Influence of *Trichoderma* species on the reduction of heavy metal levels in bean plants irrigated with wastewater: a case study from the Mezquital Valley, Hidalgo, Mexico

Corresponding authors:
Francisco Marcelo Lara-Viveros;
Email: francisco.lara@ciqa.edu.mx
Nancy Calderón-Cortés;
Email: ncalderon@enesmorelia.unam.mx

Nadia Landero-Valenzuela[1], Edith Hernández-Nataren[2], Lizeth Chávez-Cerón[3], Carlos Alejandro Granados-Echegoyen[4], Nancy Alonso-Hernández[5], Netzahualcoyotl Mayek-Pérez[6], Francisco Marcelo Lara-Viveros[7], Brenda Ponce-Lira[3] and Nancy Calderón-Cortés[8] (iD)

[1]Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, C.P. 25315, Saltillo, Coahuila, México; [2]Colegio de Postgraduados, Campus Tabasco, Periférico Carlos A. Molina S/N Km. 3.5, C.P. 86500 Cárdenas, Tabasco, México; [3]Universidad Politécnica de Francisco I. Madero, Agrotecnología, S/N, C.P. 42660, Tepatepec, Hidalgo, México; [4]CONAHCYT-Universidad Autónoma de Campeche, Centro de Estudios en Desarrollo Sustentable y Aprovechamiento de la Vida Silvestre (CEDESU), Avenida Héroe de Nacozari, 480, C.P. 24079, San Francisco de Campeche, México; [5]Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR) Unidad Oaxaca, Hornos 1003, Col. Noche Buena, C.P. 71230, Santa Cruz Xoxocotlán, Oaxaca, México; [6]Universidad Autónoma de Tamaulipas, Unidad Académica Multidisciplinaria Reynosa-Rhode, Reynosa, Tamaulipas, México; [7]Departamento de Biociencias y Agrotecnología, Centro de Investigación en Química Aplicada, Enrique Reyna H. 140, San José de los Cerritos, C.P. 25294, Saltillo, Coahuila, México and [8]Universidad Nacional Autónoma de México, Escuela Nacional de Estudios Superiores Unidad Morelia, Laboratorio de Ecología Molecular, Antigua Carretera a Pátzcuaro, 8701, Col. E × Hacienda San José de la Huerta, C.P. 58190 Morelia, Michoacán, México

Abstract

Contamination by heavy metals resulting from irrigation with wastewater is an important global concern, as it can lead to crop contamination, posing serious threats to human health. Despite the advances in bioremediation of soils, sustainable strategies that use beneficial microorganisms to reduce the accumulation of heavy metals in contaminated crops remain underexplored. The aim of this study was to evaluate the potential reduction of heavy metal concentration in different structures of bean plants cultivated in soils irrigated with untreated wastewater on an agricultural land located in Hidalgo, Mexico, through the inoculation of a ubiquitous soil fungus. Bean plants were inoculated with three *Trichoderma* spp. and distributed across eight treatments, with an additional untreated control group, following a random full design. The percentage of root colonization and concentration of heavy metals in the leaves, pods, grains, and soil were determined. The results revealed that root colonization exceeded 75%. The treatments *T. harzianum* + *T. asperellum* and *T. harzianum* with 3×10^6 spores ml^{-1} showed the greatest decrease in Cd (64.7%) and Pb (66.1%) concentrations in the grains. *T. viride* with 2×10^6 spores ml^{-1} was the most effective treatment for copper reduction (72.9%) in grains; *T. harzianum* with 3×10^6 spores ml^{-1} showed the best performance for chromium reduction (75.7%), which was below the detection limit in leaves. In conclusion, inoculation of bean plants with *Trichoderma* spp. effectively reduced the accumulation of heavy metals. Future research in this area could contribute to the development of sustainable strategies to mitigate heavy metal contamination in agricultural ecosystems, thereby promoting food and environmental safety.

CAMBRIDGE UNIVERSITY PRESS

Introduction

As a result of rapid industrialization and urbanization, heavy metal pollution in agricultural soils has become an important global concern with serious threats to human health and agricultural production (Devi et al., 2022; Hou et al., 2020). This is particularly important for agricultural soils irrigated with wastewater that usually contains high concentrations of heavy metals (Rattan et al., 2005; Khan et al., 2008; Hou et al., 2020), because heavy metals originated from fertilizers and/or wastewater irrigation can accumulate in the tissues of numerous plant species (Amin et al., 2013; Yan et al., 2020; Sun et al., 2022).

Heavy metal accumulation in plants varies among species, and even among populations and ecotypes of the same species (Rascio and Navari-Izzo, 2011). However, previous studies have reported a significant accumulation of some heavy metals in vegetables, grains, and

other edible plant structures consumed by humans as part of their daily diet (Rattan et al., 2005; Sharma, Agrawal, and Marshall, 2007; Khan et al., 2008; Amin et al., 2013). The consumption of food contaminated by heavy metals can deplete some essential nutrients in the body causing various diseases and result in a number of serious health problems in humans, including a high prevalence on gastrointestinal cancer, comma, renal damage, infertility, stroke, epilepsy, and neurodegenerative diseases, among others; with effects on children's even more severe than adults (Khan et al., 2008; Amin et al., 2013; Alengebawy et al., 2021). Consequently, there has been an increasing interest in developing technologies to reduce the accumulation of heavy metals in crops, including physical, chemical, and biological methodologies (Khalid et al., 2017). Among these, biological methodologies (i.e., bioremediation) have gained great attention because they are based on sustainable processes and more economically viable strategies (Khalid et al., 2017; Yaashikaa et al., 2022). One approach involves the use of biological materials as adsorbents for these metals, effectively decreasing their concentrations in soils and in cultivated plants, but limited information is available on this topic (Eltlbany et al., 2019; Vukelić et al., 2020). However, the use of microorganisms such as filamentous fungi has shown advantages over biological adsorbents for bioremediation of soils and for the reduction of heavy metal concentration in crops due to their efficient ability to accumulate metals in their cell walls and cellular structures (i.e., biosorption), as well as for their ability to establish stable interactions with plants (i.e., endophytic fungi) (Audet and Charest, 2007; Hildebrandt, Regvar, and Bothe, 2007; Verma and Kuila, 2019; Anand et al., 2023; Hou et al., 2020). These fungi are also known for their resilience and ability to thrive under extreme pH, temperature, and nutrient availability conditions, allowing their use under different environmental conditions in agricultural lands (Deng et al., 2020; Chugh et al., 2022). Additionally, they can also enhance plant growth and productivity in contaminated soils (O'Sullivan et al., 2019; Devi et al., 2022).

There are several fungal species identified in previous studies for bioremediation of heavy metals in soils and/or reduction of heavy metal concentration in crops, including the species in the genera *Acremonium, Alternaria, Aspergillus, Cladosporium, Fusarium, Ganoderma, Geotrichum, Monilia, Mortierella, Mucor, Penicillium, Rhizopus, Trichoderma*, among other arbuscular mycorrhizal fungi (Priyadarshini et al., 2021; Devi et al., 2022; Yaashikaa et al., 2022). Nevertheless, fungal species may be specific toward metal ions and plant species because of the variation in cellular compositions of microbial systems, the functional sites responsible for metal binding, and different cellular and molecular mechanisms employed to tolerate metals (Hildebrandt, Regvar, and Bothe, 2007; Priyadarshini et al., 2021; Devi et al., 2022). For cultivated plants, environmental and management conditions can also influence the effectiveness of heavy metal reduction by various fungal species (Priyadarshini et al., 2021; Devi et al., 2022). Hence, suitable fungal species need to be identified for specific heavy metals and cultivated plants exposed to high heavy metal concentrations in different agricultural lands.

The Mezquital Valley in Hidalgo, Mexico encompasses 80,000 hectares of land dedicated to cultivating various crops such as alfalfa, maize, wheat, oats, chili peppers, cabbage, cilantro, spinach, parsley, and beans. These crops have traditionally been irrigated with untreated domestic and industrial wastewater from Mexico City for over a century (Hernández et al., 2017; García-Salazar,

2019), and several studies have reported the presence of heavy metals in the soil of Mezquital Valley (Lara et al., 2015; Ziegler-Rivera et al., 2022). The elevated levels of heavy metals found in the Mezquital Valley soil can be attributed to their low mobility in alkaline or neutral pH soils (Plant and Raiswell, 1983). Moreover, wastewater generated by industries utilizing Pb-containing compounds is subsequently used for crop irrigation in the region, further contributing to contamination.

Among the crops produced in this valley and despite the changes in dietary habits in the last decades, beans (*Phaseolus vulgaris* L.) along with maize (*Zea mays*) continue to be essential protein sources for more than 300 million people in Mexico. For instance, in 2020, the total area of bean cultivation reached 1,676,355 hectares, with an average national yield of 0.79 tons per hectare, resulting in an approximate per capita consumption of 8.4 kg per year (Gálvez and Salinas, 2015; OECD, 2019; SIAP, 2020; Mesele et al., 2022). Given that beans have a protein content ranging from 14 to 35%, 52 to 76% of carbohydrates, 1.5 to 6.5% of lipids, 14 to 20% of fiber, 3 to 5% of minerals, and a significant content of micronutrients, such as iron, zinc, thiamine, and folic acid, their cultivation play a critical role in national food security (Abbade and Dewes, 2014; Petry et al., 2015; Chávez-Servia et al., 2016; Chávez and Sánchez, 2017). Moreover, beans are also one of the most produced and consumed food grain legumes in the world (FAO, 1997).

Previous studies focused on evaluate phytotoxicity and atmospheric deposition of heavy metals on bean plants, found significant negative effects on plant physiology and crop productivity, and high deposition rates of atmospheric heavy metals in different plant structures (Aldoobie and Beltagi, 2013; Gjorgieva et al., 2013; Cannata et al., 2015; De Temmerman et al., 2015; Hammami et al., 2022). In these studies, it was also found that the ability of bean plants to accumulate different heavy metals depends on the physicochemical conditions, heavy metal concentrations, and management of soil. However, in our knowledge there are not studies evaluating the effect of fungal inoculation on the reduction of heavy metal concentration in bean plants cultivated under a wastewater irrigation regime. Hence, it is crucial to conduct studies on microbial inoculation in bean crops to understand the impact of microorganisms on the accumulation of heavy metals in different parts of the plant, particularly in the grains, which represent the plant structure used for human consumption.

Trichoderma species are found in all types of soils and are commonly isolated from different ecosystems, since they are able to establish endophytic associations with plants (Woo et al., 2023; Racić et al., 2023). They have been used in previous studies for bioremediation because of their ability to tolerate, translocate and to act as biosorbents of different heavy metals (Babu et al., 2014; Govarthanan et al., 2018; Khan et al., 2019; Maldaner et al., 2021; Talukdar et al., 2020; Racić et al., 2023). Previous studies have also suggested that the use of a combination of *Trichoderma* species or the combination with other fungal species can increase the effectiveness of metal mobilization in soil solutions (Kacprzak et al., 2014). Based on this, the aim of this study was to evaluate the potential reduction of heavy metal concentration in bean plants cultivated in soils irrigated with untreated wastewater in Hidalgo, Mexico by inoculating different *Trichoderma* spp. and combinations of these species, in order to identify strategies to ameliorate the health impacts related to the consumption of heavy metal contaminated crops, as well to identify fungal strains that can potentially be used for bioremediation of contaminated soils in future studies.

Materials and methods

Study site

The study was conducted on an open experimental field at the Universidad Politécnica de Francisco I. Madero, located in Tepatepec, Francisco I. Madero, Hidalgo, Mexico (20° 13′ 27″ N, 99° 05′ 21″ W) at 1977 masl. Francisco I. Madero is one of the 28 municipalities conforming the Mezquital Valley, a semiarid region with a mean temperature of $16 \pm 7°C$ and an average annual rainfall of 578 mm at elevations around 2000 m (Friedel et al., 2000). This valley, located 80 km North of Mexico City, is a large agricultural production area irrigated with untreated wastewater from Mexico City by flooding soil surface with 150 to 200 mm according to crop requirements and water availability throughout the year (Guédron et al., 2014).

Fungal material

T. harzianum and *T. viride* cultures were generously provided by CEPLAC (Comissão Executiva do Plano da Lavoura Cacaueira) from Brazil. *T. asperellum* was isolated from agricultural soil in Mezquital Valley, Hidalgo, which had been irrigated with wastewater. Soil samples were collected using a widely accepted five-point sampling technique. Isolation of *T. asperellum* was conducted using the serial dilution method (ranging from 10-1 to 10-4) as described by Dhingra and Sinclair (1981). The samples were prepared in sterile distilled water and aliquots of the suspension were transferred onto potato dextrose agar (PDA) culture plates. The plates were then incubated at a controlled temperature of $28 \pm 2°C$ for a period of 10 days to facilitate sporulation. The identification process followed the guidelines of Samuels et al. (2009). The preserved strains were maintained at the Agrobiotechnology Laboratory at the Center for Research in Applied Chemistry (CIQA, Mexico).

Soil sampling

To analyze the soil in the open field of the study site where the bean crop was grown, a composite sample was collected from five sampling points, totaling 1 kg of soil taken from a depth of 1–30 cm. The sample was prepared following the guidelines of the Mexican Official Standard NMX-AA-132-SCFI (2006), which specifies the method for soil sampling to identify and quantify metals and metalloids, as well as sample handling. The collected soil samples were then dried in a forced-air oven at 75°C for 72 h, ground into fine particles, and sieved through a mesh sieve with an opening size of 0.707 mm.

Determination of physicochemical soil properties

To assess the physicochemical properties of the soil, various parameters were measured, including the pH, organic matter content, texture, and cation exchange capacity. All soil parameters were analyzed using the methods described in the Mexican Official Standard NOM-021-RECNAT (2000). The pH was determined using the 1:5 soil-to-water ratio method; organic matter content was quantified using the Walkley–Black method. The electrical conductivity was measured in the saturated extract, while the cation exchange capacity was determined using the ammonium acetate method (Jain and Taylor, 2023). The soil texture was evaluated using the Bouyoucos method (Mwendwa, 2022).

Bean cultivation

The bean crop was established using disease-free and pest-free seeds of the 'Michigan' variety (emergence and early vegetative growth) sourced from the Universidad Politecnica de Francisco I. Madero in Hidalgo, Mexico. The seeds were carefully selected to ensure their quality. Planting was conducted in an open experimental field of the study site with a total of 500 plants spread across a 100 m^2 area, ensuring proper spacing. To replicate the typical cultivation practices followed in the Mezquital Valley region, agronomic management of the crop adhered to the methods employed by local farmers. This involved the use of agricultural machinery for soil preparation and creating optimal conditions for growth. Irrigation was carried out through periodic flooding every 20 days, using wastewater sourced from Mexico City.

Inoculation of bean roots with Trichoderma spp

The experiment was conducted using a comprehensive randomized design encompassing eight distinct treatment conditions. These treatments were uniquely distinguished as follows: *T. harzianum* (T1 and T4), *T. viride* (T2 and T5), and *T. asperellum* (T3 and T6), each at two spore concentrations, 3×10^6 and 2×10^6 spores ml^{-1}. Additionally, two combinations were studied: *T. harzianum* with *T. viride* (T7) and *T. harzianum* with *T. asperellum* (T8), both administered at spore concentrations of 3×10^6 spores ml^{-1}. Finally, a control group was included, characterized by a spore concentration of zero.

For fungal inoculation in each experimental treatment, *Trichoderma* spp. were cultivated on PDA culture medium at an incubation temperature of 25°C for 10 days. Each colony was then gently flooded with 15 ml of sterile distilled water and the surface was lightly scraped using a glass rod to release the spores without disturbing the mycelium. The resulting suspension was filtered through a sterile cotton mesh to remove debris. Aliquots (0.5 ml) were collected from each plate and transferred to a Neubauer chamber for conidial counting. The spore concentration in the suspension was adjusted to either 2×10^6 or 3×10^6 spores ml^{-1}, depending on the specific treatment, using known absorbance values obtained from a UV-visible spectrophotometer (Eppendorf BioSpectrometer 6135000923, Hamburg, Germany). This ensured consistent spore concentrations for subsequent inoculation.

For the inoculation process, 50 ml of the prepared spore suspension was applied to the roots of randomly selected bean plants grown in the open field 20, 30, and 40 days after sowing. This multi-stage inoculation approach was aimed at ensuring the successful establishment of *Trichoderma* spp. on bean roots. The methodology used in this study followed the procedure outlined by Landero et al. (2019).

Percentage of bean roots colonized by Trichoderma spp

After the third inoculation (40 days later), four random root samples were selected from each of the nine treatments. To remove excess soil, roots were washed with tap water. For each root, ten 0.5 cm cuts were made, resulting in a total of 40 cuts per treatment. The cut sections were then transferred to Petri dishes, with 10 cuts placed on each dish. Each treatment had three dishes. To ensure cleanliness, cut sections were disinfected with 3% sodium hypochlorite for 1 min. Subsequently, they were rinsed with sterile distilled water and dried using sterile absorbent

paper. The dried sections were transferred to Petri dishes. The Petri dishes were incubated at a constant temperature of 25°C and the development of the samples was evaluated every 24 h. Root colonization was considered effective when fungal growth in vitro reached 50% or more, based on a previous study (Landero-Valenzuela et al., 2019).

Plant tissues sampling

For the analysis of tissues of bean plants, four samples were collected at the harvest stage, specifically 100 days after planting. Each treatment had 10 replicated samples. These samples included leaf, grain, and pod sections. Each section was separated for individual processing. Similar to the soil samples, the plant sections were dried and sieved using a previously described method.

Determination of heavy metals in leaf, pod, grain, and soil

Heavy metals were extracted from plant tissues and soil using an acid digestion method, as described by Lara-Viveros et al. (2015). The process involved taking 100 mg of each sample and adding 4 ml of nitric acid. The samples were then heated at 65°C for 60 min, followed by an increase in temperature to 120°C for an additional 30 min. Subsequently, 1.6 ml of hydrogen peroxide was added to the mixture, which was then allowed to cool to room temperature (25 ± 2°C). The resulting digested samples were filtered using Whatman 42 filter paper and adjusted to a final volume of 25 ml using deionized water (18 MΩ/cm). The quantification of Cd, Cr, Cu, and Pb was performed using the flame atomic absorption technique with an atomic absorption spectrometer (Perkin Elmer Inc., AANALYST 700, Massachusetts, USA). The wavelengths used were 228.8, 357.9, 324.8, and 283.3 nm for Cd, Cr, Cu, and Pb, respectively. The samples were measured in triplicate, including blank and blind samples. The limits of quantification (LOQ) and limits of detection (LOD) for metals were as follows: Pb (LOQ = 0.25 mg l^{-1} and LOD = 0.85 mg l^{-1}); for Cd (LOQ = 0.15 mg l^{-1} and LOD = 0.017 mg l^{-1}); for Cr (LOQ = 0.73 mg l^{-1} and LOD = 0.13 mg l^{-1}); and for Cu (LOQ = 0.07 mg l^{-1} and LOD = 0.20 mg l^{-1}). For the analysis of heavy metals, specifically Pb, Cd, Cr, and Cu, the Mexican Standard NMX-AA-132-SCFI (2006) was followed. The results were obtained by calculating the mean value based on three sets of duplicate repetitions, and the standard deviations (s.d.) were also considered.

Data analysis

The experimental design consisted of a fully random design with eight experimental treatments and one control treatment. This experimental design ensured a robust statistical approach, with each treatment being subjected to three repetitions. Within each repetition, two replicates were performed. This stringent methodology was employed to minimize potential sources of variability and to yield statistically reliable data for a comprehensive assessment of the effects of Trichoderma spp. treatments on bean plants. Rows at the edges were excluded from the analysis to reduce statistical bias.

Statistical Analysis System (SAS) version 9 was used for data analysis. The normality and homogeneity of the data were checked using the Kolmogorov–Smirnov test and Bartlett test. Subsequently, Analysis of Variance (ANOVA) was performed to

determine whether there were any significant differences between the treatments. The Tukey method was used to compare means. All statistical tests were performed at a significance level of $\alpha = 0.05$. In the case of colonization percentage, the data underwent a square root transformation before the analysis.

Results

Soil physicochemical properties

Chemical characteristics in the studied soil were: pH 7.7 ± 0.4, 1.72% of organic matter content, 27.86 cmol kg^{-1} of cation exchange capacity and a texture corresponding to silty loam soils. Meanwhile, the concentrations of Pb and Cd in the soil of the Mezquital Valley, Hidalgo, Mexico, irrigated with wastewater were higher than the allowable levels of these heavy metals in soils according to the World Health Organization, whereas the Cu and Cr concentrations were below the allowable limits (Table 1).

Root colonization by Trichoderma spp. in bean plants

In all experimental treatments, including those with the evaluation of the combination of two different species, Trichoderma spp. achieved a root colonization rate of over 75%. The highest percentage of root colonization (86.66%) was observed in T. harziamun 3×10^6 spores ml^{-1} (T1) and T. viride 3×10^6 spores ml^{-1} (T2) treatments, whereas the lowest percentage was for T. asperellum 3×10^6 spores ml^{-1} (T6) and T. harzianum + T. asperellum (T8) treatments (76.66%; Fig. 1). Control treatment showed 0% of colonization. There was no significant difference ($P = 1.0$) among the eight experimental treatments (Fig. 1), but colonization by Trichoderma spp. in all experimental treatments was significantly different from the control group ($P < 0.001$), indicating that all bean plants were successfully colonized by Trichoderma spp.

Effect of Trichoderma spp. on the accumulation of heavy metals in bean plants

In general, Trichoderma spp. inoculation resulted in a significant decrease in the accumulation of the four heavy metals in the evaluated structures (leaf, pod, and grain) of bean plants compared with the control, with the exception of Pb concentration in leaves in treatment T4, Cu concentration in leaves in treatments T3 and T8, and Cu concentration in pods in treatment T8 (Fig. 2, Table 2). Specifically, Pb concentration was higher in grains (38.5–76.00 mg kg^{-1}), followed by pods (30.08–64.16 mg kg^{-1}) and leaves (3.91–26 mg kg^{-1}) in the experimental treatments

Table 1. Concentration of heavy metals (Pb, Cr, Cu, Cd) in soils irrigated with wastewater in the Mezquital Valley, Hidalgo, Mexico

Metal	Concentration (mg kg^{-1})	Standard deviation	VPMPS (mg kg^{-1})
Pb	120.66	7.2	85
Cr	86.83	5.6	100
Cu	35.16	1.7	36
Cd	34.16	1.2	0.8

Three replicates were used to measure heavy metal levels. Pb, lead; Cr, chromium; Cu, copper; Cd, cadmium. The concentrations are expressed in mg/kg based on the dry weights of the samples. VPMPS represents the allowable levels of heavy metals in soils (mg kg^{-1}), according to the World Health Organization (WHO, 1996).
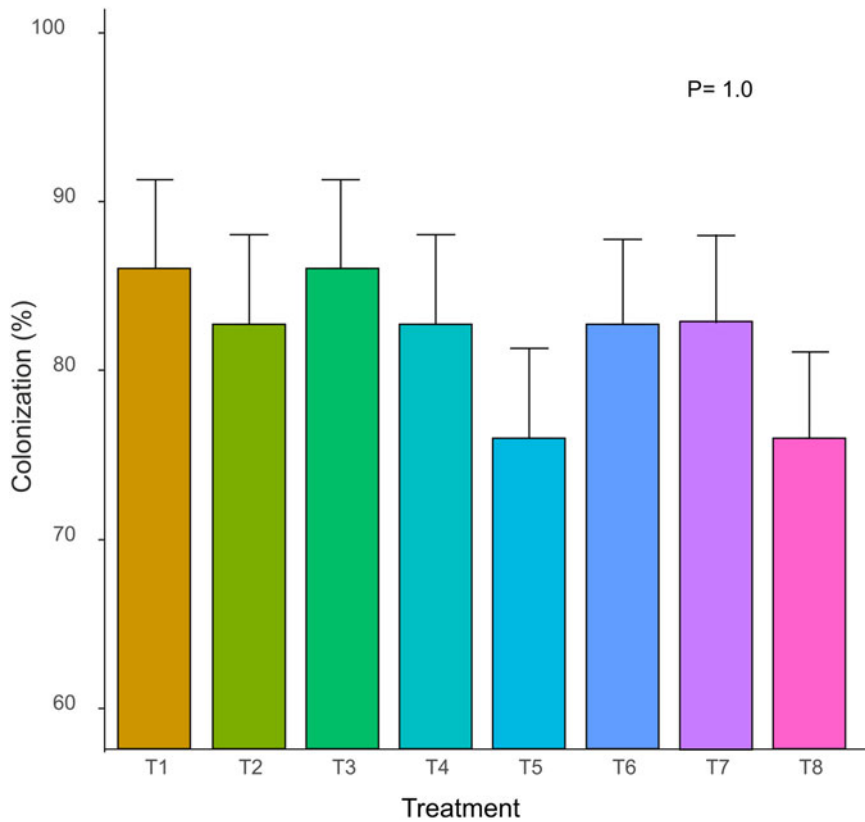
Figure 1. Effect of Trichoderma spp. strains on root colonization of bean plants. T1: inoculation with *T. harzianum* 3×10^6 spores ml^{-1}; T2: inoculation with *T. harzianum* 2×10^6 spores ml^{-1}; T3: inoculation with *T. viride* 3×10^6 spores ml^{-1}; T4: inoculation with *T. viride* 2×10^6 spores ml^{-1}; T5: inoculation with *T. asperellum* 3×10^6 spores ml^{-1}; T6: inoculation with *T. harzianum* 2×10^6 spores ml^{-1}; T7: inoculation with *T. harzianum* + *T. viride* 3×10^6 spores ml^{-1}; T8: inoculation with *T. harzianum* + *T. asperellum* 3×10^6 spores ml^{-1}. Mean (± S.E.) of the percentages of root colonization are shown. Differences between treatments were determined using Tukey's test.
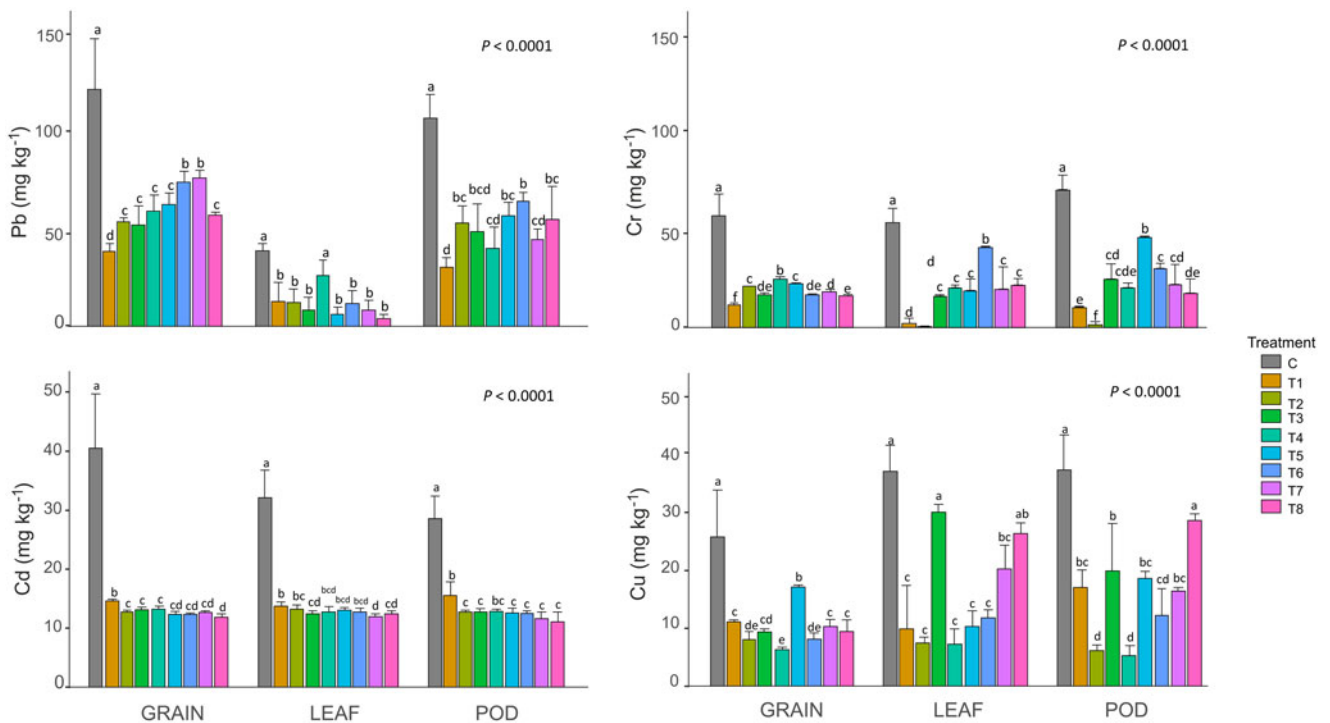

Figure 2. Concentration of lead, cadmium, chromium, and copper in bean leaf, pod, and bean grain under different treatments with *Trichoderma* spp. C, control treatment. The experimental treatments included: T1: inoculation with *T. harzianum* 3×10^6 spores ml^{-1}; T2: inoculation with *T. harzianum* 2×10^6 spores ml^{-1}; T3: inoculation with *T. viride* 3×10^6 spores ml^{-1}; T4: inoculation with *T. viride* 2×10^6 spores ml^{-1}; T5: inoculation with *T. asperellum* 3×10^6 spores ml^{-1}; T6: inoculation with *T. harzianum* 2×10^6 spores ml^{-1}; T7: inoculation with *T. harzianum* + *T. viride* 3×10^6 spores ml^{-1}; T8: inoculation with *T. harzianum* + *T. asperellum* 3×10^6 spores ml^{-1}. Pb: lead; Cd: cadmium; the concentrations of heavy metals are expressed in mg/kg based on the dry weight of the samples. Means ± standard deviations (S.D.) are provided. Means with the same letter are not significantly different according to Tukey's test ($P < 0.05$, $n = 51$).

Table 2. Treatment effect statistics for heavy metal concentration of different tissues of bean plants

	$F_{8, 55}$	P	R^2
Pb			
Grain	17.5	<0.0001	0.86
Leaf	33.7	<0.0001	0.70
Pod	12.4	<0.0001	0.77
Cr			
Grain	93.6	<0.0001	0.95
Leaf	53.5	<0.0001	0.91
Pod	57.4	<0.0001	0.92
Cd			
Grain	65.5	<0.0001	0.92
Leaf	97.1	<0.0001	0.95
Pod	46.7	<0.0001	0.90
Cu			
Grain	33.3	<0.0001	0.86
Leaf	47.2	<0.0001	0.90
Pod	32.8	<0.0001	0.86

The statistical analysis evaluated the concentration of lead (Pb), chromium (Cr), cadmium (Cd) and copper (Cu) among the control treatment and the eight experimental treatments: T1: inoculation with *T. harzianum* 3×10^6 spores ml^{-1}; T2: inoculation with *T. harzianum* 2×10^6 spores ml^{-1}; T3: inoculation with *T. viride* 3×10^6 spores ml^{-1}; T4: inoculation with *T. viride* 2×10^6 spores ml^{-1}; T5: inoculation with *T. asperellum* 3×10^6 spores ml^{-1}; T6: inoculation with *T. harzianum* 2×10^6 spores ml^{-1}; T7: inoculation with *T. harzianum* + *T. viride* 3×10^6 spores ml^{-1}; T8: inoculation with *T. harzianum* + *T. asperellum* 3×10^6 spores ml^{-1} for each plant structure.

(Fig. 2). Meanwhile, Cd and Cu showed less variation among the plant structures (11.08–15.05, 5.42–29.42 mg kg^{-1}, respectively; Fig. 2). Cr concentration ranged from 0 to 48.08 mg kg^{-1}, with the greatest values for T5 and T6 treatments for pods (48.08 mg kg^{-1}) and leaves (42.75 mg kg^{-1}), respectively (Fig. 2).

The concentration of fungal spores did not show a significant effect on Pb concentration in the leaves of plants inoculated with *T. harzianum* (T1 vs. T2; Fig. 2, Table 2), pods and grains of plants inoculated with *T. viride* (T3 *vs.* T4; Fig. 2, Table 2), or leaves and pods of plants inoculated with *T. asperellum* (T5 *vs.* T6; Fig. 2, Table 2). However, there was a significant effect associated with the increase in fungal spores on Pb concentration in the pods and grains of plants inoculated with *T. harzianum* (T1 *vs.* T2; Fig. 2, Table 2), leaves of plants inoculated with *T. viride* (T3 *vs.* T4; Fig. 2, Table 2), and grains of plants inoculated with *T. asperellum* (T5 *vs.* T6; Fig. 2, Table 2), showing lower concentrations of Pb in the structures of plants inoculated with 3×10^6 *vs* 2×10^6 spores ml^{-1}. In addition, there was no significant effect of increasing the number of fungal spores on the Cd concentration of leaves from plants inoculated with *T. harzianum, T. viride* and *T. asperellum,* or pods and grains from plants inoculated with *T. viride* and *T. asperellum* (Fig. 2, Table 2), although the increase in the concentration of fungal spores showed a significantly higher concentration of Cd in pods and grains from plants inoculated with 3×10^6 *vs* 2×10^6 spores ml^{-1} of *T. harzianum* (Fig. 2, Table 2). A similar pattern was observed for Cu, with no significant effect of fungal spore concentration in the leaves of plants inoculated with *T. harzianum* and *T. asperellum*, as well as pods of plants inoculated with the former fungal species,

and a significantly higher concentration of Cu in the structures of plants inoculated with 3×10^6 *vs* 2×10^6 spores ml^{-1} such as: pods and grains for *T. harzianum* inoculation; leaves, pods, and grains for *T. viride* inoculation; and grains for *T. asperellum* inoculation (Fig. 2, Table 2). A more variable pattern of the effect of the fungal spore concentration was found for Cr, with no significant effect on leaves from plants inoculated with *T. harzianum* (T1 and T2) and *T. viride* (T3 and T4), as well as for pods from plants inoculated with *T. harzianum* (T1 and T2), but with a significant effect on pods and grains of T1 *vs* T2, grains of T3 *vs* T4, and the three evaluated plant structures of T5 *vs* T6 (Fig. 2, Table 2). Nevertheless, in this case, inoculation with 3×10^6 spores ml^{-1} resulted in an increase (pods from plants inoculated with *T. harzianum* and *T. asperellum,* and grains from plants inoculated with *T. asperellum*) and a decrease (grains from plants inoculated with *T. harzianum* and *T. viride,* and leaves from plants inoculated with *T. asperellum*) in Cr concentration in plant structures.

The treatments with lowest concentrations of heavy metals in bean leaves were the combined treatments T7 (12 mg kg^{-1}) and T8 (3.91 mg kg^{-1}) for Cd and Pb, respectively (Fig. 2), and T4 (7.42 mg kg^{-1}) and T2 (0 mg kg^{-1}) for Cu and Cr (Fig. 2). On thother hand, for bean pods the lowest concentration was observed in the treatment with *T. harzianum* at 3×10^6 spores ml^{-1} (T1; 30.08 mg kg^{-1}) for Pb, the combined *T. harzianum* + *T. asperellum* treatment for Cd (T8; 11.08 mg kg^{-1}), *T viride* at 2×10^6 spores ml^{-1} (T4; 5.42 mg kg^{-1}) for Cu, and *T. harzianum* at 2×10^6 spores ml^{-1} (T2; 1.41 mg kg^{-1}). Finally, T1, T8, T4, and T2 treatments showed the maximum decrease in the concentration of Pb (38.50 mg kg^{-1}), Cd (11.83 mg kg^{-1}), Cu (6.50 mg kg^{-1}) and Cr (12.16 mg kg^{-1}) in bean grains, respectively (Fig. 2).

Discussion

A growing number of studies have indicated that plant endophytic fungi possess high metal tolerance and alleviation capabilities in heavy metal-contaminated soils (Lin et al., 2020; Hao et al., 2021; Priyadarshini et al., 2021; Devi et al., 2022). According to this, the results of this study indicate that inoculation of beans with different species of the fungus *Trichoderma* effectively diminishes the concentrations of Pb, Cr, Cu, and Cr in plant tissues, since in general, the plants of the experimental treatments had significantly lower concentration (47–70%) of the heavy metals than the plants in the control treatment. This is consistent with the results reported by Kredrics et al. (2001), who demonstrated that some strains of *Trichoderma* can effectively bind heavy metals, thereby reducing their toxicity to the environment.

Colonization of plants by endophytic fungi is key to the beneficial effects of these microorganisms on their host plants (Yan et al., 2019; Poveda et al., 2021). The colonization of roots by *Trichoderma* spp. was higher than 75% in all experimental treatments, indicating the successful colonization of roots. Importantly, no significant differences were observed among the experimental treatments; thus, the variation in the effects on the concentration of heavy metals among some experimental treatments could not be related to root colonization by the endophytic fungus. High root colonization by *Trichoderma* spp. under experimental conditions has been reported in several plants, including *Arabidopsis thaliana, Brassica napus, Solanum lycopersicum,* and *Capsicum annum* (Morán-Diez et al., 2012; Poveda, 2021; Sánchez-Cruz et al., 2021), and under natural conditions, *Trichoderma* spp. are ubiquitous soil fungi commonly found in

a great diversity of plant roots (Woo et al., 2023). The ability of *Trichoderma* to colonize plant roots may be linked to its capacity to tolerate various secondary metabolites present in the roots, facilitated by a KELCH-type protein (Poveda et al., 2019), and it takes some time for plants to undergo extensive genetic reprogramming in response to colonization, which likely allows the fungus to establish successful root colonization (Morán-Diez et al., 2012). Other factors that can explain the variation in the response of bean plants to the experimental treatments tested in the current study include the dynamics of heavy metals in the soil solution, the fungal species identity, variations in cellular and molecular mechanisms for tolerance to different heavy metals, and differences in metal translocation to plant structures which are discussed below (Morán-Diez et al., 2012; Haydee and Dalma, 2017; Azouzi et al., 2019; Priyadarshini et al., 2021).

Dynamics of heavy metals in soil

The mobility of heavy metals is influenced by various factors in the soil-water system, which in turn affect their toxicity and bioaccumulation in plants. These effects are particularly notable when there are changes in the pH, ionic composition, or redox conditions. The pH affects the solubility of heavy metals, with decreased solubility at higher pH levels (Antoniadis et al., 2017; Priyadarshini et al., 2021). In the current study, it is likely that pH (7.7 ± 0.4) did not significantly affect the solubility of the metals studied. However, the low organic matter content of the soil ($1.73 \pm 0.1\%$), which plays a role in metal absorption and complexation, could be involved (Sheoran, Sheoran, and Poonia, 2016; Azouzi et al., 2019). Dissolved organic matter can reduce the adsorption of heavy metals onto solid soil components, either by competing for free metal ions through the formation of soluble organometallic complexes or by adsorbing to the soil instead of metals, thereby reducing the capacity of the soil to retain metal ions (Haydee and Dalma, 2017). On the other hand, the cation exchange capacity in the studied soil was determined to be $27.86 \, \text{cmol} \, \text{kg}^{-1}$, indicating it is a loamy clay soil. Soils with high clay content might retain higher concentrations of cations than sandy soils (Antoniadis et al., 2017).

Effect of Trichoderma spp. on the accumulation of heavy metals

All the *Trichoderma* spp. used in this study reduced the concentration of heavy metals in bean plant structures, but the response in some cases was heavy metal specific and showed variation among plant structures. In general, *T. harziarium* was the fungal species that showed the highest reduction of concentration of Pb, Cr, Cu for leaves, pods, and grains, whereas the treatments that combined two *Trichoderma* spp. showed a higher reduction of Cd in the bean plants evaluated. These results indicated differences in the specificity of heavy metals among *Trichoderma* spp. Specificity and maximum tolerance limit for metals vary greatly at the genus and species levels (Priyadarshini et al., 2021; Devi et al., 2022). These differences can occur even among strains isolated from the same locality because of their different strategies to tolerate metals (Jacob, Bardhan, and Raj Mohan, 2013). For example, Cadavid-Velásquez et al. (2019) found that the fungus *Polyporus* spp. had a higher concentration of Cu ($27.54 \, \text{mg} \, \text{kg}^{-1}$) compared to other studied fungi belonging to 18 genera. *Rhizophagus intraradices* show a significant decrease in Cd content (Li et al., 2016; Parmar et al., 2022). *Penicillium*

chrysogenum and *Trichoderma viride* have been reported to tolerate Cr up to a concentration of $600 \, \text{mg} \, \text{l}^{-1}$, compared to *A. flavus* and other strains of *Fusarium* sp. and *Penicillium* sp., which can tolerate up to 400 and $100 \, \text{mg} \, \text{l}^{-1}$ of Cr, respectively (Iram et al., 2012). In this sense, some *Trichoderma* spp. are known as Pb- and Cd-tolerant fungi species (Ting and Choong, 2009; Sun et al., 2020) with a capacity for Cu removal of up to 84.6% at pH levels ranging from 5 to 8 (Kumar and Dwivedi, 2021), and have the ability to absorb high concentrations of Cr at specific pH (Narolkar and Mishra, 2022).

In general, four intracellular and extracellular strategies for metal tolerance are recognized by microorganisms: (1) biosorption (2) bioaccumulation and compartmentalization (3) metal chelation, and (4) efflux transport for metal exclusion (Priyadarshini et al., 2021). Among these, *T. harzianum* has proven to be tolerant to Cd by employing a superficial biosorption mechanism that does not involve metabolic processes. Uptake occurs through physical and chemical pathways or through metabolically mediated routes. This mechanism involves the binding of negatively charged groups in various biopolymers that make up the cell wall independent of metabolism (Ting and Choong, 2009). Indeed, it has been observed that fungi capable of capturing higher concentrations of heavy metals develop abundant mycelial masses, particularly in the top 5 cm of soil, enabling extensive contact with the soil (Allen, 1991). Hence, as the fungal cell wall plays a crucial role because it contains functional chemical groups such as chitin, proteins, phenolic polymers, melanins, and methionines which have an affinity for the uptake of heavy metals (Ayangbenro and Babaloba, 2017), even small differences in the chemical composition of cell wall among fungal species can result in differences of metal biosorption (Priyadarshini et al., 2021).

Most of the available information focuses on the individual properties of the fungus itself, and there is limited knowledge regarding the specific effects of inoculating microorganisms on the reduction of heavy metal bioaccumulation in plants. It has been observed that some heavy metals, such as Pb and Cd, are distributed unevenly in different parts of the plant (Lara-Viveros et al., 2015; Abdelgawad et al., 2020; Sassykova et al., 2020; Rasool et al., 2021). Specifically, some studies with grasses and mung beans have reported decreased translocation as the heavy metal moves away from the roots, leading to a higher concentration of Pb in leaves than in grains (Rasool et al., 2021). Nonetheless, previous studies on bean plants reported the opposite pattern for Pb (De Temmerman et al., 2015; Govarthanan et al., 2018) and higher concentrations of Cd in leaves than in grains; however, the high concentration of Cd in bean leaves was related to atmospheric deposition rather than translocation from the soil (De Temmerman et al., 2015). Similarly, the results of this study only indicated an uneven distribution of Pb in bean plants, with a higher concentration in the grains, followed by pods and leaves, suggesting that bean plants show a consistent pattern of Pb translocation from the soil that differs from the Pb translocation pattern of other studied plants. Nonetheless, besides the plant species effect and even cultivar effect, other factors such as solubility and the presence of chelating agents and transporters also play a significant role in the uptake of heavy metals (Sharma, Dietz, and Mimura, 2016). In addition, many plant species possess genes that encode proteins responsible for transporting metals to different plant structures (Tian et al., 2021). ATPase proteins also use energy derived from ATP hydrolysis to transport cations, such as Pb, throughout the plant (Williams and Mills, 2005; Haque et al., 2022). Therefore, further research is needed

to elucidate the specific mechanisms involved in heavy metal translocation to plant tissues.

Health implications of heavy metal accumulation in soil and bean plants

In line with the World Health Organization (WHO, 1996), the maximum allowable limits for Cd, Pb, Cr, and Cu in agricultural soils are 0.8, 85, 10, and 36 mg kg^{-1}, respectively. It is evident from the results of this study that the concentrations of Pb and Cd in soil exceeded these limits, indicating a significant health risk to the local population. A previous study at the Mezquital Valley reported maximum permissible limits for Pb and Cd in soil as 3.5 and 3.7 mg kg^{-1}, respectively, considering factors such as human body weight, assimilation rate, and daily food consumption by the population in this region (Vázquez-Alarcón et al., 2005). Based on this, the studied soil should not be used for agricultural purposes.

In the case of heavy metals accumulated in plant tissues, it is important to note that bean grain, which is consumed by humans, is the plant structure with the highest concentrations of some heavy metals, especially Pb. Notably, both the control group and the treatment with *T. harzianum* and *T. asperellum* showed the highest values of Pb concentration (113.58 and 76 mg kg^{-1}, respectively), while the treatment with *T. harzianum* at 3×10^6 spores ml^{-1} had the lowest Pb concentration (38.5 mg kg^{-1}). These findings are particularly important because of the potential health risks associated with Pb ingestion such as kidney failure, liver damage, mental retardation, hearing impairment, and pregnancy complications (Chen et al., 2021; Bazié et al., 2022). According to the World Health Organization (WHO, 1996), the maximum allowable levels of Pb in plants, particularly in grains, are 2.0 and 0.2 mg kg^{-1}, respectively. However, even the lowest Pb concentrations obtained in this study exceeded these limits by more than two orders of magnitude. This also applies for Cd concentration in bean grains (11.83–14.66 mg kg^{-1}), since the maximum permissible limit for Cd in legumes is 0.1 mg kg^{-1} based on the Codex Alimentarius, and Cr with high concentrations found in grains (12.16–25.75 mg kg^{-1}). Cr, in particular, is classified as a highly carcinogenic substance when it exceeds the recommended limit of 1.30 mg kg^{-1} by the WHO and the Food and Agriculture Organization of the United Nations (Paweena et al., 2022). Finally, Cu is essential for plant growth, but excessive levels in plant tissues can deactivate enzymes involved in important metabolic processes, such as photosynthesis and cellular respiration (Schmitt et al., 2022). According to the World Health Organization (WHO, 1996), the maximum permissible limit for Cu in plant tissues is 10 mg kg^{-1}. In the human body, excess Cu can lead to liver damage or even death, with daily intake exceeding 0.9 mg. In this study, the control group exhibited a Cu content of 26.5 mg kg^{-1} in the grains, surpassing the permitted limit. However, with the treatment of *T. viride*, *T. harzianum* and *T. asperellum* at 3×10^6 spores ml^{-1}, the Cu content in this structure was below permissible limits.

Conclusions

In light of the limited information available on the reduction of heavy metals through the inoculation of microorganisms in cultivated plants, the findings of the current study revealed that selected *Trichoderma* strains have the ability to decrease the concentrations of heavy metals in agriculturally important crops. The

results demonstrated that the levels of Pb, Cd, Cu, and Cr in the edible part of the bean plants, namely the grain, were effectively reduced when the plants were inoculated with *Trichoderma*, although the concentrations of heavy metals were still above the permissible limits for human consumption. The observed high concentrations of heavy metals in the studied soil might explain these results, emphasizing the need for further research using not only new isolates but also different spore concentrations. The risk to human health is minimized by reducing the metal content of the cultivated species. Therefore, investigating the inoculation of *Trichoderma* in plant species with high consumption rates will provide a deeper understanding of the mechanisms involved in fungus-plant interactions, ultimately leading to improved efficiency.

References

Abbade, E.B. and Dewes, H. (2014) 'Brazilian dry-beans and food security in developing countries', *Journal of Agribusiness in Developing and Emerging Economies*, 4, pp. 115–32. doi: 10.1108/JADEE-06-2012-0015

Abdelgawad, H., Zinta, G., Hamed, B.A., Selim, S., Beemster, G., Hozzein, W.N., Wadaan, M.A.M., Asard, H. and Abuelsoud, W. (2020) 'Maize roots and shoots show distinct profiles of oxidative stress and antioxidant defense under heavy metal toxicity', *Environmental Pollution*, 258, pp. 113705. doi: 10.1016/j.envpol.2019.113705

Aldoobie, N.F. and Beltagi, M.S. (2013) 'Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris* L.) plants to heavy metals stress', *African Journal of Biotechnology*, 12, pp. 4614–22. doi: /10.5897/AJB2013.12387

Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R. and Wang, M.Q. (2021) 'Heavy metals and pesticides toxicity in agricultural soil and plants: ecological risks and human health implications', *Toxics*, 9, pp. 42. doi: 10.3390/toxics9030042

Allen, M.F. (1991) *The ecology of mycorrhizae*. Cambridge, UK: Cambridge University Press. 200 pp.

Amin, N.U., Hussain, A., Alamzeb, S. and Begum, S. (2013) 'Accumulation of heavy metals in edible parts of vegetables irrigated with waste water and their daily intake to adults and children, District Mardan, Pakistan', *Food Chemistry*, 136, pp. 1515–23. doi: 10.1016/j.foodchem.2012.09.058

Anand, U., Pal, T., Yadav, N., Singh, V.K., Tripathi, V., Choudhary, K.K., Shukla, A.K., Sunita, K., Kumar, A., Bontempi, E., Ma, Y., Kolton, M. and Singh, A.K. (2023) 'Current scenario and future prospects of endophytic microbes: promising candidates for abiotic and biotic stress management for agricultural and environmental sustainability', *Microbial Ecology*, 86, pp. 1455–86. doi: 10.1007/s00248-023-02190-1

Antoniadis, V., Levizou, E., Shaheen, M.S., Ok, Y.S., Sebastian, A., Baum, C., Prasad, N.V.M., Wenzel, W.W. and Rinklebe, J. (2017) 'Trace elements in the soil-plant interface: phytoavailability, translocation, and phytoremediation a review', *Earth Science Reviews*, 172, pp. 621–45. doi: 10.1016/j.earscirev.2017.06.005

Audet, P. and Charest, C. (2007) 'Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation: meta-analytical and conceptual perspectives', *Environmental Pollution*, 147, pp. 609–14. doi: 10.1016/j.envpol.2006.10.006

Ayangbenro, A.S. and Babalola, O.O. (2017) 'A new strategy for heavy metal polluted environments: a review of microbial biosorbents', *International Journal of Environment Research and Public Health*, 14, pp. 94. doi: 10.3390/ijerph14010094

Azouzi, R., Charef, A., Ayed, L. and Khadhar, S. (2019) 'Effect of water quality on heavy metal re-distribution and mobility in polluted agricultural soils in a semi-arid region', *Pedosphere*, 29, pp. 730–9. doi: 10.1016/S1002-0160(17)60367-9

Babu, A.G., Shim, J., Bang, K., Shea, P.J. and Oh, B. (2014) '*Trichoderma virens* PDR-28: a heavy metal-tolerant and plant growth-promoting fungus

for remediation and bioenergy crop production on mine tailing soil', *Journal of Environmental Management*, **132**, pp. 129–34. doi: 10.1016/j.jenvman.2013.10.009

Bazié, B.S.R., Compaoré, M.K.A., Bandé, M., Kpoda, S.D., Méda, N.R., Kangambega, T.M.O., Ilboudo, I., Sandwidi, B.Y., Nikiema, F., Yakoro, A., Bassolé, I.H.N., Hien, H. and Kabré, E. (2022) 'Evaluation of metallic trace elements contents in some major raw foodstuffs in Burkina Faso and health risk assessment', *Scientific Reports*, **12**, pp. 4460. doi: 10.1038/s41598-022-08470-z

Cadavid-Velásquez, J., del Socorro, Pérez-Vásquez, N. and Marrugo-Negrete, J. (2019) 'Contaminación por metales pesados en la bahía Cispatá en Córdoba-Colombia y su bioacumulación en macromicetos', *Gestión y Ambiente*, **22**, pp. 43–53. doi: 10.15446/ga.v22n1.76380

Cannata, M.G., Bertoli, A.C., Carvalho, R., Augusto, A.S., Bastos, A.R.R., Freitas, M.P. and Carvalho, J.G. (2015) 'Stress induced by heavy metals Cd and Pb in bean (*Phaseolus vulgaris* L.) grown in nutrient solution', *Journal of Plant Nutrition*, **38**, pp. 497–508. doi: 10.1080/01904167.2014.934476

Chávez, M. and Sánchez, E. (2017) 'Bioactives compounds from Mexican varieties of the common bean (*Phaseolus vulgaris*): implications for health', *Molecules*, **22**, pp. 1360. doi: 10.3390/molecules22081360

Chávez-Servia, J.L., Heredia-García, E., Mayek-Pérez, N., Aquino-Bolaños, E.N., Hernández-Delgado, S., Carrillo-Rodríguez, J.C., Hill-Langarica, H.R. and Vera-Guzmán, A.M. (2016) 'Diversity of common bean (Phaseolus vulgaris) landraces and the nutritional value of their grains' in Goyal A.K. (ed.) *Grain legumes*. Rijeka: IntechOpen, pp. 1–33.

Chen, J., Wang, N., Yuan, Y., Zhang, W., Xia, F.Z., Chen, B., Dong, R.H. and Lu, Y.L. (2021) 'Blood lead, nutrient intake, and renal function among type 2 diabetic patients', *Environmental Science of Pollution Resources*, **28**, pp. 49063–73. doi: 10.1007/s11356-021-13623-0

Chugh, M., Kumar, L., Bhardwaj, D. and Bharadvaja, N. (2022) 'Bioaccumulation and detoxification of heavy metals: An insight into the mechanism' in Maulin, P.S., Rodriguez-Couto, S. and Riti, T.K. (eds) *Development in wastewater treatment research and processes*. Amsterdam: Elsevier, pp. 243–64.

De Temmerman, L., Waegeneers, N., Ruttens, A. and Vandermeiren, K. (2015) 'Accumulation of atmospheric deposition of As, Cd and Pb by bush bean plants', *Environmental Pollution*, **199**, pp. 83–8. doi: 10.1016/j.envpol.2015.01.014

Deng, J., Fu, D., Hu, W., Lu, X., Wu, Y. and Bryan, H. (2020) 'Physiological responses and accumulation ability of *Microcystis aeruginosa* to zinc and cadmium: implications for bioremediation of heavy metal pollution', *Bioresources Technology*, **303**, pp. 122963. doi: 10.1016/j.biortech.2020.122963

Devi, R., Behera, B., Raza, M.B., Mangal, V., Altaf, M.A., Kumar, R., Kumar, A., Tiwari, R.K., Lal, M.K. and Singh, B. (2022) 'An insight into microbes mediated heavy metal detoxification in plants: a review', *Journal of Soil Science and Plant Nutrition*, **22**, pp. 914–36. doi: 10.1007/s42729-021-00702-x

Dhingra, O.D. and Sinclair, J.B. (1981) *Basic plant pathology methods*. Boca Ratón, USA: CRC Press. 448 pp.

Eltlbany, N., Baklawa, M., Ding, G.C., Nassal, D., Weber, N., Kandeler, E., Weber, N., Kandeler, E., Neumann, G., Ludewig, U., Van Overbeek, L. and Small, A.K. (2019) 'Enhanced tomato plant growth in soil under reduced P supply through microbial inoculants and microbiome shifts', *FEMS Microbiology Ecology*, **95**, pp. 124. doi: 10.1093/femsec/fiz124

Food and Agriculture Organization of the United Nations. (1997) *FAOSTAT statistical database*. Rome: FAO.

Friedel, J.K., Langer, T., Siebe, C. and Stahr, K. (2000) 'Effects of long-term waste water irrigation on soil organic matter, soil microbial biomass and its activities in central Mexico', *Biology and Fertility of Soils*, **31**, pp. 414–21. doi: 10.1007/s003749900188

Gálvez, A. and Salinas, G. (2015) 'El papel del frijol en la salud nutrimental de la población mexicana', Revista Digital Universitaria 16. Available at http://www.revista.unam.mx/vol.16/num2/art12/index.html

García-Salazar, E.M. (2019) 'Wastewater as generator of the space of the agricultural activity in the Mezquital Valley, Hidalgo, Mexico. Estudios

Sociales', *Revista de Alimentación Contemporánea y Desarrollo Regional*, **29**, pp. e19741. doi: 10.24836/es.v29i54.741

Gjorgieva, D., Kadifkova, P.T., Ruskovska, T., Bačeva, K. and Stafilov, T. (2013) 'Mineral nutrient imbalance, total antioxidants level and DNA damage in common bean (*Phaseolus vulgaris* L.) exposed to heavy metals', *Physiology and Molecular Biology of Plants*, **19**, pp. 499–507. doi: 10.1007/s12298-013-0196-0

Govarthanan, M., Mythili, R., Selvankumar, T., Kamala-Kannan, S. and Kim, H. (2018) 'Myco-phytoremediation of arsenic- and lead-contaminated soils by *Helianthus annuus* and wood rot fungi, *Trichoderma* sp. isolated from decayed wood', *Ecotoxicology and Environmental Safety*, **151**, pp. 279–84. doi: 10.1016/j.ecoenv.2018.01.020

Guédron, S., Duwig, C., Prado, B.L., Point, D., Flores, M.G. and Siebe, C. (2014) '(Methyl) mercury, arsenic, and lead contamination of the world's largest wastewater irrigation system: the Mezquital Valley (Hidalgo State—Mexico)', *Water, Air & Soil Pollution*, **225**, pp. 1–19. doi: 10.1007/s11270-014-2045-3

Hammami, H., Parsa, M., Bayat, H. and Aminifard, M.H. (2022) 'The behavior of heavy metals in relation to their influence on the common bean (*Phaseolus vulgaris*) symbiosis', *Environmental and Experimental Botany*, **193**, pp. 104670. doi: 10.1016/j.envexpbot.2021.104670

Hao, L., Zhang, Z., Hao, B., Diao, F., Zhang, J., Bao, Z. and Guo, W. (2021) 'Arbuscular mycorrhizal fungi alter microbiome structure of rhizosphere soil to enhance maize tolerance to La', *Ecotoxicology and Environmental Safety*, **212**, pp. 111996. doi: 10.1016/j.ecoenv.2021.111996

Haque, A.F.M.M., Gohari, G., El-Shehawi, A.M., Dutta, A.K., Elseehy, M.M. and Kabir, A.H. (2022) 'Genome-wide identification, characterization and expression profiles of heavy metal ATPase 3 (HMA3) in plants', *Journal of King Saud University*, **34**, pp. 101730. doi: 10.1016/j.jksus.2021.101730

Haydee, K.M. and Dalma, K.E. (2017) 'Concerning organometallic compounds in environment: Occurrence, fate, and impact' in Rahman, M.M. and Asiri, A.M. (eds) *Recent progress in organometallic chemistry*, Rijeka: IntechOpen, pp. 47–67.

Hernández, I.O., Kampfner, O., Rodríguez, F. and Meneses, J. (2017) 'Uso múltiple del agua en las cuencas del Valle de México y Río Tula', *Revista de Ingeniería Civil*, **1**, pp. 1–11.

Hildebrandt, U., Regvar, M. and Bothe, H. (2007) 'Arbuscular mycorrhiza and heavy metal tolerance', *Phytochemistry*, **68**, pp. 139–46. doi: 10.1016/j.phytochem.2006.09.023

Hou, D., O'Connor, D., Igalavithana, A.D., Alessi, D.S., Luo, J., Tsang, D.C., Sparks, D.L., Yamauchi, Y., Rinklebe, J. and Ok, Y.S. (2020) 'Metal contamination and bioremediation of agricultural soils for food safety and sustainability', *Nature Reviews Earth & Environment*, **1**, pp. 366–81. doi: 10.1038/s43017-020-0061-y

Iram, S., Parveen, K., Usman, J., Nasir, K., Akhtar, N., Arouj, S. and Ahmad, I. (2012) 'Heavy metal tolerance of filamentous fungal strains isolated from soil irrigated with industrial wastewater', *Biologija*, **58**, pp. 107–16. doi: 10.6001/biologija.v58i3.2527

Jacob, J.M., Bardhan, S.K. and Raj Mohan, B. (2013) 'Selenium and lead tolerance in fungi isolated from sea water', *International Journal of Innovative Research in Science', Engineering and Technology*, **2**, pp. 2975–82.

Jain, A. and Taylor, R.W. (2023) 'Determination of cation exchange capacity of calcareous soils: comparison of summation method and direct replacement method', *Communications in Soil Science and Plant Analysis*, **54**, pp. 743–8. doi: 10.1080/00103624.2022.2118765

Kacprzak, M.J., Rosikon, K., Fijalkowski, K. and Grobelak, A. (2014) 'The effect of *Trichoderma* on heavy metal mobility and uptake by *Miscanthus giganteus*, *Salix* sp., *Phalaris arundinacea*, and *Panicum virgatum*', *Applied and Environmental Soil Sciences*, **2014**, pp. 506142. doi: 10.1155/2014/506142

Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I. and Dumat, C. (2017) 'A comparison of technologies for remediation of heavy metal contaminated soils', *Journal of Geochemical Exploration*, **182**, pp. 247–68. doi: 10.1016/j.gexplo.2016.11.021

Khan, I., Ali, M., Aftab, M., Shakir, S., Qayyum, S., Haleem, K.S. and Tauseef, I. (2019) 'Mycoremediation: a treatment for heavy metal-polluted soil using indigenous metallotolerant fungi', *Environmental Monitoring and Assessment*, **191**, pp. 622. doi: 10.1007/s10661-019-7781-9

Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z. and Zhu, Y.G. (2008) 'Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China', *Environmental Pollution*, **152**, pp. 686–92. doi: 10.1016/j.envpol.2007.06.056

Kredrics, L., Dóczi, I., Antal, Z. and Manczinger, L. (2001) 'Effect of heavy metals on growth and extracellular enzyme activities of mycoparasitic *Trichoderma strains*', *Bulletin of Environmental Contamination and Toxicology*, **66**, pp. 249–54. doi: 10.1007/s0012800231

Kumar, V. and Dwivedi, S.K. (2021) 'Bioremediation mechanism and potential of copper by actively growing fungus *Trichoderma lixii* CR700 isolated from electroplating wastewater', *Journal Environmental Management*, **27**, pp. 111370. doi: 10.1016/j.jenvman.2020.111370

Landero-Valenzuela, N., Lara-Viveros, F.M., Rodríguez-Ortega, A., Pérez-Vite, A. and Ortíz-Hernández, A. (2019) '*Trichoderma* posible parásito de *Sporisorium reilianum* y su influencia en el rendimiento del maíz', *Entreciencias Diálogos en la Sociedad del Conocimiento*, **7**, pp. 13–23. doi: 10.22201/enesl.20078064e.2019.20.67345

Lara-Viveros, F.M., Ventura-Maza, A., Muhammad, E., Rodríguez-Ortega, A., Vargas-Monter, J. and Landero-Valenzuela, N. (2015) 'Contenido de Cd y Pb en suelo y planta en diferentes cultivos irrigados con aguas residuales en el Valle del Mezquital', *Revista Internacional de Contaminación Ambiental*, **32**, pp. 127–32.

Li, H., Luo, N., Zhang, L.J., Zhao, H.M., Li, Y.W., Cai, Q.Y., Wong, M.H. and Mo, C.H. (2016) 'Do arbuscular mycorrhizal fungi affect cadmium uptake kinetics, subcellular distribution and chemical forms in rice?', *Science of Total Environmental*, **571**, pp. 1183–90. doi: 10.1016/j.scitotenv.2016.07.124

Lin, Y., Xiao, W., Ye, Y., Wu, C., Hu, Y. and Shi, H. (2020) 'Adaptation of soil fungi to heavy metal contamination in paddy fields: a case study in eastern China', *Environmental Science and Pollution Research*, **27**, pp. 27819–30. doi: 10.1007/s11356-020-09049-9

Maldaner, J., Steffen, G.P.K., Missio, E.L., Saldanha, C.W., de Morais, R.M. and Nicoloso, F.T. (2021) 'Tolerance of Trichoderma isolates to increasing concentrations of heavy metals', *International Journal of Environmental Studies*, **78**, pp. 185–97. doi: 10.1080/00207233.2020.1778290

Mesele, T., Dibaba, K., Garbaba, C.A. and Mendesil, E. (2022) 'Effectiveness of different storage structures for the management of Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) on stored common bean, *Phaseolus vulgaris* L. (Fabaceae)', *Journal of Stored Products Research*, **96**, pp. 101928. doi: 10.1016/j.jspr.2022.101928

Morán-Diez, E., Rubio, B., Domínguez, S., Hermosa, R., Monte, E. and Nicolás, C. (2012) 'Transcriptomic response of *Arabidopsis thaliana* after 24 h incubation with the biocontrol fungus *Trichoderma harzianum*', *Journal of Plant Physiology*, **169**, pp. 614–20. doi: 10.1016/j.jplph.2011.12.016

Mwendwa, S. (2022) 'Revisiting soil texture analysis: practices towards a more accurate Bouyoucos method', *Heliyon*, **8**, pp. e09395. doi: 10.1016/j.heliyon.2022.e09395

Narolkar, S. and Mishra, A. (2022) 'Biosorption of chromium by fungal strains isolated from industrial effluent contaminated area', *Pollution*, **8**, pp. 159–68. doi: 10.22059/POLL.2021.326818.1132

Norma Mexicana NMX-AA-132-SCFI (2006) *Muestreo de suelo para identificación y la cuantificación de metales y metaloides, y manejo de la muestra*. México: Secretaría de Medio Ambiente y Recursos Naturales. Available at https://dof.gob.mx/nota_detalle.php?codigo=5475373&fecha=06/03/2017#gsc.tab=0 (Accessed 11 June 2023).

Norma Oficial Mexicana NOM-021-RECNAT (2000) *Especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreos y análisis*. México: Secretaría de Medio Ambiente y Recursos Naturales. Available at https://www.sinec.gob.mx/SINEC/Vista/Normalizacion/DetalleNorma.xhtml?pidn=N2lrSU9jS05YUm5oc2Y3L2FLdmxSZz09 (Accessed 11 June 2023).

Organization for Economic Cooperation and Development. (2019) *Safety assessment of food and feeds derived from transgenic crops. Volume 3: Common bean, rice, cowpea and apple compositional considerations*. Paris, France: OECD Publishing.

O'Sullivan, C.A., Bonnett, G.D., McIntyre, C.L., Hochman, Z. and Wasson, A.P. (2019) 'Strategies to improve the productivity, product diversity and profitability of urban agriculture', *Agricultural Systems*, **174**, pp. 133–44. doi: 10.1016/j.agsy.2019.05.007

Parmar, S., Daki, S., Bhattacharya, S. and Shrivastav, A. (2022) 'Microorganism: An ecofriendly tool for waste management and environmental safety in development' in Shah, M.P., Rodriguez-Couto, S. and Thapar, R.K. (eds) *Wastewater treatment research and processes*. Amsterdam: Elsevier, pp. 175–93.

Paweena, A., Ramnaree, N., Piriyaporn, T., Sutha, K. and Phitsanu, T. (2022) 'Carcinogenic risk of Pb, Cd, Ni, and Cr and critical ecological risk of Cd and Cu in soil and groundwater around the municipal solid waste open dump in Central Thailand', *Journal of Environmental Public Health*, **2022**, pp. 3062215.

Petry, N., Boy, E., Wirth, J.P. and Hurrell, R.F. (2015) 'Review: the potential of the common bean (*Phaseolus vulgaris*) as a vehicle for iron biofortification', *Nutrients*, **7**, pp. 1144–73. doi: 10.3390/nu7021144

Plant, J.A. and Raiswell, R. (1983) 'Principles of environmental geochemistry' in Thornton, I. (ed.) *Applied environmental geochemistry*. San Diego: Academic Press, pp. 1–39.

Poveda, J. (2021) 'Glucosinolates profile of Arabidopsis thaliana modified root colonization of Trichoderma species', *Biological Control*, **155**, pp. 104522. doi: 10.1016/j.biocontrol.2020.104522

Poveda, J., Eugui, D., Abril-Urías, P. and Velasco, P. (2021) 'Endophytic fungi as direct plant growth promoters for sustainable agricultural production', *Symbiosis*, **85**, pp. 1–19. doi: 10.1016/j.micres.2017.02.001

Poveda, J., Hermosa, R., Monte, E. and Nicolás, C. (2019) 'The *Trichoderma harzianum* Kelch protein ThKEL1 plays a key role in root colonization and the induction of systemic defense in Brassicaceae plants', *Frontiers in Plant Science*, **10**(1478), pp. 2–14. doi: 10.3389/fpls.2019.01478

Priyadarshini, E., Priyadarshini, S.S., Cousins, B.G. and Pradhan, N. (2021) 'Metal-fungus interaction: review on cellular processes underlying heavy metal detoxification and synthesis of metal nanoparticles', *Chemosphere*, **274**, pp. 129976. doi: 10.1016/j.chemosphere.2021.129976

Racić, G., Vukelić, I., Kordić, B., Radić, D., Lazović, M., Nešić, L. and Panković, D. (2023) 'Screening of native Trichoderma species for nickel and copper bioremediation potential determined by FTIR and XRF', *Microorganisms*, **11**, pp. 815. doi: 10.3390/microorganisms11030815

Rascio, N. and Navari-Izzo, F. (2011) 'Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting?', *Plant Science*, **180**, pp. 169–81. doi: 10.1016/j.plantsci.2010.08.016

Rasool, B., Ur-Rahman, M., Adnan Ramzani, P.M., Zubair, M., Khan, M.A., Lewińska, K., Turan, V., Karczewska, A., Khan, S.A., Farhad, M., Tauqeer, H.M. and Iqbal, M. (2021) 'Impacts of oxalic acid-activated phosphate rock and root induced changes on Pb bioavailability in the rhizosphere and its the distribution in mung bean plant', *Environmental Pollution*, **280**, pp. 116903. doi: 10.1016/j.envpol.2021.116903

Rattan, R.K., Datta, S.P., Chhonkar, P.K., Suribabu, K. and Singh, A.K. (2005) 'Longterm impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater: a case study', *Agriculture, Ecosystems and Environment*, **109**, pp. 310–22. doi: doi:10.1016/j.agee.2005.02.025

Samuels, G.J., Chaverri, P., Farr, D.F. and McCray, E.B. (2009) *Trichoderma online. Systematic mycology and microbiology laboratory*, ARS, USDA. Available at http://nt.arsgrin.gov/taxadescriptions/keys/TrichodermaIndex.cfm (Accessed 11 June 2023).

Sánchez-Cruz, R., Mehta, R., Atriztán-Hernández, K., Martínez-Villamil, O., del Rayo Sánchez-Carbente, M., Sánchez-Reyes, A., Lira Ruan, V., González-Chávez, C.A., Tabche-Barrera, M.L., Bárcenas-Rodríguez, R.C., Batista-García, R.A., Herrera-Estrella, A., Balcázar-López, E. and Folch-Mallol, J.L. (2021) 'Effects on *Capsicum annuum* plants colonized with *Trichoderma atroviride* P. karst strains genetically modified in Taswo1, a gene coding for a protein with Expansin-like activity', *Plants*, **10**, pp. 1919. doi: 10.3390/plants10091919

Sassykova, L.R., Aubakirov, Y.A., Akhmetkaliyeva, M.S., Sassykova, A.R., Sendilvelan, S., Prabhahar, M., Prakash, S., Tashmukhambetova, Z.K., Abildin, T.S. and Zhussupova, A.K. (2020) 'Heavy metal accumulation in plants of the dry-steppe zone of the East Kazakhstan region', *Materials Today: Proceedings*, **33**, pp. 1187–91. doi: 10.1016/j.matpr.2020.07.660

Schmitt, O.J., Andriolo, J.L., Silva, I.C.B., Tiecher, T.L., Chassot, T., Tarouco, C.P., Lourenzi, C.R., Nicoloso, F.T., Marchezan, C., Casagrande, C.R., Drescher, G.L., Kreutz, M.A. and Brunettom, G. (2022) 'Physiological responses of beet and cabbage plants exposed to copper and their potential insertion in human food chain', *Environmental Sciences and Pollution Research*, **29**, pp. 44186–98. doi: 10.1007/s11356-022-18892-x

Servicio de Información Agroalimentaria y Pesca (SIAP) (2020) *Avance de siembra y cosechas, resumen por estado*. México: SIAP. Available at http://infosiap.siap.gob.mx:8080/agricola_siap_gobmx/ResumenProducto.do (Accessed 7 April 2023).

Sharma, R.K., Agrawal, M. and Marshall, F. (2007) 'Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India', *Ecotoxicology and Environmental Safety*, **66**, pp. 258–66. doi: 10.1016/j.ecoenv.2005.11.007

Sharma, S.S., Dietz, K.J. and Mimura, T. (2016) 'Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants', *Plant Cell Environment*, **39**, pp. 1112–26. doi: 10.1111/pce.12706

Sheoran, V., Sheoran, A.S. and Poonia, P. (2016) 'Factors affecting phytoextraction: a review', *Pedosphere*, **26**, pp. 148–66. doi: 10.1016/S1002-0160(15)60032-7

Sun, X., Sun, M., Chao, Y., Wang, H., Pan, H., Yang, Q., Cui, X., Lou, Y. and Zhuge, Y. (2020) 'Alleviation of lead toxicity and phytostimulation in perennial ryegrass by the Pb-resistant fungus *Trichoderma asperellum* SD-5', *Functional Plant Biology*, **48**, pp. 333–41. doi: 10.1071/FP20237

Sun, S., Zhang, H., Luo, Y., Guo, C., Ma, X., Fan, J., Chen, J. and Geng, N. (2022) 'Occurrence, accumulation, and health risks of heavy metals in Chinese market baskets', *Science of the Total Environment*, **829**, pp. 154597. doi: 10.1016/j.scitotenv.2022.154597

Talukdar, D., Jasrotia, T., Sharma, R., Jaglan, S., Kumar, R., Vats, R., Kumar, R., Mahnashi, M.H. and Umar, A. (2020) 'Evaluation of novel indigenous fungal consortium for enhanced bioremediation of heavy metals from contaminated sites', *Environmental Technology Innovation*, **20**, pp. 101050. doi: 10.1016/j.eti.2020.101050

Tian, W., He, G., Qin, L., Li, D., Meng, L., Huang, Y. and He, T. (2021) 'Genome-wide analysis of the NRAMP gene family in potato (*Solanum tuberosum*): identification, expression analysis and response to five heavy metals stress', *Ecotoxicology and Environmental Safety*, **208**, pp. 111661. doi: 10.1016/j.ecoenv.2020.111661

Ting, A.S.Y. and Choong, C.C. (2009) 'Bioaccumulation and biosorption efficacy of *Trichoderma* isolates SP2F1 in removing copper (Cu II) from aqueous solutions', *World Journal of Microbiology and Biotechnology*, **25**, pp. 1431–7. doi: 10.1007/s11274-009-0030-6

Vázquez-Alarcón, A., Cajuste, J., Carrillo, R., Zamudio, B. and Álvarez, E.C.Z. (2005) 'Límites permisibles de acumulación de cadmio, níquel y plomo en suelos del Valle del Mezquital, Hidalgo', *Terra Latinoamericana*, **23**, pp. 447–55. Available at http://www.redalyc.org/articulo.oa?id=57311146003

Verma, S. and Kuila, A. (2019) 'Bioremediation of heavy metals by microbial process', *Environmental Technology & Innovation*, **14**, pp. 100369. doi: 10.1016/j.eti.2019.100369

Vukelić, I.D., Racić, G.M., Bojović, M.M., Ćurčić, N.Z., Mrkajić, D.Z., Jovanović, L.B. and Panković, D.M. (2020) 'Effect of *Trichoderma harzianum* on morpho-physiological parameters and metal uptake on tomato plants', *Journal of Natural Sciences*, **139**, pp. 61–71. doi: 10.2298/ZMSPN2039061V

Williams, L.E. and Mills, R.F. (2005) 'P1B-ATPases: an ancient family of transition metal pumps with diverse functions in plants', *Trends in Plant Science*, **10**, pp. 491–502. doi: 10.1016/j.tplants.2005.08.008

Woo, S.L., Hermosa, R., Lorito, M. and Monte, E. (2023) '*Trichoderma*: a multipurpose, plant-beneficial microorganism for eco-sustainable agriculture', *Nature Reviews Microbiology*, **21**, pp. 312–26. doi: 10.1038/s41579-022-00819-5

World Health Organization (1996) *Permissible limits of heavy metals in soil and plants*. Geneva, Switzerland: World Health Organization. Available at https://www.omicsonline.org/articles-images/2161-0525-5-334- (Accessed 11 June 2023).

Yaashikaa, P.R., Kumar, P.S., Jeevanantham, S. and Saravanan, R. (2022) 'A review on bioremediation approach for heavy metal detoxification and accumulation in plants', *Environmental Pollution*, **301**, pp. 119035. doi: 10.1016/j.envpol.2022.119035

Yan, L., Zhu, J., Zhao, X., Shi, J., Jiang, C. and Shao, D. (2019) 'Beneficial effects of endophytic fungi colonization on plants', *Applied Microbiology and Biotechnology*, **103**, pp. 3327–40. doi: 10.1007/s00253-019-09713-2

Yan, S., Wu, Y., Fan, J., Zhang, F., Paw U, K.T., Zheng, J., Qiang, S., Guo, J., Zou, H., Xiang, Y. and Wu, L. (2020) 'A sustainable strategy of managing irrigation based on water productivity and residual soil nitrate in a no-tillage maize system', *Journal of Cleaner Production*, **262**, pp. 121279. doi: 10.1016/j.jclepro.2020.121279

Ziegler-Rivera, F., Prado, B., Mora, L., Ponce de León-Hill, C. and Siebe, C. (2022) 'Mobilization of arsenic and heavy metals in polluted soil due to changes in the soil-water management system'. Available at SSRN: https://ssrn.com/abstract=4043400