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Pb and Cr Content in an Agricultural Soil Irrigated with Wastewater and Their Bioaccumulation in Alfalfa

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Abstract

Urban wastewater contains various pollutants, with heavy metals being common, posing risks to the environment and public health, especially when used for irrigation in agricultural systems. This study aimed to assess the total and bioavailable content of Pb and Cr in agricultural soil irrigated with wastewater and their accumulation in alfalfa crops. Five alfalfa sites were sampled, and ten surface soil samples were collected from the top 30 cm of each plot. Plant samples were also collected at each sampling point. A composite sample of irrigation water from the main channels was also collected and prepared. The concentration of heavy metals was determined using atomic absorption spectrophotometry. The results revealed concentrations of Pb and Cr (VI) in the irrigation water above the maximum permissible limits according to environmental regulations. The soil had high organic matter content, neutral pH, elevated electrical conductivity (EC), and Pb and Cr contents of up to 103 and 89 mg/kg, respectively. In alfalfa tissues, Pb and Cr concentrations were recorded at 26 mg/kg and 4.6 mg/kg, respectively (Cr (VI), with lower concentrations observed in the roots. Cr (VI) was higher at all sites, with ranges from 2.25 to 43.56, while lead (Pb) had an AR ranging from 0.64 to 27.04. The Pb presented a mean of 1.45 of the Bioaccumulation factor values, which can be considered a metal-accumulating plant.

Keywords

Heavy Metals, Bioavailability, Lead, Bioaccumulation Factor

1. Introduction

The use of wastewater for agricultural irrigation has been implemented for several years. The availability of low-cost water, nutrient contribution, and improvement in yields have made it an increasingly common practice. According to Hanseok et al. (2016), more than 10% of the world's population consumes agricultural products irrigated with wastewater. It is recommended that wastewater used in agriculture be treated; however, in most cases, untreated wastewater is used (Mendoza-Retana et al., 2021). Wastewater provides benefits, but it can contain heavy metals, fecal coliforms, oils, fats, hydrocarbons, etc., which can affect soil balance, alter soil properties, and potentially impact the health of consumers of the cultivated products (Mateo-Sagasta et al., 2015).

Mexico ranks second globally, after China, in the use of wastewater for agricultural irrigation. According to Jiménez & Asano (2008), 350,000 hectares of agricultural land are irrigated with wastewater, with 130,000 hectares using treated wastewater and 190,000 hectares using untreated wastewater. Near Mexico City alone, 100,794 hectares are irrigated with wastewater in the Mezquital Valley and Zumpango, where basic consumption products such as corn, wheat, oats, alfalfa, and others are grown (Jiménez & Asano, 2008). Furthermore, Alfalfa (*Medicago sativa* L.) is one of the most widely cultivated forage crops for cattle feed in Mexico (Mendoza et al., 2010). Mexico produces over 3 million tons of alfalfa annually (CONAGUA, 2011), with the State of Mexico being one of the leading alfalfa-producing regions in the country. Zumpango is the region with the highest annual production, covering an area of 1148 hectares, and accounting for nearly 80% of the state's production (SIAP, 2021). Therefore, in wastewater without treatments, contaminants can be absorbed by plants, leading to their subsequent integration into food chains (Kumar et al., 2019), which makes important the implementation of this type of project.

The use of wastewater in agriculture should involve treated wastewater, but there are still many regions in the world, including Mexico, that use untreated wastewater (Mendoza-Retana et al., 2021). According to Siebe (1994), the metals introduced through irrigation accumulate in the arable layer, and over time, their levels increase, potentially reaching levels that could pose a risk to the environment. Similarly, Vázquez et al. (2001) reported that the concentration of extractable lead (Pb), nickel (Ni), and cadmium (Cd) in the soil depends on the duration of wastewater use, with Ni and Pb showing a higher annual accumulation rate, while Cd has a rate 4.3 times lower compared to Ni.

Knowing the total or bioavailable concentration of heavy metals in the soil is essential to assess the risk they pose. Interpretation is done through parameters established in the relevant environmental regulations (Norma Oficial Mexicana NOM-147-SEMARNAT/SSA1-2004) or with the use of certain indicators to measure risk (Tijani & Onodera, 2009). This study evaluated the degree of contamination with lead (Pb) and chromium (Cr) in five plots in the agricultural area of Zumpango, State of Mexico, where the total, bioavailable, and accumulated content in alfalfa (*Medicago sativa*) was analyzed.

2. Materials and Methods

2.1. Study Area Description

The study area is in the municipality of Zumpango, State of Mexico, at geographic coordinates 19˚47'49"N 99˚05'57"W. The area has a subhumid temperate climate, with an average annual temperature of 17˚C and an average annual precipitation of 436 mm. Native flora include agave, prickly pear cactus, mesquite, pine, and more. Native fauna that can be observed in uninhabited areas include opossums, and rabbits, among others (**Figure 1**).

2.2. Sampling Design and Sample Collection

Soil samples were taken from five alfalfa plots in the Zumpango area (**Figure 2**). A random surface soil sampling was conducted at a depth of 30 cm, obtaining 10 sub-samples to form a composite sample at each site. Additionally, a 60 cm deep

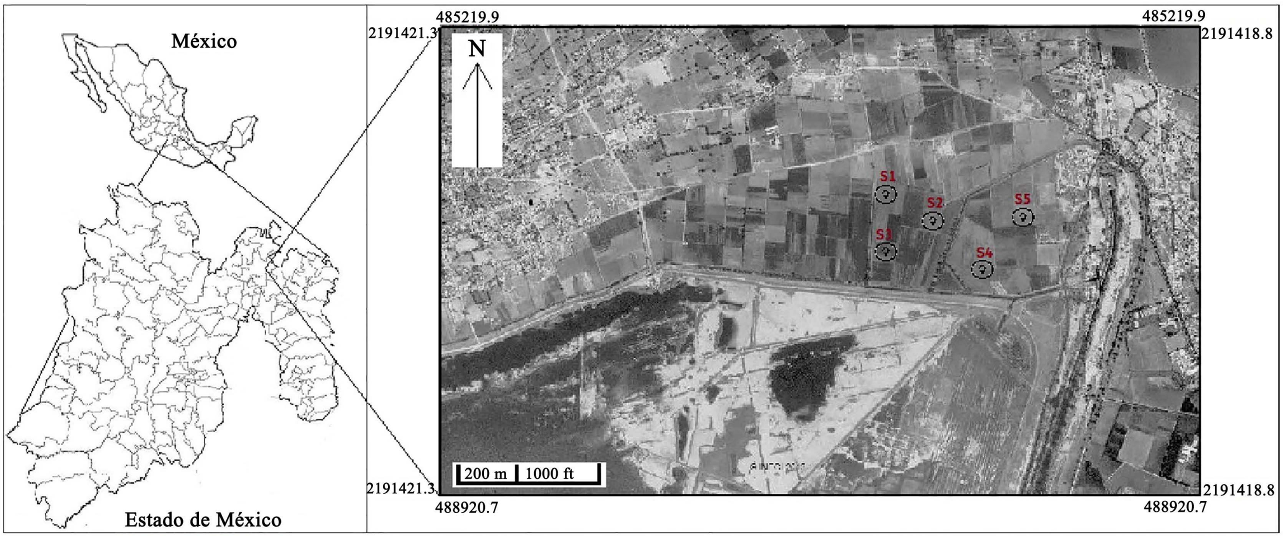

Figure 1. Location of the study area and sampling sites. Source: Own elaboration based on INEGI map (2023).

Figure 2. Sampling of irrigation wastewater (a); and field sampling for plant and soil (b). Source: Own elaboration.

profile was created at each site, collecting samples at 10 cm intervals. Soil samples were placed in polyethylene bags for transportation and subsequent laboratory analysis. For sampling, a GPS was used for georeferencing, a metal shovel for sample collection, polyethylene bags, and a polystyrene cooler for transportation. Plant samples were placed in kraft paper bags for transportation and subsequent analysis. Water was collected from the main irrigation channels, with 1 liter taken from each channel and stored for study in glass bottles and placed in a polystyrene cooler for transportation. Five alfalfa samples were collected from the same sites where soil sampling was conducted. Complete plants were collected from their roots, placed in paper bags for transportation to the laboratory, and then prepared by washing with distilled water and acidified water with HCl (1%). They were dried in an oven at 60˚C for 48 hours and ground in a stainless-steel blade mill for subsequent sieving with a No. 32 mesh.

2.3. Characterization of Physical and Chemical Parameters of Irrigation Water and Soil Samples

The pH was determined by potentiometry (Palmer, 1979) in a soil:water ratio of 1:4. In the same solution, the electrical conductivity (EC) was measured after 20 hours using conductometry (López-Aguilar et al., 2002). Organic matter (OM) and organic carbon (OC) were determined using the modified Walkey & Black method by Nelson & Sommers (1982). Total nitrogen (TN) was determined using the micro Kjeldalh method (Palmer, 1979). Texture analysis was performed using the Bouyoucos hydrometer technique (Gee & Bauder, 1986). The cation exchange capacity (CEC) was determined using the 1N ammonium acetate method at pH 7 (Rowell, 1994). The pseudo-total content of Pb and Cr was determined by Atomic Absorption Spectrometry (AAS) with the Thermo Scientific ICE 3000 Series AA Spectrometer. The analysis was performed in triplicate after acid digestion (HNO_3:$HClO_4$:H_2O_2; 4:1:1). Atomic absorption spectrometry equipment was calibrated using a certified standard of 1000 mg/L of Pb (Perkin Elmer Pure: PartN9304239) with a calibration curve in the range of 0 - 10 mg/L and Cr (Perkin Elmer Pure: PartN9303736) with a calibration curve in the range of 0.2 - 1.6 mg/L. For water samples, 100 mL of each water sample was heated almost to dryness, digested with 8M HNO_3 for 6 hours, and the following parameters were determined: pH, EC, OM, soluble cations, soluble heavy metal contents, and total concentration of Fe, Mn, Pb, Cr, and Cd.

2.4. Determination of Bioavailable Metals

To measure the concentration of bioavailable metals, the exchangeable fraction was extracted from the soil using a 100 mM $CaCl_2$ solution and a 0.05 M DTPA solution adjusted to pH 7.0 (Berrow & Mitchell, 1980). Heavy metals were quantified by AAS.

2.5. Determination of Heavy Metals in Plant Tissue

The collected alfalfa individuals were washed and sectioned into roots, stems, leaves, and fruits. They were then dried, ground in a stainless steel blade mill, and sieved through a No. 32 mesh. For analysis, 0.5 g of the ground material was digested in a block digester with a concentrated $H_2SO_4^-$ HCl mixture and 2 mL of H_2O_2 at 270˚C for 48 hours. Heavy metals were quantified by AAS.

2.6. Data Analysis for Metal Concentration

For the analysis of Pb and Cr concentration data, the following indicators were determined:

2.6.1. Availability ratio (AR)

The AI was calculated based on the relationship between the DTPA-extractable concentration of metals and their total concentration in the soil, calculated as follows:

$$AR = (C_{available}/C_{total}) \times 10^2 \quad (1)$$

where $C_{available}$ represents the metal concentration in the soil extracted with the DTPA solution, and C_{total} is the total metal concentration in the analyzed soil samples (Usman et al., 2012).

2.6.2. Bioaccumulation Factor (BAF)

This factor is defined as the ratio of the accumulated concentration in plant tissue to the bioavailable concentration of the element in the soil. It can be expressed with the following equation:

$$BAF = (C_{plant})/(C_{soil}) \quad (2)$$

where C_{plant} represents the total concentration of the analyzed element in plant tissue, and C_{soil} represents the bioavailable concentration in the soil. A BAF value closer to or greater than one indicates that the plant has a greater capacity to absorb elements from the soil (Yoon et al., 2006).

2.7. Statistical Data Analysis

Statistical data analysis was conducted using SAS V9.2 software. Descriptive statistics were performed, as well as one-way analysis of variance (ANOVA) for a completely randomized experimental design, and mean comparisons were made using the Tukey method.

3. Results and Discussion

3.1. Characteristics and Heavy Metal Content in Irrigation Water

The wastewater used for irrigation in the studied agricultural area had a neutral pH, with an electrical conductivity (EC) of 2.13 dS/m and concentrations of soluble cations for Na, Ca, Mg, and K at 8.78, 4.0, 1.5, and 0.93 meq/L, respectively. Additionally, the average concentration of total heavy metals was 0.48 mg/L of Mn, 0.40 mg/L of Cr, 0.31 mg/L of Fe, 0.23 mg/L of Pb, 0.027 mg/L of Cu, and 0.008 mg/L of Cd. The conditions found in the water are similar to those reported by other authors. Méndez et al. (1997) recorded concentrations of Cd, Cr, and Pb in water for agricultural irrigation of 0.03, 0.04, and 0.21 mg/L, respectively. In both cases, the recorded concentrations exceeded the permissible maximum values established in the Norma Oficial Mexicana NOM-001-SEMARNAT-2021, which sets the maximum permissible limits of contaminants in urban or municipal wastewater for agricultural irrigation. Although metals like Pb, Cu, Cd, and Fe were within the norm's limits, they were higher than those reported by Vázquez, González, Cajuste, Bauer, & Siebe (2001) in irrigation water in the Mezquital Valley in the state of Hidalgo. The chemical composition of wastewater indicates that the electrical conductivity and sodium levels exceed permissible limits according to the Norma Oficial Mexicana NOM-001-SEMARNAT-2021. Comparing the results of Carrillo González et al. (2018) in the same location where this study was conducted, they reported an EC in the range of 0.422 to 1.011 dS/m, indicating lower soluble salt content in the Hidalgo soil irrigated with wastewater.

3.2. Physical and Chemical Characteristics of Agricultural Soil in Zumpango

Table 1 presents the physical and chemical characteristics corresponding to the topsoil layer of the analyzed soil. It can be observed that, in all five sampled sites, the pH remained in the range of 6.7 to 8.0, indicating neutral to slightly alkaline soils (Norma Oficial Mexicana NOM-021-SEMARNAT-2000). The soils showed low EC, except for site 5, which, according to the same norm, indicates salinity problems. Regarding organic matter (OM) content, all sites had high levels, classifying them as soils with abundant OM content. Sites 3 and 5 had the highest OM content, with levels from high to very high %OM in the soil according to the Norma Oficial Mexicana NOM-021-SEMARNAT-2000.

According to Carrillo González et al. (2018), higher organic matter content increases the cation exchange values, enhancing the soil's ability to retain trace elements present in the soil solution. Wastewater represents a continuous source of organic matter. Rascón-Alvarado et al. (2008) reported that organic matter content in soil irrigated with wastewater can vary from 1.8% to 2.4% in the top 20 cm and may decrease to values near 1% in deeper layers, which is similar to the values recorded in the topsoil layer of the analyzed soils in this study. Differences were observed in other variables like pH, possibly due to their proximity to the irrigation canals, with sites farther from the canals showing lower pH values, potentially related to the volume of water received.

3.3. Total Content of Pb and Cr in Agricultural Soil

All five sites presented very similar total concentrations of heavy metals. The concentration in the top 20 cm of each site was recorded as follows: site 1 registered an average concentration of 87 mg/kg of Pb, site 2 had 96 mg/kg, site 3 had 93 mg/kg, site 4 had 122 mg/kg, and site 5 had 113 mg/kg. In addition, a total content of 36 mg/kg of total Cr was found in site 1, 22 mg/kg in site 2, 28 mg/kg in site 3, 49 mg/kg in site 4, and 37 mg/kg in site 5 (**Figure 3**).

Table 1. Physical and chemical characteristics in surface soil from Zumpango.

Site	Parameter							
	pH	EC	OM	Clay	Silt	Sand	N_{total}	CIC
		(dSm^{-1})	----------------(%)----------------					(Cmol/kg)
1	6.8	365	4.26	35	22	43	2.1	12
2	7.5	452	6.50	32	24	44	1.2	11
3	7.6	861	7.33	32	25	43	1.9	13
4	8.0	546	6.85	29	18	53	2.2	8
5	7.4	1856	7.82	32	21	47	2.7	7

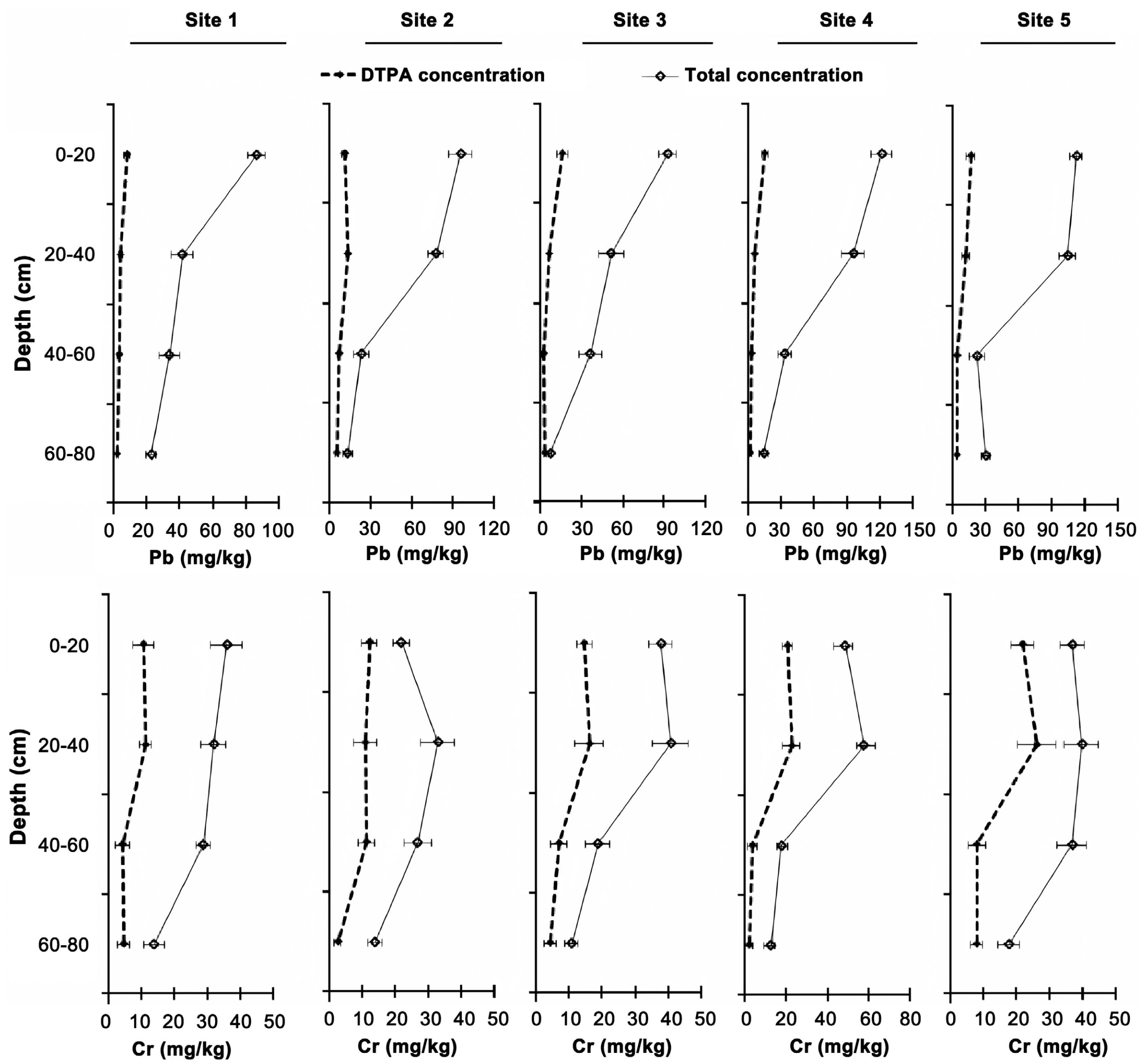

Figure 3. Total and DTPA-Extractable Pb and Cr concentration in soil from the sampling sites in Zumpango, State of Mexico. Source: Own elaboration.

From the soil profiles conducted at each site, it was observed that the metal concentrations varied in subsurface layers. For lead (Pb), the top layer showed higher concentrations and decreased with depth. In the case of chromium (Cr), in three of the sites, the layer with the highest concentration was at a depth of 20 to 40 cm. The DTPA-extractable content of Pb and Cr in the topsoil (0 to 20 cm depth) recorded the following concentrations: Pb concentrations ranged from highest to lowest as follows: Site 5 > Site 3 > Site 4 > Site 2 > Site 1, with 18, 17, and 15 mg/kg of Pb, respectively. As for Cr, the concentrations were as follows: Site 4 > Site 5 > Site 3 > Site 1 > Site 2, with concentrations of 23, 21, 16, 12, and 10 mg/kg of Cr, respectively.

3.4. Availability Ratio (AR)

The total concentrations of Pb were higher than those of Cr in all sites; however, in terms of proportion, Cr had higher extractable values, as shown in **Table 2** through the relationship between the two determined concentrations. Among the statistical parameters, it can be noted that the means for Cr were higher at all sites, with ranges from 2.25 to 43.56, while lead (Pb) had an AR ranging from 0.64 to 27.04.

The AR of heavy metals in soil reflects the relationship between the content of an element available for the plant and its total content in the soil sample. An AR value close to one indicates the rate of availability of the element to plants and other organisms. According to Massas et al. (2013), the AR are metal availability indexes normalized in percentage for the total metal concentrations in soil. In this work, although Cr had lower total concentrations than Pb, it had higher AR values, suggesting a potentially more significant ecological risk. In other studies analyzing Pb and other metals (Zhao et al., 2022), Cr also had lower availability

Table 2. Descriptive statistics of the available ratio for Pb and Cr in agricultural soil.

	Site 1	Site 2	Site 3	Site 4	Site 5
			Lead		
Min.	1.0	1.69	0.64	7.0	1.44
Max.	1.44	17.64	5.2	27.04	4.41
Mean	1.21	6.72	2.3	1.69	2.56
Median	1.21	6.25	1.6	1.44	2.56
Desv. est.	0.01	1.69	2.0	0.36	0.16
C.V.	0.49	6.24	8.5	21.16	5.29
			Chromium		
Min.	2.25	4.0	14.44	3.61	4.84
Max.	12.96	31.36	16.81	18.41	43.56
Mean	8.41	14.44	16.0	9.61	23.04
Median	10.24	14.44	16.0	9.61	28.09
Desv. est.	0.81	12.25	0.01	1.44	3.61
C.V.	10.81	16.0	0.16	16.0	16.0

values, which may be due to its affinity for soil components like organic matter or clays (Pagnanelli et al., 2004). On the other hand, Cr has different characteristics from Pb, with its most stable chemical forms in the soil being Cr (III) and Cr (VI), which can shift between each other depending on redox changes. Cr (VI) is more toxic and mobile under alkaline or slightly acidic conditions, whereas Cr (III) precipitates as $Cr(OH)_3$ and can also be retained by organic ligands (Kozuh et al., 2000).

Through ANOVA, no significant difference was observed in the total content of Pb and Cr in different sites ($F = 1.38$ and $F = 0.751$, with $\alpha = 0.05$, respectively). However, compared to the work carried out by Carrillo González et al. (2018) in the Mezquital Valley, Hidalgo, they reported Pb content ranging from 44.4 to 98.1 mg/kg, while Méndez et al. (1997) reported total lead concentrations in agricultural soil ranging from 56.2 to 94.3 mg/kg in Atoyac, Puebla. These values are very similar to those found in this work and are considered normal according to Kabata-Pendias & Pendias (2000). In agricultural soil, such concentrations could potentially lead to toxicity issues in crops (Kloke et al., 1994).

3.5. Pb and Cr Content in Alfalfa

As can be seen in **Figure 4**, sites 3, 4, and 5 presented the highest concentrations accumulated in the above-ground parts in alfalfa, with 23 mg/kg, 18 mg/kg, and 26 mg/kg of Pb, respectively. For the roots, the highest Pb concentrations were recorded in sites 1, 4, and 5. Although **Figure 4** shows differences in concentrations between roots and above-ground parts, no statistically significant difference was found between both parts of the plant for any element. On the other hand, Cr was accumulated in lower concentrations, with root concentrations ranging from 2.4 to 4.6 mg/kg, with one site showing a statistically different value, but low concentrations in site 4. Meanwhile, in the above-ground parts, concentrations ranged from 1.98 to 4.47 mg/kg, with the highest concentrations.

According to Cao et al. (2003), a plant can be considered a phytoremediator when it can absorb and accumulate high concentrations of heavy metals in roots or above-ground parts. However, in this study, alfalfa did not exhibit characteristics to be considered as such. Nevertheless, it absorbs lead (Pb), which can be ingested by animals consuming the plant. As mentioned by Guevara et al. (2005) in their studies on the absorption of different plants, especially the absorption of Cr by alfalfa, this plant can absorb up to 41% of this metal, a high percentage compared to this study, and observed that unlike other metals when using Cr solutions, the plants did not show any damage to stems or leaves.

3.6. Determination of Bioaccumulation Factor (BAF) for Pb and Cr in Alfalfa

In **Figure 5**, the bioaccumulation factor (BAF) of the analyzed metals in the alfalfa plant can be observed. Each box represents the maximum value in the upper line of the box, the minimum at the bottom, and the median is represented by the transversal line. The values of BAF for Pb were generally higher than

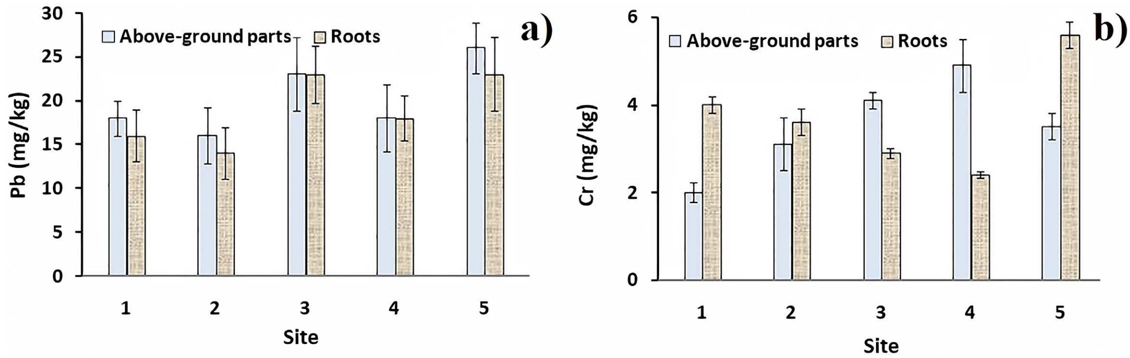

Figure 4. Pb and Cr content in roots and above-ground parts of alfalfa from the five sampled sites. Source: Own elaboration.

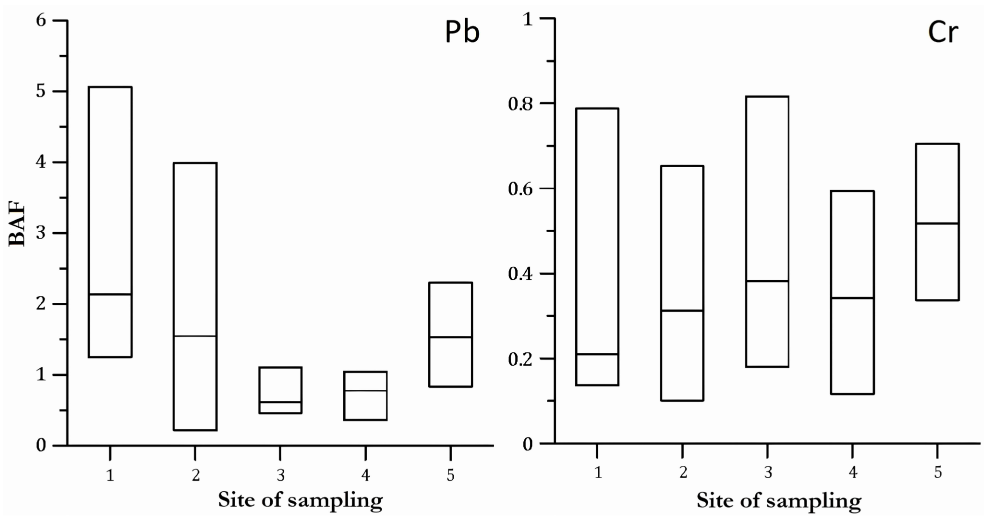

Figure 5. Bioaccumulation factor of Pb and Cr in alfalfa at each sampled site.

those for Cr. The BAF for Pb in alfalfa ranged from 0.21 to 5.08, with a mean of 1.54 and a standard deviation of 1.36. For Cr, the BAF ranged from 0.10 to 0.82, with a mean of 0.42 and a standard deviation of 0.25. Pb had the highest BAF values in sites 1 and 2, while Cr had relatively similar maximum values among most sites.

According to Alarcón & Peláez (2019), the Bioaccumulation Factor (BAF) indicates a plant's ability to accumulate heavy metals from the soil by comparing the concentration of the element in plant tissues to its concentration in the soil. If the value is greater than one, the plant is considered an accumulator of the heavy element, and if it is less than one, the plant is not considered an accumulator. Although it is the same area, there were different trends in results among metals and even among the different sampled sites. The nature of the analyzed metals plays a crucial role in the diversity of trends, as well as other factors such as the volume and frequency of irrigation, soil management, the use of agrochemicals, etc. In three out of the five sites, the BF was greater than one in the following order: Site 1 > Site 2 > Site 5, with average values of 2.1, 1.5, and 1.47, respectively. However, for Cr, the BAF values were below one, indicating that alfalfa cannot be considered an accumulator of this element.

4. Conclusion

Urban-origin wastewater contributes heavy metals to agricultural soils, where they accumulate and migrate through their different layers. The levels of Pb and Cr (VI) in the irrigation water under study were recorded above the limits established in environmental regulations, representing a continuous source of metals in agricultural soil. This is confirmed by the soil analysis, where up to 103 and 89 mg/kg of Pb and Cr (VI) were recorded, respectively. The soils exhibited a loamy texture, high organic matter content, neutral pH, and low to moderately high electrical conductivity (EC). Lead (Pb) showed higher concentrations in the top layer and decreased with depth. In the case of chromium (Cr), the second layer (20 to 40 cm) exhibited higher levels, indicating the greater mobility of Cr (VI). Finally, chromium registered higher values for the availability ratio (AR); however, alfalfa accumulated a higher concentration of Pb, considered a lead-accumulating plant based on BAF values, which was not observed with Cr (VI).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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