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# BIOACCUMULATION OF HEAVY METALS IN CUCUMBER PLANTS INOCULATED WITH *Trichoderma* STRAINS (HYPOCREACEAE) IN THE MEZQUITAL VALLEY, HIDALGO, MEXICO

Bioacumulación de metales pesados en plantas de pepino inoculadas con cepas de *Trichoderma* (Hypocreaceae) en el Valle del Mezquital, Hidalgo, México

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## ABSTRACT

In the Mezquital Valley, Hidalgo, Mexico, wastewater is often used in agricultural systems which have caused negative environmental effects, such as edaphic heavy metal pollution. Consequently, plants in the area begin to absorb heavy metals. Therefore, the objective of this experiment was to evaluate the effects of *Trichoderma harzianum* and *Trichoderma viride* strains on bioaccumulation of Pb, Cd and Cr in inoculated cucumber plants (*Cucumis sativus*). The soil used in this study contains Pb, Cd and Cr (34.63, 0.24, and 32.32 mg/kg, respectively). In *Trichoderma* root colonization, the highest measurement was *Trichoderma harzianum* (100 %). The cucumber plants translocated Pb, Cr, and Cd to their fruits and leaves. Concentrations of Pb and Cd were higher in the basal leaves of the cucumber plant, whereas Cr was more concentrated in the cucumber fruits. Pb was not found in the cucumber skin. For Cd concentration in the skin, the *T. harzianum* and *T. viride* treatments at $2.34 \times 10^6$ and $2.34 \times 10^6$ CFU/mL, respectively, resulted in a 20.2 % reduction of this metal. *Trichoderma*, with $2.34 \times 10^6$ CFU/mL, had the lowest concentration of Cr in the pulp (5.572 mg/kg). No differences were found in metal concentrations in the skin or leaves.

Palabras clave: microorganismos benéficos, Pb, Cd, Cr, cultivos agrícolas.

**RESUMEN**

En el Valle del Mezquital, Hidalgo, México, se han utilizado aguas residuales en sistemas agrícolas, causando efectos ambientales negativos como la contaminación edáfica por metales pesados. Como consecuencia, las plantas los absorben. Por lo tanto, el objetivo del presente trabajo fue evaluar el efecto de las cepas de *Trichoderma harzianum* y *Trichoderma viride* en la bioacumulación de Cr, Cd y Pb en plantas de pepino (*Cucumis sativus*) inoculadas. El suelo usado en este estudio mostró Pb, Cd y Cr (34.63, 0.24 y 32.32 mg/kg, respectivamente). En cuanto a la colonización de raíces por *Trichoderma*, el valor más alto lo mostró *Trichoderma harzianum* (100 %). Las plantas de pepino traslocaron Pb, Cd y Cr a frutos y hojas. El Pb y el Cd se encontraron en concentraciones más altas en las hojas basales, mientras que el Cr estuvo más concentrado en los frutos de pepino. El Pb no fue encontrado en la cáscara de pepino. En cuanto a la concentración de Cd en la cáscara, los tratamientos *T. harzianum* y *T. viride*, a $2.34 \times 10^6$ y $2.34 \times 10^6$ UFC/mL, respectivamente, redujeron la concentración en un 20.2 %. *Trichoderma*, con $2.34 \times 10^6$ UFC/mL, mostró la concentración más baja de Cr en pulpa (5.572 mg/kg). No se encontraron diferencias significativas en la concentración de metales en la cáscara y hojas.


## INTRODUCTION

Demographic growth, urbanization, industrialization, and climate change are exerting pressure on water resources, causing scarcity (González and Chiroles 2011). Therefore, countries in the Middle East, as well as India, Israel, Germany, Australia, the United States, France, England, Poland, and Mexico, have used wastewater as an alternative in the agricultural sector (Carter et al. 2019).

In Mexico, wastewater has been utilized for irrigation in agriculture since 1890, mainly in the Mezquital Valley, Hidalgo (García-Salazar 2019). This water comes from the discharges of Mexico City. Heavy metal contamination, as one of the most serious environmental problems in soil, has a great magnitude because of its toxicity, persistence, bioaccumulation, and biomagnification throughout the food chain, which can pose a serious threat to human health even at low concentrations (Tchounwou et al. 2012, Sarwar et al. 2016). Although soil fertility and crop yields have increased (Zamora et al. 2008), there are potentially negative effects on the environment (Silva et al. 2008, Robledo-Zacarías et al. 2017), such as soil and groundwater contamination by Pb, Cd, Cu, and Cr, among others. Thus, Huerta et al. (2002) reported that Cr, Cu, Zn, As, Pb, Cd, Ag, and Hg concentrations exceeded critical levels for agricultural soils in the region. It should be noted that of these heavy metals, Cu and Zn are essential elements for the plants, but when present in high concentrations, they represent a toxic risk to living organisms.

Therefore, crops grown in soils contaminated with heavy metals can produce dangerous food, which is a threat to human health (Zhu et al. 2007). As a result, lines of research to decrease heavy metals in plants have been pursued. These alternatives are related to the absorption of heavy metals by microorganisms (Rajkumar et al. 2012, Tak et al. 2013). It was found that in *Trichoderma harzianum* Rifai inoculated tomato plants, the contents of Cr and Ni were reduced in roots, while contents of Cd decreased in all plant parts (Vukelyk et al. 2020). Few studies were executed on the ability of cucumbers to uptake and accumulate heavy metals from the soil when inoculated with *Trichoderma.* The hypothesis is that the inoculation of two species of *Trichoderma* in cucumber plants irrigated with wastewater will decrease the bioaccumulation of Pb, Cd, and Cr.

Based on these findings, the objective of this research was to evaluate the effect of *Trichoderma* strains on the bioaccumulation of Pb, Cd, and Cr in cucumber (*Cucumis sativus*) plants established in soil clay loam irrigated with wastewater in the Mezquital Valley, Hidalgo, Mexico.

## MATERIALS AND METHODS

The experiment was conducted in the experimental unit of the Universidad Politécnica de Francisco I. Madero (UPFIM), located at 20º14’ 432 N, 99º 5’ 28” W at an elevation of 1980 masl, with an average annual temperature of 17 ºC and annual precipitation of 540 mm. The experiment was conducted in two crop cycles.

Cucumber seedlings of the 'Thunderbird' variety were obtained and transplanted 20 days after sowing into beds with a plastic mulching system. The crop was selected for study as it is a commonly established crop (open field) in the region. Drip irrigation using wastewater from Mexico City was employed. To avoid interference with response variables, especially root colonization by experimental strains, no fungicides or biological control agents were applied during the experiment.

The experimental design was a full 3 × 2 factorial, with an interaction model, in a randomized complete block arrangement. The study factors were *Trichoderma* strains and conidial concentrations, resulting in seven treatments with negative control (untreated). **Table I** shows the treatments evaluated. There were four repetitions for each treatment, with 25 experimental units for each repetition (each experimental unit was one cucumber plant).

**TABLE I.** TREATMENTS EVALUATED TO DETERMINE THE EFFECT OF *Trichoderma viride* AND *Trichoderma harzianum* ON THE BIOACCUMULATION OF PB, CD, AND CR IN CUCUMBER PLANTS (*Cucumis sativus*).

| Treatment | Strain | Conidial concentration (CFU/mL) |
|---|---|---|
| T1 | *Trichoderma harzianum* | $2.34 \times 10^6$ |
| T2 | *Trichoderma harzianum* | $2.15 \times 10^7$ |
| T3 | *Trichoderma viride* | $2.34 \times 10^6$ |
| T4 | *Trichoderma viride* | $2.15 \times 10^7$ |
| T5 | *Trichoderma harzianum* (CS) | $2.34 \times 10^6$ |
| T6 | *Trichoderma harzianum* (CS) | $9.35 \times 10^6$ |
| T7 | Control (untreated) | 0 |

CS: commercial strain; CFU: colony forming units.

## Chemical and physical properties of the soil

The soil properties analyzed were carried out under NOM-021-RECNAT-2000 (SEMARNAT 2000): organic matter, by the Walkley and Black method; pH with the 1:5 radio method (soil:water); electrical conductivity by the measurement method in the saturation extract; cation exchange capacity by the ammonium acetate method; texture by the Bouyoucos method.

## Fungal material

Two isolates of *T. harzianum* and *T. viride* Pers. provided by the Executive Committee of the Cacao Farming Plan (CEPLAC) and a commercial strain of *T. harzianum* (BioControlSoil, Agribest) were used. The strains were plated on potato dextrose agar (PDA) and incubated at 28 ± 2 ºC for seven days. When the colonies were old, fungal masses were extracted and mixed separately with sterile water to obtain propagule suspensions.

Suspension concentrations were determined, a conidial count was performed using a Neubauer chamber, and absorbance was measured using a UV-vis spectrophotometer (Hach, Dr 6000, Germany). Linear regression models employing the function concentration (conidia) = f (absorbance, nm) were then used to determine the concentrations to be applied in the field.

## Inoculation of cucumber plants

Inoculations of each treatment were carried out at two different times. The first inoculation was performed 10 days after transplantation, as follows: 50 mL of the suspension were injected using a syringe directly into the rhizosphere of each of the seedlings. The second inoculation was performed using the same procedure 30 days after the first inoculation. To determine the percentage of colonization (PC) of *Trichoderma* strains in the root system of cucumber plants, one gram of roots of four plants was weighed per treatment and washed with common water. The samples were disinfested in 3 % sodium hypochlorite for three minutes, rinsed with sterile distilled water, and dried to be inoculated in a PDA culture medium and incubated in a growth chamber at a temperature of 28 ± 2 ºC. The colonies were daily evaluated from the observation of mycelium and spores in the culture medium (Tlapal et al. 2014).

## Determination of Pb, Cd, and Cr in soil, leaves, and fruits (pulp and skin)

To obtain soil (loamy clay) samples an auger was used. These were taken from the first 30 cm from each treatment, where the roots of cucumber plants were, and the surface was removed. Random zigzag sampling was carried out. The sampling was made according to NOM 021-RECNAT-2000 (SEMARNAT 2000) (**Table II**).

**TABLE II.** CONTENTS OF PB, CD, AND CR IN SOIL.

| Metal | Concentration in soil (mg/kg) |
|---|---|
| Pb | 34.63 |
| Cd | 0.24 |
| Cr | 32.32 |

For analysis of plant tissue (leaves and fruits), samples were taken from each of the treatments by randomly collecting five fruits and five leaves per replicate (analyses were performed in triplicate). The fruits were washed with 3 % sodium hypochlorite and distilled water; thin longitudinal sections were then cut, separating the skin from the mesocarp. The sections were dried at room temperature on Styrofoam plates. A similar process was applied for leaves, except that the samples were dehydrated in an oven at 70 ºC for 48 h. After drying, both samples (fruits and leaves) were ground using an electric mill and sieved through 0.5-mm mesh. Fruits were collected in the harvest stage, in physiological maturity, while the leaves were cut from the middle part of the plant, 50 days after sowing.

Extraction of heavy metals from plant tissue and soil was performed using the acid digestion method, as described in Lara et al. (2015). The procedure consisted of digesting 100 mg of sample, adding 4 mL of nitric acid, and heating at 65 ºC for 60 min, after which the temperature was increased to 120 ºC for an additional 30 min. Next, 1.6 mL of hydrogen peroxide were added, and the mixture was cooled to room temperature (25 ± 2 ºC). The samples were filtered through Whatman 42 paper, and the volume was adjusted to 25 mL with deionized water (18 MΩ/cm).

Quantification of Pb, Cd, and Cr was carried out using an atomic absorption spectrometer (AAS) (PerkinElmer precisely, model Analyst 700, Shelton, Connecticut, USA) in the Analytical Instrumentation Area of the Central Laboratory of Postgraduate School, Tabasco Campus. The wavelengths used were 283.3, 228.8, and 357.9 nm for Pb, Cd, and Cr, respectively.

**Statistical analysis**

Statistical analysis was performed using the Statistical Analysis System v. 9 (SAS 2004). The normality and homogeneity of the data were verified with the Kolmogorov-Smirnov ($n > 20$) and the Bartlett ($n > 20$) tests, respectively. For variables that did not comply with normality, Johnson's transformations were conducted to determine the best fit function. Then, ANOVA was performed to determine significant differences between treatments, and means were compared with the Tukey test. For all tests $p = 0.05$ was considered significant. The data used to determine the colonization percentage were transformed using the square root function to obtain continuous variables.

## RESULTS AND DISCUSSION

Regarding the values corresponding to the analyzed physical and chemical parameters of the soil, loamy clay soil was found, with a low content of organic matter, moderately alkaline due to its pH of 7.63. The concentrations of Pb, Cd, and Cr in the soil were 34.63, 0.247 y 32.32 mg/kg, respectively.

**Root colonization by *T. viride* and *T. harzianum* strains**

Cucumber root colonization by the two *Trichoderma* strains was different ($p = 0.0001$). Higher colonization in undisinfected roots was found in T2 (*T. harzianum*, $2.15 \times 10^7$ CFU/mL), compared to the other treatments (**Table III**), whereas T5 and T6, corresponding to the commercial strain of *T. harzianum*, exhibited the lowest percentage. This trend was also

**TABLE III.** ROOT COLONIZATION BY TWO *Trichoderma* STRAINS IN CUCUMBER PLANTS (*Cucumis sativus*)

| Treatment | Strain | Conidial concentration (CFU/mL) | Root colonization* (%) | |
|---|---|---|---|---|
| | | | Undisinfected | Disinfected |
| T1 | *T. harzianum* | $2.34 \times 10^6$ | 9.665 ba† | 8.99 ba |
| T2 | *T. harzianum* | $2.15 \times 10^7$ | 10.58 a | 10.915 a |
| T3 | *T. viride* | $2.34 \times 10^6$ | 8.598 ba | 8.66 ba |
| T4 | *T. viride* | $2.15 \times 10^7$ | 9.268 ba | 9.33 ba |
| T5 | *T. harzianum* (C.S.) | $2.34 \times 10^6$ | 6.433 b | 6.553 b |
| T6 | *T. harzianum* (C.S.) | $9.35 \times 10^6$ | 6.035 b | 6.035 bc |
| T7 | Control | 0 | 0 c | 2.5 c |
| P | | | <.0001 | <.0001 |
| S.E. | | | 2.05 | 2.4 |

*Reported colonization values are square root transformed.
CS: commercial strain; SE: standard error; CFU: colony forming units.
†Different letters in a column indicate significant difference (Tukey test, $p \leq 0.05$).

observed for disinfected roots, which is indicative of the infectiveness of pure *T. harzianum* isolate. Regarding the colonization, it is widely studied that *Trichoderma* colonizes the roots of several plant species (Viterbo et al. 2004, Zhang et al. 2014, Kai et al. 2020, Eslahi et al. 2020)

Regarding the commercial control (BioControlSoil, *T. harzianum*), it presented a low root colonization efficiency possibly because commercial product spores were inactive or dead according to a germination test carried out, therefore the infection was minimal compared with the rest of the treatments based on pure strains of *Trichoderma.*

### Translocation of Pb, Cd, and Cr in cucumber plants

Cucumber plants differentially translocated Pb, Cr, and Cd to fruits and leaves. The average concentration of Pb (5.78 mg/kg) found in basal leaves was significantly different from that found in fruit pulp (1.38 mg/kg) and skin, which tended to the baseline (0 mg/kg). These results are similar to studies about cereals (maize [*Zea mays* L.] and oats [*Avena sativa* L.]) and legumes bean (*Phaseolus vulgaris* L.) and alfalfa (*Medicago sativa* L.), which show the greatest bioaccumulation capacity for Pb in leaves and roots (García et al. 2011, Prabagar et al. 2021).

Significant differences were also found concerning the contents of Cd in the skin, pulp, and leaves. The highest average concentration of Cd, 2.17 mg/kg, was found in basal leaves, which was significantly different from that found in pulp and skin (0.165 and 0.169 mg/kg, respectively). The above results suggest that cucumber plants differentially translocate this element towards fruits and leaves. Similar studies in lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.), reported a greater accumulation of Cd in basal leaves than in old leaves (Martin et al. 2006). Lara et al. (2015) found the highest Cd content in the basal and middle parts of maize plants.

There were highly significant differences in Cr contents for pulp, skin, and basal leaves. The highest average concentration of Cr was found in skin (8.79 mg/kg), which was significantly different from that found in pulp and basal leaves (6.20 and 5.44 mg/kg, respectively). The results of our study showed that cucumber plants translocate the largest amount of Cr to fruits, which was similar to those reported by Yongan et al. (2010), who found the following Cr content in *Cucurbita maxima* Duchesne after exogenous application of Cr: fruits, 44.3 %; leaf, 36.1 %; root, 13.3 %; stem, 4.2 %, and flower, 2.1 %.

In general, it is observed that the green parts were those with the highest concentrations of the heavy metal studied, but Pb in the skin, whose values were, zero was the exception. This behavior is because heavy metals are associated in the form of organometallic complexes with chlorophyll (Kabata-Pendias and Pendias 2000).

### Effect of *Trichoderma* strains on Pb, Cd, and Cr accumulation

There was no significant effect of *Trichoderma* strains on Pb bioaccumulation in the aerial part of the plant (leaves and fruits) (**Fig. 1**), though other studies show that multiple fungi with symbiotic activity are effective in removing Pb from contaminated soils (Babu et al. 2014, Eutrópio et al. 2016). In our study, although the colonization capacity of the *T. harzianum* and *T. viride* strains was demonstrated at both concentrations, there was no significant effect of the plant-fungus association in inhibiting Pb bioaccumulation. Nonetheless, the plant absorbed and translocated Pb mainly towards leaves.

Highly significant differences ($p \leq 0.0001$) were found for the bioaccumulation of Cd in fruit pulp and skin, but not in basal leaves ($p > 0.216$; **Fig. 1**). The result for treatment T6 (*T. harzianum* commercial strain at $9.35 \times 10^6$ CFU/mL) was significantly different from that of the other treatments concerning pulp and resulted in the highest value of Cd bioaccumulation observed (0.186 mg/kg). Contrary to what was expected, all the treatments in pulp showed higher values of bioaccumulation of Cd compared to the control. Sun et al. (2017) found that with the addition of *Mucor circinelloides* Tiegh., 45.77 % higher Pb was accumulated by *Solanum nigrum* L. compared with single plant treatment. These microorganism associations can also modify the chemical composition of root exudates and the bioavailability of heavy metals in the soil (Sheng and Xia 2006). Many fungi are known to alleviate heavy-metal toxicity to plants and influence their accumulation and transportation. Additionally, Cd is an element with high mobility, which is why it accumulates in high concentrations in plants (Arduini et al. 2006, Matusik et al. 2008, Li et al. 2009).

In the case of Cd bioaccumulation in the skin, T1 (*T. harzianum* at $2.34 \times 10^6$ CFU/mL) and T3 (*T. viride* at $2.34 \times 10^6$ CFU/mL) treatments showed a reduction of 20.2 %, though highly significant differences from the absolute control were not found. The literature reports several species of fungi that have the potential to remove Cd, for example, *Aspergillus terreus* Thom, *Aspergillus flavus* Link, *Cladosporium, Fusarium oxysporum* Schltdl., *Gliocladium*

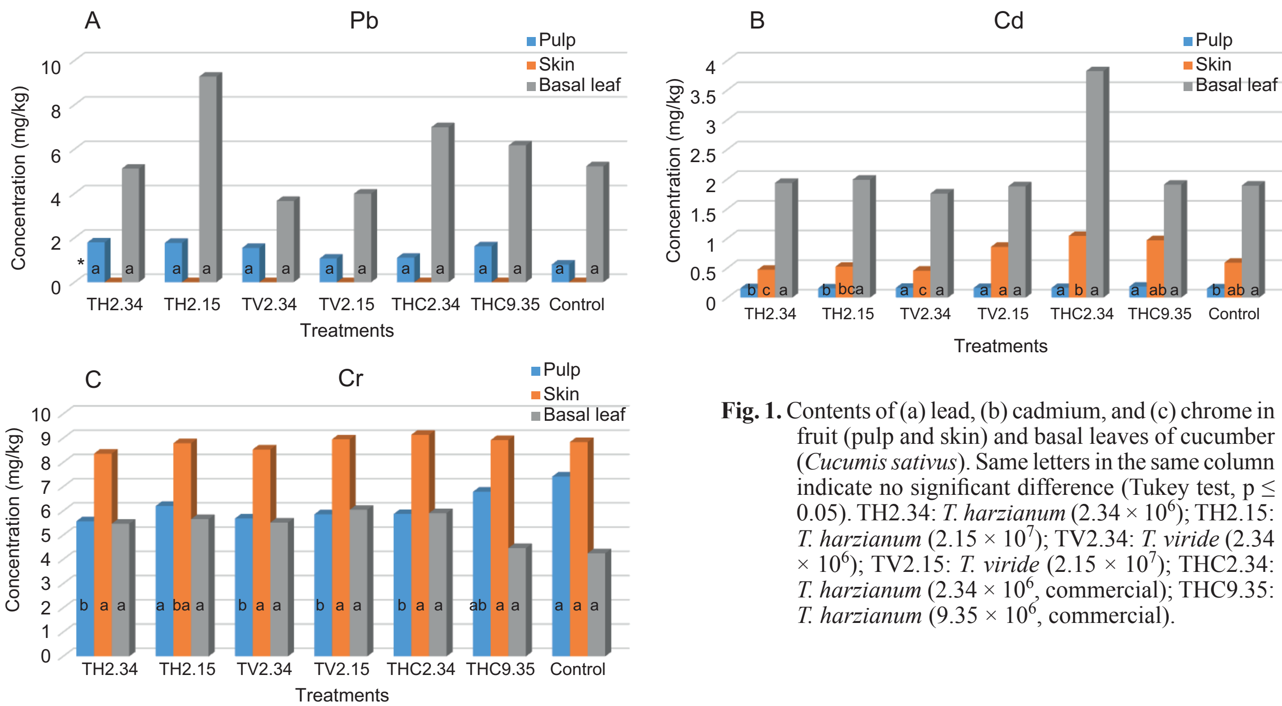


**Fig. 1.** Contents of (a) lead, (b) cadmium, and (c) chrome in fruit (pulp and skin) and basal leaves of cucumber (*Cucumis sativus*). Same letters in the same column indicate no significant difference (Tukey test, $p \leq 0.05$). TH2.34: *T. harzianum* ($2.34 \times 10^6$); TH2.15: *T. harzianum* ($2.15 \times 10^7$); TV2.34: *T. viride* ($2.34 \times 10^6$); TV2.15: *T. viride* ($2.15 \times 10^7$); THC2.34: *T. harzianum* ($2.34 \times 10^6$, commercial); THC9.35: *T. harzianum* ($9.35 \times 10^6$, commercial).

*roseum* Bainier, *Penicillium* spp., *Rhyzopus arrhizus* A. Fisch., *Helminthosporium* sp., *Talaromyces helices* Raper y Fennell, *Trichoderma koningii* Rifai, *T. harzianum,* and *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (Lima et al. 2011). This removal capacity is mainly because mycelium can sequester inassimilable heavy metals and prevent translocation to plants. The mechanism by which some heavy metals accumulate in the mycelium remains unclear. Some reports suggest that they may be associated with a high activity of o-acetylserine(thiol)lyasa, which is involved in cysteine biosynthesis (Raspanti et al. 2019).

Concerning the control (T7), treatments T1, T3, T4, and T5 exhibited a significant effect ($p \leq 0.002$) in reducing Cr at the pulp level (**Fig. 1**). However, for leaf and skin bioaccumulation, no significant effect of the treatments for the control was observed. In particular, *T. harzianum* caused a 24.8 % decrease in Cr bioaccumulation in the pulp at a concentration of $2.34 \times 10^6$ CFU/mL. *T. viride* at two concentrations ($2.34 \times 10^6$ and $2.15 \times 10^7$ CFU/mL) also resulted in a significant reduction of 24 % relative to the control. Studies have shown the effectiveness of *Trichoderma* for the bioabsorption of Cr ions (Shukla and Vankar 2014). Rhamrakhiani et al. (2011) suggested that carboxyl, hydroxyl, phosphoryl, imidazole, and amine functional groups are the binding sites of Cr on the fungal surface.

Heavy metals such as Cd, Cr, As, Hg, and Pb are toxic and regarded as the most harmful to human health (Balali-Mood et al. 2021), and they are considered as the priority control pollutants by the United States Environmental Protection Agency (US-EPA 1989). According to the World Health Organization and the Food and Agriculture Organization (WHO/FAO 2007) of the United Nations, the permissible limit of Pb, Cd, and Cr in plant tissues should not exceed 2, 0.02, and 1.30 mg/kg, respectively. In the present research, maximum values of 1.794 mg/kg of Pb were found in fruit pulp and 9.27 mg/kg in leaf tissue. However, the cucumber leaf is not an organ used by consumers, so it does not constitute a risk. Regarding Cd, the fruit pulp accumulated 0.186 mg/kg, whereas leaf tissue showed the highest bioaccumulation, 3.82 mg/kg, exceeding the maximum limit indicated by WHO/FAO (2007), which leads to a high risk for consumers because Cd is a toxic heavy metal that can cause renal tubular dysfunction or skeletal damage from anemia (Liu et al. 2014). On the other hand, Cr presented a similar behavior. It was found in the pulp at 5.2 mg/kg and in foliar tissue at 6.04 mg/kg, exceeding the maximum limit allowed by WHO, therefore it is considered as a high risk because it has carcinogenic, mutagenic, and teratogenic effects (Verma et al. 2014).

Studies by Vázquez et al. (2005) showed that to determine the permissible limits of Cd and Pb accumulation, other variables such as daily intake of metals, type of food consumed, transfer of metals from soil to crops, and food produced in non-contaminated areas and areas contaminated with heavy metals need to be considered. In the future, these models will provide a better approximation of the permissible limits of accumulation of these metals in the soil.

Regarding the mobility of heavy metals, it is conditioned by the characteristics of the soil affecting the toxicity and translocation or bioaccumulation of these in plants or crops (Sauquillo et al. 2003). The pH is the main factor affecting their solubility, which decreases at high pH (Sheoran et al. 2016). In the present research, a pH of 7.63 was observed in the soil; this influenced the low solubility of these metals, which is shown in the absorption of these in different treatments. Cr and Pb were found in the soil at concentrations of 32.32 and 34.63 mg/kg, whereas the highest bioaccumulated concentrations in the plant tissue barely reached 6.78 and 6.99 mg/kg, respectively, although other factors such as plant species, environmental factors, chemical properties of the contaminant, environmental condition and root zone are involved (Tangahu et al. 2011). These data can also be related to the high concentrations of organic matter (4.06 %) in the studied soil, because this affects the absorption and complex formation of heavy metals (Zeng et al. 2011, Bravo et al. 2014). On the other hand, the capacity of cationic exchange also plays an important role for soil to release or retain the cations, including heavy metals. The cationic exchange capacity in the studied soil was 30.5 cmol/kg, which indicates it is a clayey soil (loam-clay) and explains why the number of heavy metals in the soil was not absorbed in high concentrations by the cucumber plants.

## CONCLUSIONS

The results of this study suggest that *C. sativus* bioaccumulates Pb and Cd mainly in leaves, whereas Cr bioaccumulates in fruit skin. Besides, pure strains of *Trichoderma* had higher colonization of roots.

The concentrations of Pb and Cr in plant organs were lower than those found in soil, and the content of Cd in leaves was higher than that in soil. Non-commercial strains of *T. harzianum* and *T. viride* showed significant effects on the reduction of bioaccumulation of Cd in the skin (except for the highest *T. viride* spore concentration) and of Cr in the pulp. Pb was not found in cucumber fruit skin.

Once other microorganisms (such as *Trichoderma*) are involved, symbiotic associations are generated that can inhibit the accumulation of metal ions, or their absorption of them can be stimulated. In this study, the inhibition of Cr was observed in the organs of the plant that are of economic interest, whereas there was greater absorption of Cd in the skin, and of Pb in pulp and leaves. Cd and Cr exceed the maximum limit indicated by WHO/FAO in fruit pulp, whereas bioaccumulation of Pb is below the established limits, but it is still greater than the control. Therefore, it is proposed to continue with the generation of knowledge through the research of such strains on cucumber and several crops of agricultural interest irrigated with wastewater. The association of *Trichoderma* with plants that have phytoremediation effects should also be further studied, and their possible synergism for the remediation of soils evaluated.

For cucumber crops and under the conditions of this study, it is recommended inoculation with *T. harzianum* at a concentration of $2.34 \times 10^6$ on plants, which reduces Cr levels in fruit pulp consumed by people.

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