3, except for Iron, which represents a high value in soil S3 during T3 in the fruiting phase. Statistical analysis did not indicate any statistically significant difference between the periods of fava bean development for each metal, but its existence can be noted by comparing the three metals with each other

Heavy metals in fava bean

The results for heavy metal concentrations (Cd, Cr, and Fe) in the different organs of fava bean plants are presented in **Table 2**.

Table 2: Concentration of heavy metals in the various fava bean organs

Heavy metal	Time	Organ	S1	S2	S3
Cd (mg/Kg)	T1	Roots	$0.005\pm0.00^{(c.a)}$	$0.013\pm0.00^{(c.a)}$	$0.011\pm0.00^{(b.a)}$
		Stems	$0\pm0.00^{(c.a.b)}$	$0\pm0.00^{(c.a.b)}$	$0\pm0.00^{(b.a)}$
		Leaves	$0\pm0.00^{(c.a)}$	$0\pm0.00^{(c.a.b)}$	$0\pm0.00^{(b.a.c)}$
	T2	Roots	$0.014\pm0.00^{(c.a)}$	$0.018\pm0.00^{(c.a)}$	$0.022\pm0.00^{(b.a)}$
		Stems	$0\pm0.00^{(c.a.b)}$	$0\pm0.00^{(c.a.b)}$	$0\pm0.00^{(b.a)}$
		Leaves	$0\pm0.00^{(c.a)}$	$0\pm0.00^{(c.a.b)}$	$0.011\pm0.00^{(b.a.c)}$
		Flowers	$0\pm0.00^{(c.a)}$	$0\pm0.00^{(c.a.b)}$	$0\pm0.00^{(b.a.c)}$
	T3	Roots	$0.028\pm0.00^{(c.a)}$	$0.036\pm0.00^{(c.a)}$	$0.105\pm0.00^{(b.a)}$
		Stems	$0\pm0.00^{(c.a.b)}$	$0\pm0.00^{(c.a.b)}$	$0\pm0.00^{(b.a)}$
		Leaves	$0\pm0.00^{(c.a)}$	$0\pm0.00^{(c.a.b)}$	$0.023\pm0.00^{(b.a.c)}$
		Fruits	$0\pm0.00^{(c.a)}$	$0\pm0.00^{(c.a.b)}$	$0.021\pm0.00^{(b.a.c)}$
Cr (mg/Kg)	T1	Roots	$1.04\pm0.09^{(b.a)}$	$2.67\pm0.107^{(b.a)}$	$2.42\pm0.21^{(b.a)}$
		Stems	$0.021\pm0.00^{(b.a)}$	$0.212\pm0.01^{(b.a)}$	$0.274\pm0.01^{(b.a)}$
		Leaves	$0.001\pm0.00^{(b.a.c)}$	$0.007\pm0.00^{(b.a)}$	$0.263\pm0.01^{(b.a.c)}$
	T2	Roots	$1.367\pm0.01^{(b.a)}$	$3.47\pm0.109^{(b.a)}$	$3.171\pm0.108^{(b.a)}$
		Stems	$0.097\pm0.00^{(b.a)}$	$0.244\pm0.01^{(b.a)}$	$0.232\pm0.01^{(b.a)}$
		Leaves	$0.039\pm0.00^{(b.ac)}$	$0.114\pm0.04^{(b.a)}$	$0.142\pm0.05^{(b.a.c)}$
		Flowers	$0\pm0.00^{(b.a.c)}$	$0.085\pm0.00^{(b.a)}$	$0.051\pm0.00^{(b.a.c)}$
	T3	Roots	$1.535\pm0.01^{(b.a)}$	$3.072\pm0.23^{(b.a)}$	$3.81\pm0.11^{(b.a)}$
		Stems	$0.078\pm0.00^{(b.a)}$	$0.205\pm0.02^{(b.a)}$	$0.223\pm0.02^{(b.a)}$

		Leaves	0.038±0.00$^{(b.a.c)}$	0.141±0.01$^{(b.a)}$	0.094±0.00$^{(b.a.c)}$
		Fruits	0±0.00$^{(b.a.c)}$	0.107±0.00$^{(b.a)}$	0.034±0.00$^{(b.a.c)}$
Fe (mg/Kg)	T1	Roots	46.12±1.09$^{(a,b)}$	88.47±1.05$^{(a)}$	88.43±1.004$^{(a)}$
		Stems	39.56±2.01$^{(a.c)}$	34.31±1.66$^{(a.c)}$	39.52±1.89$^{(a.c)}$
		Leaves	36.8±1.01$^{(a.c)}$	55.26±0.9$^{(a.b)}$	56.93±0.9$^{(a.b.)}$
	T2	Roots	59.98±1.03$^{(a,b)}$	88.70±1.05$^{(a)}$	97.54±1.08$^{(a)}$
		Stems	31.20±1.02$^{(a.c)}$	59.68±1.01$^{(a.b)}$	54.85±0.91$^{(a.b)}$
		Leaves	42.51±1.02$^{(a.b)}$	70.09±1.0$^{(a.b.c)}$	72.14±1.02$^{(a.c)}$
		Flowers	39.55±0.109$^{(a.c)}$	53.46±1.09$^{(a.b)}$	29.17±0.89$^{(a.c)}$
	T3	Roots	63.70±2.01$^{(a,b,c)}$	89.63±1.99$^{(a)}$	97.34±2.02$^{(a)}$
		Stems	33.82±0.108$^{(a.c)}$	48.41±0.107$^{(a.b)}$	62.84±1.08$^{(a.b,c)}$
		Leaves	45.37±0.9$^{(a.b)}$	54.47±1.01$^{(a.b)}$	66.39±1.04$^{(a.b.c)}$
		Fruits	38.33±0.9$^{(a.c)}$	43.58±1.01$^{(a.b)}$	46.28±1.02$^{(a.b)}$

From the results obtained, we can see that Cd levels in the roots increase as a function of time, in other words, from 0.005 to 0.028 for S1; from 0.013 to 0.036 for S2; and, in the case of S3, concentrations varied between 0.011 and 0.105 mg/kg. For bioaccumulation in the roots, they can be ranked in descending order, S3 > S2 > S1, as confirmed by their transfer factors (TF) in **Table 4**, which are ranked respectively 52.75; 18.81 and 15.45. For chromium (Cr), significant levels were observed in the roots, varying from 1.035 to 1.535 for S1, from 2.674 to 3.469 for S2 and finally from 2.424 to 3.803 (mg/kg) for S3, these values being higher than those detected in the aerial part. Bioaccumulation is ranked in decreasing order in the three soil types S3 > S2 > S1, as confirmed by their (TF) 42.26; 40.81 and 30.70. Iron (Fe) is also accumulated in the roots, where its content increases progressively during the development of the bean. Its content is slightly higher in the aerial part. Based on the results obtained in **Table 2**, there was a significant difference between the parameters studied, which was justified by the ANOVA statistical analysis followed by Fisher's test.

4. Discussion

4.1. Soils physicochemical parameters

Our results for pH are almost close to those found by Yong and Phadungchewit, (1993). They investigated the effect of pH on the selectivity and retention of heavy metals in specific clay soils and found a value of 7.8 for natural clay soils. Heavy metals can affect soil pH to either higher or lower values. For example, cadmium can give acidic pH values as well as alkaline pH values. (Zhu et al., 2021). These kinds of results can have an influence on nutrient richness, microbial activity and soil health.

Organic matter content depends mainly on soil texture. Clay soils contain high levels of organic matter. The nature and quantity of organic matter in the soil can have an influence in determining metal bioavailability in contaminated farmland soils the bioavailability of metals in contaminated agricultural soils. An excess of heavy metals affects the concentration of organic matter in the soil and the rate of its decomposition. High levels can slow down microbial activity, which is responsible for decomposition. (Li et al., 2018). This can lead to a build-up of organic matter in the soil, which is detrimental to nutrient availability and soil fertility.

An increase in heavy metal contamination can reduce soil porosity, thereby limiting air circulation, water infiltration, and nutrient availability. Once the soil pores become obstructed, water penetration is significantly reduced, ultimately hindering root development. Statistical analysis using ANOVA followed by Fisher's test revealed significant differences in soil characteristics. The presence of heavy metals and variations in particle size distribution can alter the soil texture. In particular, metals such as cadmium (Cd) and chromium (Cr) have been shown to influence the proportions of sand, silt, and clay, thereby modifying the overall textural composition of the soil. (Fatna et al., 2022).

4.2. Heavy Metal Levels in Selected Soils

Heavy metal concentrations were found to vary among the three soil types increased progressively with time during the development of the fava bean, suggesting that this phenomenon was due to treated urban wastewater irrigation (Carlos et al., 2005). Additionally, it was observed that the accumulation of these heavy metals are below the toxicity threshold in soils (Baize, 1997).

The presence of elevated levels of heavy metals in soil can negatively impact its quality and lower its fertility potential and agricultural production, and even cause damage to human and animal health. Heavy metals are classified in order of toxicity, the most dangerous being mercury, lead, cadmium and arsenic. Heavy metal accumulation in soils is largely attributed to intensive agricultural inputs, particularly the excessive use of chemical fertilizers and irrigation using untreated urban wastewater (Tang et al., 2019).

4.3. Heavy metals in fava beans

It is evident that the concentrations of these heavy metals in the tissues increase as the fava bean develops. These levels remain below the toxicity threshold in the various organs of the fava bean (Fageria et al., 2002). It can be seen that the accumulation of these metals accumulates more in the roots compared to the aerial parts in all types of soil (S1, S2 and S3). These characteristics corroborate the results of Rafati et al., (2011) and Adki et al., (2013). Fava beans have a good capacity for sequestering heavy metals in their roots, thus limiting contamination of the fruit in the event of soil pollution. The roots retain most of the trace elements collected and fix them largely to the

cell walls, which explains why the levels are often higher there than in the aerial parts. The soil perimeter serves as a favorable zone for the accumulation of heavy metals, which plants are able to absorb through their roots.
Iron is the only major element that has been identified because of its importance for plant growth. The same findings were reported by EL Hassani et al., (2024), whose sampling was carried out in urban and peri-urban agricultural settings, irrigation water, soil, and various vegetable samples were systematically collected (beets, cardoons, broad beans, lettuce, radishes and courgettes). They found a value of 4.575 mg/kg for iron in broad beans. Iron is not very mobile in S1 soil as a result of the clay content (clay soil 38.96%). In this soil, clay regulates its mobility by adsorbing or desorbing these elements at the surface of the particles. In addition, this low mobility is accentuated by the organic matter (4.15%) present in soil S1, which is richer than S2 and S3, and by the alkaline pH of this soil (7.82). As the availability of H^+ ions in the soil decreases, the binding capacity of soil complexes for metal ions increases and the lower the mobility of these ions. Thus, the bioavailable fraction of this element is greater in S3 and S2, which are less rich in OM (1.02% OM) and (1.39% OM).
Chromium is not very mobile in S1 soil and according to (INERIS, 2011), Cr is adsorbed on to soil components, which prevents its uptake by plants. Its mobility is high under alkaline and calcareous soil conditions, which explains its high bioavailable fraction in S2, or its limestone content (9.89%), and its pH (7.89). The results obtained for chromium are relatively identical to those found by Karam et al., (2016), who studied six vegetables (various crops such as cabbage, onion, garlic, fava bean, wheat, and peppermint) and found a value of 2 mg/kg of chromium in fava bean stems.
Our cadmium values do not exceed the permissible values of the standard; they are significantly lower than those found by Chaoua et al., 2019, who analyzed a range of vegetables, including broad beans irrigated with wastewater, and found high values for different parts of the broad bean, for example, a value of 13 mg/kg in the roots of the plant. Cadmium (Cd) mobility is influenced by soil pH: when the pH increases, its adsorption by the solid phase can be multiplied by a factor of 3, and migration of this element is fairly easy in predominantly sandy and silty environments, less rich in OM and poor in clay, hence its high bioavailable fraction in S3 (pH 7. 49, 1.02% OM its sandy texture) then S2 (pH 7.89, 1.39% OM its silty texture) and less important in S1 (pH 7.82, 4.15% OM its clay texture) (Baldantoni et al., 2015). It can be concluded that the mobility of these trace metals is different in each soil type in decreasing order S3 > S2 > S1.

4.4. Evaluation of Potential Risks to Human Health

The parameters characterising the risk of consumption of fava beans in Algeria: EDI, NCRQ and CRQ for heavy metals are presented in **Table 3**.

Table 3. EDI. NCRQ and CRQ heavy metal values

Heavy metal	Time	Organ	S1	S2	S3
			Estimated daily intake (EDI) (mg/Kg)		
Cd	T3	Fruits	0.00±0.00	0.00±0.00	0.007±0.0001
Cr			0.012±0.00	0.038±0.00	0.00±0.00
Fe			1.55±0.037	1.36±0.032	1.65±0.037
			Non-carcinogenic risk quotient (NCRQ)		
Cd	T3	Fruits	0.00±0.00	0.00±0.00	0.008±5.77E-04
Cr			0.003±5.31E-5	0.010±0.00	0.00±0.00
Fe			0.002±5.21E-05	0.002±4.57E-05	0.002±5.21E-05
			Carcinogenic risk (CRQ)		
Cd	T3	Fruits	0.00±0.00	0.00±0.00	2.88E-08±1.36E-09
Cr			4.51E-08±0.00	1.43E-07±0.00	0.00±0.00
Fe			5.85E-06±1.37E-07	5.15 E-06±1.20E-07	6.22E-06±2.63E-06

Significant levels of heavy metals have been documented in some developing countries, particularly in urban areas. The main cause is irrigation with waste water. Daily consumption of fava beans in Algeria is considerable, estimated at 2.5×10^4 mg/day. Non-carcinogenic risk factors (NCRQ) have been highlighted to evaluate the health hazards resulting from chronic exposure to heavy metals. A NCRQ<1 indicates that exposed individuals are safe, but that the risk exists and that measures must be taken if the NCRQ >1 (Akele et al., 2017).
In this work, the result obtained for the carcinogenic risk factor (NCRQ) for the selected metals is less than 1 (**Table 3**) for the different soil types. They are also lower than those found by (Xiang et al., 2022), who conducted a study on 1,999 agricultural soil samples gathered from 11 cities across Zhejiang Province during the period 2016–2020 and found an NCRQ value for Cd equal to 6.07×10^{-4} and for Cr equal to 3.51×10^{-2} respectively. However, even if the NCRQ value does not exceed 1, the risk is probably there and appropriate safety measures should be implemented to ensure consumer protection.
For the carcinogenic risk factor (CRQ), the results obtained in the samples for iron vary between (5.15 and 6.22×10^{-6}) and exceed the permissible values set by the United States Environmental Protection Agency (USEPA), which are 10^{-6} (USEPA, 2022).

Heavy metals are harmful compounds that are not biodegradable and come from industrial waste, particularly Cd and Cr (Guowei et al., 2021). Certain commonly consumed foods, including fresh vegetables and fruits, may contain elevated levels of heavy metals, posing risks such as kidney dysfunction, carcinogenesis, immune system disruption, and in some cases, potentially fatal health effects due to their bioaccumulative nature to bioaccumulate and biomagnify (Shamsudduha et al., 2020).

Principal component analysis (PCA)

Principal component analysis (PCA) results are shown in **Figure 4**.

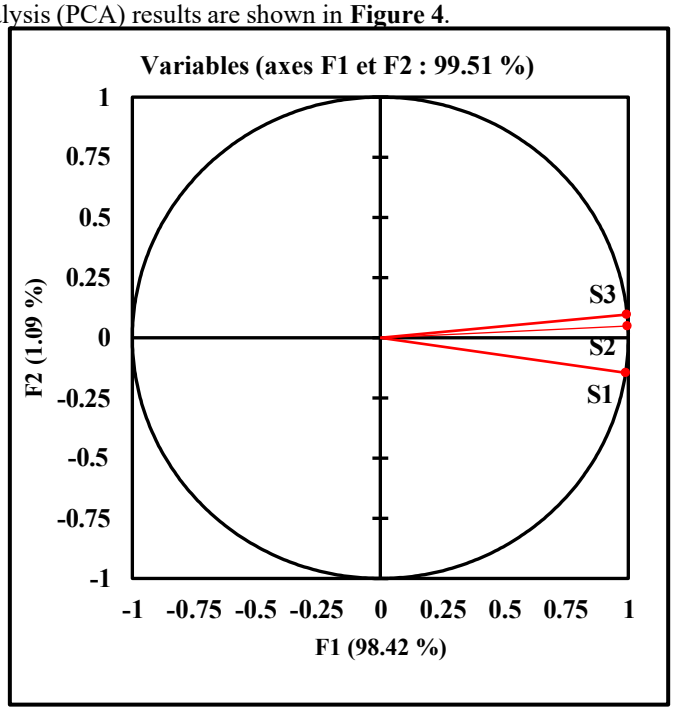


Figure 4. Correlations circle.

PCA was performed on a data matrix comprising 3 soils, 3 metals and 4 fava bean development stages during which the three variables (S1, S2 and S3) were determined. Figure 4 displays the eigenvalues for principal components F1 and F2 and their roles in explaining total inertia. The two axes alone capture 99.51% of the total dataset variability associated with spatial structures, 98.41% for the F1 axis and 1.09% for the F2 axis. The first component (axis F1) partially describes the contamination of different parts of the plant by metals in S3 and S2 soils, with an inertia of 98.41%. The second component (axis F2) describes the enrichment of the fava bean in Cd and Cr. From left to right, The F1 axis displays an increasing gradient for beans and their iron (Fe) content. Meanwhile, the F2 axis reveals a rising gradient of cadmium (Cd) and chromium (Cr) contamination in beans from bottom to top (**Figure 5**).

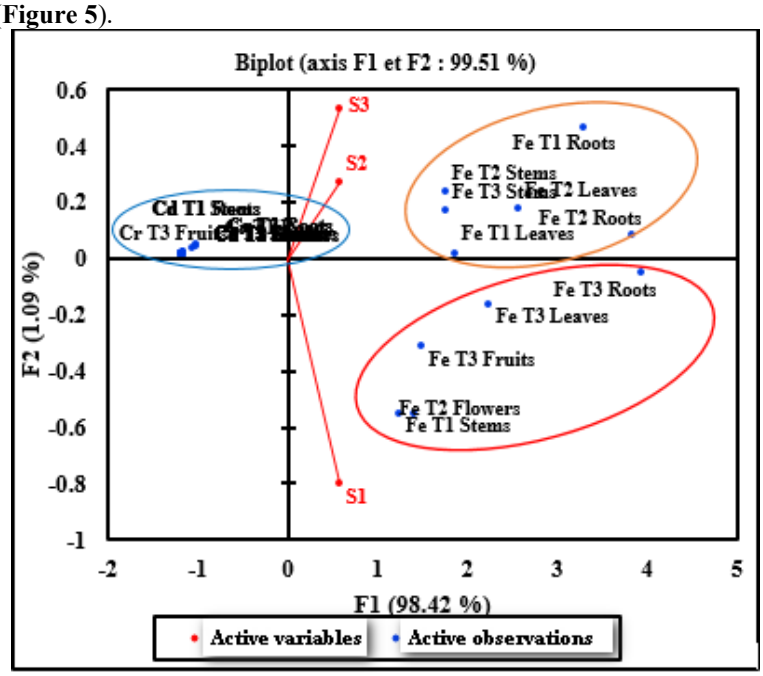


Figure 5. Observations graph.

The factorial map (F1 × F2) illustrates the spatial distribution of individuals into three distinct groups according to contamination intensity (**Figure 5**). The first group includes individuals (Plant-Iron) where there is considerable

Fe-S2-S3 pollution. Area formed by individuals (Plant-Iron) characterised by moderate Fe- S1 pollution. Finally, area formed by individuals (Cd-Cr plant) with high Cd, Cr-S2-S3 pollution.

Ascending hierarchical classification (AHC)

The results of the hierarchical ascending classification (HAC) are presented in **Figure 6**.

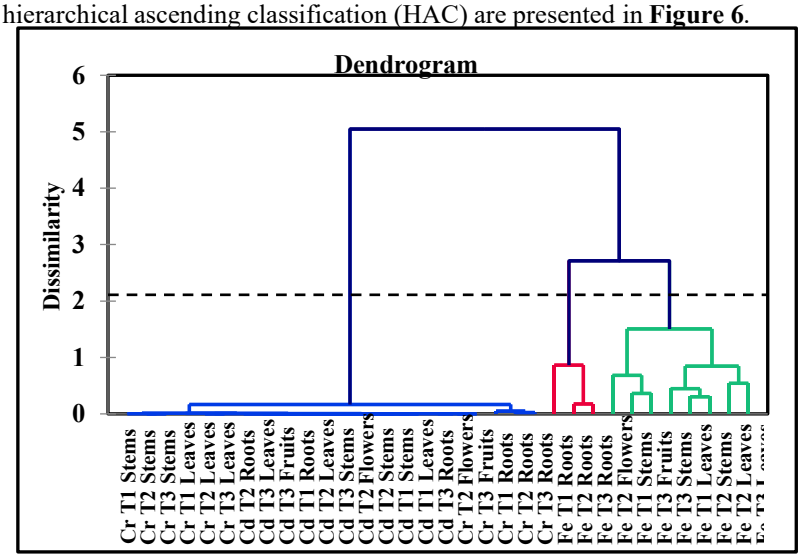


Figure 6. Dendrogram of different plant organ absorption of heavy metal.

The hierarchical ascending classification (HAC) enabled the grouping of heavy metal accumulation in the various organs of the fava bean into distinct classes. Among these, Classes 2 and 3 demonstrated the lowest levels of heavy metals in the plant, indicating minimal contamination (**Table 4)**. These findings are consistent with those reported by Mohamed et al. (2022), who investigated twelve heavy metals in agricultural soils from the town of Kafr El-Zayat, Egypt and found three clusters.

Table 4. Hierarchical bottom-up classification of fava bean plant heavy metal.

	Class 1	**Class 2**	**Class 3**
Objects	22	3	8
Sum of weights	22	3	8
Intra-class variance	2.558	113.280	342.564
Minimum distance to centre of gravity	0.362	4.595	4.726
Average distance to centre of gravity	1.059	8.128	15.711
Maximum distance to centre of gravity	4.421	12.088	26.010
	Cd T1 Roots	Fe T1 Roots	Fe T1 Stems
	Cd T1 Stems	Fe T2 Roots	Fe T1 Leaves
	Cd T1 Leaves	Fe T3 Roots	Fe T2 Stems
	Cd T2 Roots		Fe T2 Leaves
	Cd T2 Stems		Fe T2 Flowers
	Cd T2 Leaves		Fe T3 Stems
	Cd T2 Flowers		Fe T3 Leaves
	Cd T3 Roots		Fe T3 Fruits
	Cd T3 Stems		
	Cd T3 Leaves		
	Cd T3 Fruits		
	Cr T1 Roots		
	Cr T1 Stems		
	Cr T1 Leaves		
	Cr T2 Roots		
	Cr T2 Stems		
	Cr T2 Leaves		
	Cr T2 Flowers		
	Cr T3 Roots		
	Cr T3 Stems		
	Cr T3 Leaves		

5. Conclusions

The present study contributes significant data regarding the concentrations of metal contamination (cadmium, chromium and iron) in agricultural soils and different parts of the bean. Iron and chromium are the metals most commonly found in soils and plants, while cadmium is present in smaller quantities. A notable increase in iron content was observed in plant organs. The measured values indicate that consumption of the fruit (fava bean) contributes significantly to the tolerable daily intake of heavy metals. Although consuming this fruit in the studied region currently does not present a health risk, the bioaccumulation of these heavy metals within this food matrix or through other food chains must be considered. Concentrations exceeding the tolerable daily intake pose potential public health risks and should be promptly reduced to acceptable levels during processing.

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