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HEAVY METALS PHYTOEXTRACTION POTENTIAL OF *Medicago sativa* L. IRRIGATED WITH WASTE AND GROUNDWATER

Potencial de fitoextracción de metales pesados de *Medicago sativa* L. irrigada con agua residual y subterránea

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Key words: contamination, copper, lead, soil, remediation.

ABSTRACT

Wastewater is generally used for crop irrigation purposes it since provides nutrients and organic matter to the soil. However, it can also add contaminants such as heavy metals. The objective of the present study was to evaluate the absorption and accumulation of lead and copper in the aerial and root parts of alfalfa (*Medicago sativa* L.) irrigated with raw wastewater, treated wastewater, and groundwater using the bioconcentration (BCF) and translocation (TF) factors. Three treatments (raw wastewater, treated wastewater, and groundwater) were tested in a randomized block design with four replicates. The results showed significant differences between treatments. The highest concentrations of lead and copper occurred in the aerial part of the alfalfa irrigated with raw wastewater. The TF factor for lead and copper was higher than one in all treatments; something similar was observed for the BCF, except in the groundwater treatment, indicating that alfalfa has the potential for phytoextraction.

Palabras clave: contaminación, cobre, plomo, suelo, remediación.

RESUMEN

Las aguas residuales son utilizadas, por lo general, para regar cultivos agrícolas, ya que contienen nutrimentos y materia orgánica de importancia para el suelo; sin embargo, también pueden contener contaminantes, como metales pesados. El objetivo del presente estudio fue evaluar la absorción y acumulación de plomo y cobre en la parte aérea y la raíz de la alfalfa (*Medicago sativa* L.) irrigada con agua residual cruda, agua residual tratada y agua subterránea, mediante los factores de bioconcentración (FBC) y translocación (FT). Los resultados de tres tratamientos (agua residual cruda, agua residual tratada y agua subterránea) se analizaron según un diseño de bloques al azar. Cada tratamiento se repitió cuatro veces. Los resultados mostraron diferencias significativas entre los tratamientos; las mayores concentraciones de plomo y cobre se encontraron en la parte aérea de la planta regada con agua residual cruda. El FT para plomo y cobre fue mayor a la unidad en todos los tratamientos; asimismo, para el FBC, a excepción del tratamiento con agua subterránea, lo que indica que el cultivo de la alfalfa tiene potencial para la fitoextracción.

INTRODUCTION

Worldwide, wastewater is used for crop irrigation purposes; the global annual production of wastewater is estimated 1500 billion m^3, and 20 million ha of agricultural land are irrigated with contaminated water. In Mexico, 345 083 ha are reported irrigated with wastewater, which represents 13.22% (Cisneros-Estrada y Saucedo-Rojas 2016) of the total irrigated area of 2.61 million ha (CONAGUA 2019). There are 2526 municipal wastewater treatment plants in operation, treating 135.6 m^3/s, 63% of the 215.2 m^3/s, collected through sewerage systems (CONAGUA 2018).

In the Comarca Lagunera, from Torreón, Coahuila, México, 31 treatment plants were identified, treating 2.42 m^3/s flow, used to irrigate agricultural crops and green areas (CONAGUA 2014). Fodder is the main crop irrigated with this type of water, particularly alfalfa. In the Comarca Lagunera 38 860 ha of alfalfa were in production in 2019 (SIAP 2021). At the national level, approximately 392 183 ha of this crop were under production in 2020, representing 0.8% more than in 2019.

The use of residual water for the irrigation of crops has certain advantages since it provides nutrients and organic matter to the soil (García-Orenes et al. 2015), favoring an increase in crop yields (Jung et al. 2014); however, it can cause problems in the medium and long terms, on some physical and chemical properties of the soil (Zenginbal et al. 2017, García-Carrillo et al. 2020). Residual water may contain heavy metals, which accumulate in the soil to be absorbed by plants (Antoniadis et al. 2019) and incorporated into the food chain with the potential danger of causing health problems for living organisms (Oppong et al. 2018, Yu et al. 2022).

The agricultural goods produced with the help of residual water are a risk for humans, in addition to the damage caused to the environment by the possible presence of heavy metals in the wastewater used to irrigate soils (Lugo-Morin 2009). Wastewater treatments through oxidation ponds and activated sludge do not eliminate heavy metals. Galindo-Pardo et al. (2021) have reported Pb and Cd concentrations of 11.75 and 2.29 mg/L, respectively, values that exceed the maximum limits allowed in NOM-001-SEMARNAT-1996. Concentration of metals like the above reported can restrict the application of residual water to soils due to the high potential for accumulation in the soil and possible translocation to the crops.

Phytoremediation uses plants to reduce the concentrations or toxic effects of pollutants in the environment. It is a relatively recent technology perceived as profitable, efficient, and respectful of the environment; phytoremediation is an active research area (Ashraf et al. 2019). In this same sense, phytoextraction, also known as phytoaccumulation, phytoabsorption or phytosequestration, is understood as the absorption of contaminants from the soil or water by plant roots. The accumulation of these contaminants into the aerial biomass, is a crucial biochemical process desirable in effective phytoextraction because harvesting biomass from roots is generally not feasible (Ali et al. 2013).

Plants that offer multiple harvests in a single growing period may have great potential for heavy metal phytoremediation (Ali et al. 2013). Also, some native species can absorb and accumulate these metals in their tissues (Opoku et al. 2020, Kafil et al. 2019) as well, and some ornamentals and agroforestry trees are reported as phytoremediators (Kankan et al. 2020, Hussain et al. 2022). Certain cultivated species also present this characteristic, such as some vegetables (Fonge et al. 2021, Yu et al. 2022), perennial crops such as alfalfa (Myriam et al. 2018), fruit trees (Zenginbal et al. 2017) or annual crops like wheat (Khan et al. 2018).

From the above, the study's objective was to evaluate the potential of phytoextraction of lead and copper from soil irrigated with raw wastewater, treated wastewater, and groundwater through the bioconcentration and translocation factor of the alfalfa (*M. sativa* L.) crop.

MATERIALS AND METHODS

Study area

This research was conducted in three locations in the Comarca Lagunera, Coahuila, Mexico, planted with alfalfa and irrigated with different kinds of water: raw residual water, treated water, and groundwater. The geographical location is presented in **Table I**.

TABLE I. GEOGRAPHICAL LOCATION OF THE SAMPLING.

Treatment	Latitude	Longitude	Altitude (m asl)
Raw wastewater	25º 31’ 16.7”	103º 20’ 27.5”	1119
Treated wastewater	25º 30’ 41.3”	103º 19’ 20.2”	1122
Groundwater	25º 33’ 23.3”	103º 21’ 57”	1123

Soil and water sample collection and preparation

Samples of groundwater, treated, and raw wastewater were collected in plastic containers at the entrance to the plots cultivated with alfalfa. The pH and electrical conductivity were immediately determined with a potentiometer (Orion model 420-A, USA) and a conductivity meter (Orion model 162-A, USA). All samples were transported to the lab and stored at 4 ºC until analysis. The following cations were determined: calcium (Ca), magnesium (Mg), sodium (Na), and anions: carbonates, bicarbonates, sulfates, and chloride. In addition, sodium absorption ratio (RAS), lead (Pb) and copper (Cu) concentration, using the volumetric method (USDA 1954, SEMARNAT 2007) were evaluated.

From a representative pasture, four soil subsamples were collected from the 0-30 cm depth increment at each plot irrigated with raw wastewater, treated wastewater, and groundwater to conform a composite soil sample. The samples were placed in plastic bags, identified, and transported to the soil laboratory of the Antonio Narro Autonomous Agrarian University, air dried, and passed through a 2 mm mesh sieve inox steel. Heavy metal analysis was performed after solubilization carrying out the following procedure: 5 grams of soil in a container treated to 50 mL of nitric acid 4 M, put in a water bath for 12 hours at 70 degrees Celsius, cooled, shaken for one hour, filter and finally quantify by atomic absorption.

Plants sampling

For each water quality, a hectare of alfalfa was chosen as the reference for random sample collection, serving as a standard for comparison. Sampling site selection was done using a zigzag scheme to ensure comprehensive coverage. Alfalfa plants from a one-square-meter area were harvested, packed in polyethylene bags, and transported to the laboratory. After being washed with distilled water, the plants were separated into root and aerial parts, left to dry at room temperature, and then in an oven at 80 ºC for 40 hours before grinding and storing in plastic bags for analysis.

Sample digestion

0.5 g of the ground plant tissue sample was placed in a digestion flask with 2 mL of hydrogen peroxide and 6 mL of nitric acid (HNO_3); the flask was sealed and placed in a microwave digestion system (Ethos One Microwave Digestion System) for 50 minutes at 250 ºC. After digestion, the digest was taken to 100 mL with distilled water.

Analysis of water, soil, and plant

The water samples were filtered, and the concentrations of anions, cations, RAS, and heavy metals were determined using the volumetric methods (USDA 1954) and a Perkin Elmer model 2380 atomic absorption equipment (SE 2001), respectively. Lead and copper concentrations were determined considering four repetitions using atomic absorption (SE 2001) after solubilization in nitric acid. The content of heavy metals in the root and aerial part of the plant was determined using atomic absorption spectrophotometry equipment (Perkin Elmer model AAnalyst200). To perform the calibration curve, a standard of 1000 ppm Perkin Elmer of each heavy metal (Pb and Cu) was used to calibrate the equipment.

Analysis of data

For the statistical analysis of the results, a randomized block design was used with three treatments and four repetitions through a means test by the Tukey method, using the Statistical Analysis System V 9.1.3 program (SAS 2008).

Quantification of efficiency in phytoextraction

Phytoextraction efficiency was quantified by the procedure of Ali et al. (2013) used to estimate the translocation and bioaccumulation factors. The procedure used was Translocation factor (TF) = Concentration in the aerial part / Concentration in the root, and Bioconcentration factor (BCF) = Concentration in the harvested tissue / Concentration in the soil.

RESULTS AND DISCUSSION

Table II shows the average results of the chemical analysis of the water. The USDA (1954) classifies the EC of the raw and treated wastewater as high and the RAS as medium. Based on the CE and RAS, these wastewaters contain a high concentration of soluble salts (C3) and a medium Sodium Adsorption Ratio (S2). These results coincide with a previous report by Galindo-Pardo et al. (2021), who analyzed treated wastewater in the Laguna Region, classifying it in the same class regarding salinity and sodicity. The groundwater was classified as quality C2S1. The EC values were higher than those reported by Zenginbal et al. (2017) and Papaioannou et al. (2018).

TABLE II. AVERAGE RESULTS OF THE CHEMICAL ANALYSIS OF THE WATER.

Parameter	Raw wastewater	Treated wastewater	Groundwater
Ca_2^+ (meq/L)	2.32	2.96	2.00
Mg^{2+} (meq/L)	3.48	2.80	0.56
Na^+ (meq/L)	17.62	18.32	6.69
CO_3^- (meq/L)	ND	0.96	0.20
HCO_3^- (meq/L)	8.86	8.00	2.08
SO_4^- (meq/L)	100	100	34.5
Cl^- (meq/L)	2.14	3.96	1.70
RAS	10.35	10.79	6.69
pH	7.48	8.07	7.29
EC (µs/cm)	1762	1833	670
Cu (mg/L)	0.50	0.06	0.06
Pb (mg/L)	0.20	0.29	0.42

ND: not detected; EC = electric conductivity; Cu = copper; Pb = lead

The heavy metal concentration at the three kinds of water assayed was within the maximum permissible limits of NOM-001-ECOL-1996 (SEMARNAT 1997). These values are lower than those reported by Galindo-Pardo et al. (2021), who found lead concentrations of 11.75 mg/L, which exceed the maximum permissible limits established in the previous standard; a clear explanation still needs to be provided. **Table III** shows the concentration of heavy metals in the soil at a depth of 0-30 cm, which is within the maximum permissible limits established in NOM-147-SEMARNAT/SSA-2004 (SEMARNAT 2007).

The presence of lead was detected in both the roots and aerial parts of the plant (**Table IV**). Significant differences ($p < 0.05$) in lead concentration were not observed in the aerial and root portions of alfalfa irrigated with raw and treated wastewater, however, both of them differed from those irrigated with groundwater. The lead concentration in the aerial part of the alfalfa irrigated with groundwater was 0.49 mg/kg, as compared to 5.41 and 4.65 mg/kg found in the aerial part of alfalfa irrigated with raw and treated wastewater, respectively. The lead concentration was significantly higher in the aerial part of alfalfa irrigated to raw and treated wastewater than in those irrigated to groundwater. These results are coincident with those reported by Papaioannou et al. (2018), who reported higher lead concentrations in the aerial part (3.59 mg/kg) in beets irrigated with poor-quality water and the lowest (0.54 mg/kg) when irrigated with good quality water.

TABLE IV. AVERAGE CONCENTRATION OF LEAD IN THE ROOT AND AERIAL PART OF ALFALFA DUE TO THE EFFECTS OF THE TREATMENTS.

Treatment	Lead concentration (mg/kg)		
	Root	Aerial	Total
Raw wastewater	1.94 ± 0.72 a	3.47 ± 0.89 a	5.41 ± 1.23 a
Treated wastewater	1.25 ± 0.46 a	3:40 ± 0.87 a	4.65 ± 0.95 b
Groundwater	0.13 ± 0.05 b	0.37 ± 0.09 b	0.49 ± 0.11 b
CV	37.11	25.63	22.84
DHS	0.81	1.22	1.58

CV = Coefficient of variation. DHS = Honestly-significant-difference. Different letters in the same column indicate statistically significant differences, according to Tukey's test ($p < 0.05$).

TABLE III. THE AVERAGE CONCENTRATION OF COPPER AND LEAD IN THE SOIL (mg/kg).

Soil irrigated with raw wastewater		Soil irrigated with treated Wastewater		Soil irrigated with groundwater	
Copper	Lead	Copper	Lead	Copper	Lead
1.48	2.90	1.39	1.70	1.45	2.17

However, lead concentration in the roots of this crop was higher than in the aerial part (16.89 and 6.30 mg/kg, respectively).

Similarly, Galal et al. (2018) found significant differences between the accumulation of heavy metals in cabbage plants tissue irrigated with wastewater. The lead concentration in cabbage was in leaves and root 606 and 423 mg/kg, respectively, when irrigated with wastewater but leaves and roots concentrations of this metal were lower in the control treatment. Elouear et al. (2016) also found a higher concentration of lead in the aerial part of alfalfa compared to the root, these results show that lead was easily transferred from the root to the aerial part of the crop. In the case of potato irrigated with wastewater, a crop with subterranean tubers, Nzediegwu et al. (2018) found lead concentrations of 35.4 and 3.3 in root and leaf, respectively, attributing these results to the longer contact time of the product with wastewater.

Table V shows the concentrations of copper in the aerial part of the plant and root due to the effect of the treatments studied. Significant differences ($p < 0.05$) were observed for the harvested part of the plant in all treatments and the root in raw and treated wastewater. A 29% reduction in copper concentration was observed in the aerial part of alfalfa irrigated to raw wastewater compared to treated wastewater, and a nearly 14% reduction was observed when compared to the groundwater treatment. Likewise, the copper concentration in the alfalfa root was 22% less in the raw wastewater treatment than in treated wastewater, and the latter 14% less than irrigated with groundwater. The highest copper concentrations were found in the aerial part of the alfalfa plant. The lowest in the root in all treatments. Reductions in the copper concentration of the aerial part compared to the root are observed as a result of 61, 46, and 48% for treatments of raw wastewater, treated wastewater, and groundwater, respectively.

TABLE V. CONCENTRATION OF COPPER IN THE ROOT AND AERIAL PART OF ALFALFA DUE TO THE EFFECT OF THE TREATMENTS.

Treatment	Copper concentration (mg/kg)		
	Root	Aerial	Total
Raw wastewater	3.73 ± 0.96 a	6.12 ± 0.62 a	9.85 ± 0.94 a
Treated wastewater	0.83 ± 0.21 b	1.79 ± 0.18 b	2.63 ± 0.25 b
Groundwater	0.12 ± 0.03 b	0.25 ± 0.02 c	0.37 ± 0.03 c
CV	25.49	10.22	9.58
DHS	0.78	0.55	0.81

CV = Coefficient of variation. DHS = Honestly-significant-difference. Different letters in the same column indicate statistically significant differences, according to Tukey's test ($p < 0.05$).

These results agree with Khan et al. (2018), who state that crops irrigated with raw wastewater accumulate more heavy metals. In this regard, they report 1.21 mg/kg of copper concentrations in wheat grains irrigated with raw wastewater and 0.68 for groundwater. Galal et al. (2018) reported copper concentrations of 3.83 and 0.77 mg/kg in leaves and roots, respectively, in cabbage crops. However, Nzediegwu et al. (2018) found concentrations considerably higher, 26.0 and 13.9 mg/kg of lead in the roots and leaves of the potato crop irrigated with wastewater, explaining this behavior to the longer contact time of the product with wastewater.

The efficiency of alfalfa for the extraction and accumulation of lead and copper is presented in **table VI**. These results indicate a translocation factor of>1 for lead and copper in all evaluated treatments. According to Ali et al. (2013) and Egendorf et al. (2020), plant species with a translocation factor higher than one can potentially be used for phytoextraction. The translocation factor was the highest for lead (3.08) and copper (2.15) in alfalfa irrigated by groundwater and treated wastewater, followed by treated wastewater and groundwater (2.72, 2.08, for lead and copper, respectively). Finally, the lowest values were for the raw wastewater treatment (1.78, 1.64).

This last finding might seem contradictory and might induce us to think that according to this result, the use of raw wastewater is better for the safety of the agricultural crops. However, the translocation factor indicates the potential of the plant species for phytoextraction, not the concentration of the heavy metals in the aerial part, since this factor is the quotient resulting from dividing the concentration of the metal in the aerial part of the plant by the concentration in the root; that is, at higher values in the aerial part and lower in the root the translocation factor will be higher. For this reason, the translocation factor is higher in the treatment of groundwater than in raw wastewater for lead and copper in the cultivated alfalfa in this site (**Table VI**).

Our results are opposite to what was reported by Galal et al. (2018) who mention a translocation factor for the cultivation of cabbage (*Brassica oleracea* L. var. Capitata) for lead and copper of 1.43 and 5.00 for a contaminated area; and 0.24 and 1.40 for the uncontaminated. However, our results agree with Elouear et al. (2016) who reported a translocation factor

TABLE VI. TRANSLOCATION FACTOR FOR LEAD AND COPPER IN ALFALFA CULTIVATION.

Treatment	(TF) Lead	(TF) Copper
Raw wastewater	1.78	1.64
Treated wastewater	2.72	2.15
Groundwater	3.08	2.08

greater than one for lead in the alfalfa crop, irrigated with wastewater, suggesting that this metal is easily transported from the root to the aerial part of the plant.

The bioconcentration factor (BCF) shows the efficiency of a plant species in accumulating a metal in its tissues from the surrounding environment. **Table VII** shows that the highest bioconcentration factor was for copper in treatment raw wastewater, possibly because the raw wastewater has the highest concentration of copper (0.5 mg/L) and the soil presented the highest concentration of copper (1.48 mg/kg). Only plant species with BCF and TF greater than one have the potential to be used for phytoextraction (Ali et al. 2013). **Table VII** shows that the bioconcentration factor in the alfalfa irrigated with raw and treated wastewater has a value greater than one. This result coincides with the results reported by Elouear et al. (2016) showing a BCF greater than one in the cultivation of *M. sativa* L. irrigated with raw wastewater.

TABLE VII. BIOCONCENTRATION FACTOR (BCF) FOR LEAD AND COPPER IN ALFALFA CULTIVATION.

Treatment	Lead	Copper
Raw wastewater	1.19	4.14
Treated wastewater	2.00	1.28
Groundwater	0.17	0.17

Alemú et al. (2022) conducted a phytoremediation study of lead-contaminated soils in Ethiopia using the species *Phytolacca dodecandra* L., reporting an average bioconcentration factor for plant regrowth in the dry season that varied from 0.87 to 1.74 for lead and in the wet season between 1.1 and 1.53. The translocation factor for this plant varied from 0.84 to 3.49 in the dry season, while for the humid season, it varied between 1.34 and 2.01, mean values of BCF and TF were >1, concluding that *P. dodecandra* L. has considerable potential for lead phytoextraction in contaminated sites. Likewise, Khan et al. (2022) evaluated the bioaccumulation of copper in five wheat varieties irrigated with unconventional water sources, the accumulation and translocation factor was >1 in all samples, concluding that wheat varieties have desirable traits that are vital for processes of phytoremediation.

CONCLUSIONS

The present study showed significant differences ($p < 0.05$) in the concentration of lead and copper in the aerial part and root of the alfalfa plant irrigated with raw and treated wastewater and groundwater. The highest concentrations of heavy metals were found in the aerial part of the alfalfa plant treated to raw wastewater for irrigation. Lower concentrations of heavy metals occurred in the root of the alfalfa as compared to the aerial part in all treatments. The extraction efficiency for both metals expressed regarding the translocation and bioconcentration factors were higher than unity in all the evaluated treatments. Therefore, it is concluded that the alfalfa crop has the potential to be used for phytoextraction of lead and copper in soils irrigated with raw wastewater and treated wastewater.

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