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Research Article

Soil properties and bioavailability of heavy metals in soils of orange orchards in north-eastern Mexico

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Abstract

Soil pollution by heavy metals has increased due to anthropogenic activities, particularly agriculture, where intensive agrochemical use contributes to contaminant accumulation. Evaluating metal bioavailability is essential to understand potential risks in cultivated soils. This study aimed to assess the presence of heavy metals (Cu, Cd, Cr and Pb) in orange orchard soils by analysing their bioavailable concentrations and the soil physicochemical properties influencing their bioavailability. An exploratory soil sampling was conducted, collecting 60 soil samples from six orchards in the Municipality of Hidalgo. Samples were processed using diethylenetriaminepentaacetic acid extraction and quantified by Flame Atomic Absorption Spectrophotometry. Six soil physicochemical properties were also evaluated. Descriptive statistical parameters and Spearman's correlation analysis were applied to assess the relationship between variables. The bioavailable concentrations of HMs followed the order Cu > Cr > Pb > Cd, with ranges of 1.1–3.2, 0.1–0.8, 0.2–0.4 and 0.04–0.2 mg/kg, respectively. Significant correlations were observed between particle density and bulk density, porosity, pH and electrical conductivity and the bioavailable concentrations, suggesting a significant association between these properties and heavy metal bioavailability. These findings demonstrate that soil physicochemical properties strongly influence metal mobility and highlight the need for continuous monitoring in agricultural areas. Changes in management practices could alter these properties and increase metal bioavailability, potentially raising toxicological risks.

Key words: Agrochemicals, *Citrus sinensis*, contamination, DTPA, environmental risk, potentially toxic elements

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Introduction

Environmental contamination by heavy metals (HMs) is a growing global concern due to their increasing concentrations reported in environmental compartments, such as soils, water and plant tissues. Although these elements occur naturally, anthropogenic activities, such as mining, industrial processes and agriculture, have significantly elevated their levels (Adimalla et al. 2019). Agriculture represents one of the major long-term sources of soil HM

contamination, due to the intensive use of agrochemicals (Quinteros et al. 2016). In particular, inputs such as phosphate fertilisers, fungicides such as copper sulphate and zinc oxide, herbicides such as glyphosate and 2,4-D and insecticides such as cypermethrin and fipronil can contribute to the accumulation of metals such as Cu, Cr, Pb, Cd and Zn. These products contain organic and inorganic compounds with varying degrees of persistence and toxicity, frequently including HMs (Rashid et al. 2023).

The continuous application of agrochemicals, combined with the limited implementation of good agricultural practices such as crop rotation, promotes the retention and accumulation of heavy metals in the soil due to their low degradability and high persistence (Salem et al. 2020). This problem is particularly critical in perennial cropping systems, where repeated inputs over time favour their gradual accumulation in soil (Andreeva et al. 2025). This is because these contaminants can persist in the soil for hundreds of years, even after their application has ceased, reaching toxic concentrations and, consequently, rendering the soils unsuitable for agricultural use (Andrade et al. 2020).

The mobility and bioavailability of HMs in soil are dynamic processes regulated by the chemical characteristics of each element, the physicochemical properties of the soil and prevailing climatic conditions (Ye et al. 2022). The concentration and mobility of HMs are governed by soil characteristics such as pH, texture, organic matter, redox potential and moisture, which regulate their solubility and bioavailability (Kim et al. 2015; Martínez et al. 2017; Salem et al. 2020). Once in a bioavailable form, HMs can be absorbed by plant roots and translocated to stems, leaves and fruits, resulting in contamination of agricultural products (Xiang et al. 2021). It is through this soil-plant transfer process that agricultural products become contaminated, posing a risk to organisms consuming them via the food chain (Zhang et al. 2018). This poses a risk to human health, as ingestion of contaminated food represents one of the primary exposure pathways to HMs (Zhang et al. 2018; Cantoral et al. 2024). Numerous studies have shown that consuming food contaminated with HMs can lead to a broad range of adverse health effects, ranging from mild intoxication and neurological or muscular impairment to chronic diseases, such as chronic kidney disease (CKD), Parkinson's disease, muscular dystrophy, cancer and even death (Londoño-Franco et al. 2016; Calderon et al. 2023).

In the State of Tamaulipas, agriculture is one of the most important economic sectors, with predominant crops, such as sorghum, sugarcane, lemons and oranges (Rinconada-Carbajal et al. 2021). Orange cultivation is particularly relevant due to its economic and social importance at both regional and national levels. Mexico is one of the world's leading citrus producers and exporters and, in 2023, it ranked as the fourth-largest producer of oranges globally. In this context, Tamaulipas was the second-largest producing State with an estimated output of 600,000 tonnes, representing approximately 13% of national production of this crop (SIAP 2024). The objective of this study was to evaluate the presence of heavy metals Cu, Cd, Cr and Pb in orange orchard soil by determining their bioavailable concentrations and assessing the soil physicochemical properties that influence their bioavailability.

Materials and methods

Study area

The Municipality of Hidalgo is located in the central region of the State of Tamaulipas, Mexico, at coordinates 24°01'19.2"N, 99°14'30.8"W. The area has an elevation of 329 m a.s.l. and is characterised by a semi-arid subhumid climate with summer rainfall. The average annual temperature is 24 °C, and annual precipitation ranges from 800 to 1,000 mm (INEGI 2021). In this area, Valencia Orange (*Citrus sinensis*) is the most important crop, followed by Italian lemon (*Citrus limon*), grapefruit (*Citrus paradisi*) and Mandarin (*Citrus reticulata*). The main citrus-producing zone in the Municipality is Santa Engracia, with 5,940 ha of cultivated land. Along with the municipalities of Güémez, Padilla, Llera and Ciudad Victoria, Hidalgo forms the citrus-producing zone of Tamaulipas (SIACON 2023).

Agronomic management of the orchards varied amongst sites due to differences in production cycles and specific technical requirements, resulting in diverse management strategies for orange cultivation, strongly influenced by experience, soil characteristics and the specific needs of each orchard. Agronomic practices and agrochemical use during the five years prior to sampling were documented, including agrochemical inputs and management practices (Appendix 1). However, reconstructing the complete history is limited due to the long productive lifespan of the orchards, changes in ownership and a lack of systematic record-keeping.

Soil sampling and sample conditioning

Exploratory sampling was conducted during the summer of 2024 in six orange orchards (*C. sinensis*), following a simple random sampling design in accordance with the Mexican standard NMX-AA-132-SCFI-2016 to evaluate HM concentrations in the soil (SE 2016). The location of the soil sampling sites in the study area is shown in Fig. 1. At each site, 10 composite surface-soil samples were collected. Approximately 1 kg of soil was obtained per sample using a stainless-steel shovel previously decontaminated with deionised water and dried between samplings to avoid cross-contamination. Samples were placed into pre-labelled polyethylene bags and transported to the Laboratory of Soil Sciences of the Faculty of Engineering and Sciences (FIC) of the Autonomous University of Tamaulipas.

In the laboratory, soil samples were air-dried at room temperature for 72 h and sieved through a 2 mm mesh to remove plant residues, roots and large aggregates. This conditioning process improved sample homogenisation and minimised potential chemical alterations prior to subsequent analyses (SE 2016).

Determination of heavy metals

Bioavailable assessment of Cu, Cd, Cr and Pb was determined according to the AS-14 method of NOM-021-RECNAT-2000, which employs DTPA (diethylenetriaminepentaacetic acid) as a chelating agent. This method extracts the fraction of HMs that are bioavailable for plant uptake and associated with organic matter (SEMARNAT 2002). The extraction solution consisted of 0.005 mol DTPA, 0.1 mol triethanolamine (TEA) and 0.01 mol calcium chloride ($CaCl_2$), adjusting the final pH to 7.3 with 6 mol HCl (Lindsay and Norvell 1978). For each sample (including blanks), 10 g of air-dried and sieved soil was placed into a 125 ml Erlenmeyer flask and

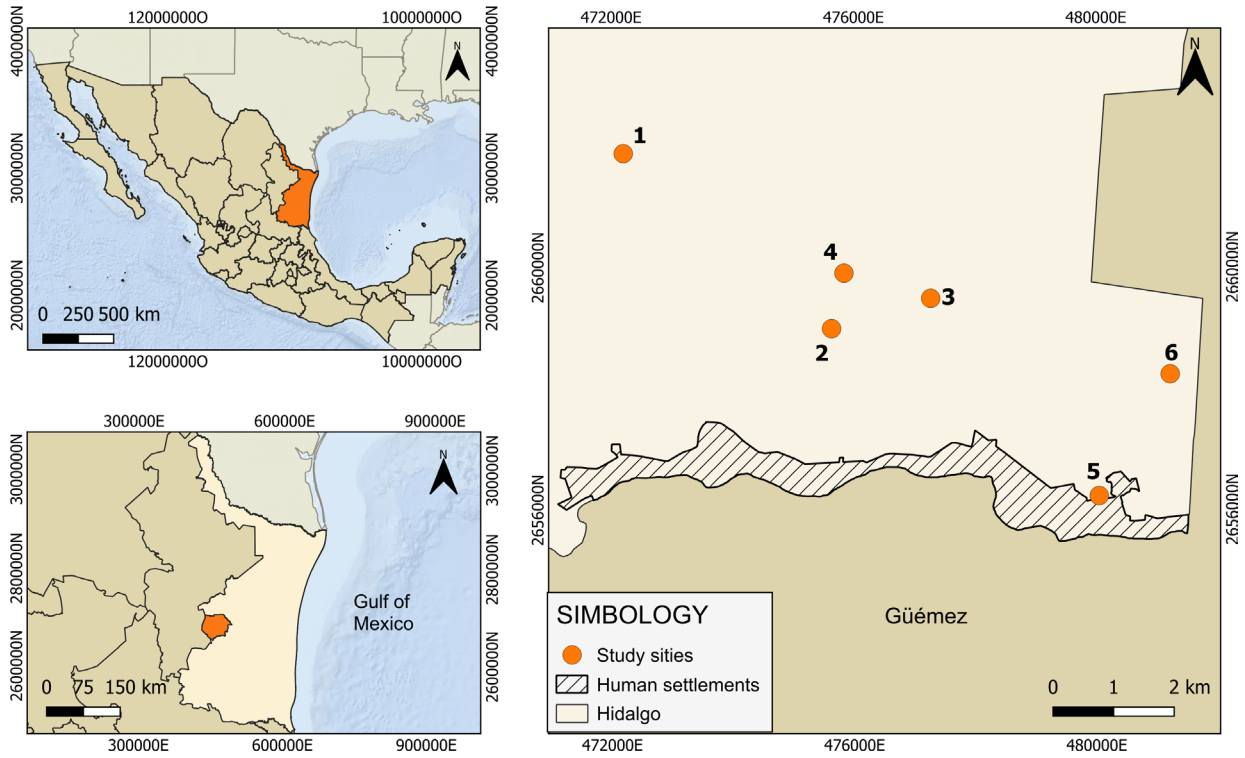

Figure 1. Location map of soil sampling sites in the study area.

mixed with 20 ml of DTPA solution. The samples were agitated at 120 rpm for 2 h. The suspension was then filtered through Whatman No. 42 filter paper and the HMs concentrations in the extracts were quantified using Flame Atomic Absorption Spectroscopy (PerkinElmer model PinAAcle 900H, USA), following the operating conditions specified in appendix B of NOM-147-SEMARNAT/SSA1-2004.

Physicochemical analysis of the soil

The physicochemical characterisation of the soil was conducted by evaluating six properties, following the method described in NOM-021-RECNAT-2000, which established technical specifications for soil sampling and analysis (SEMARNAT 2002). Soil texture was determined using the AS-09 method, which uses the Bouyoucos hydrometer (Bouyoucos 1927). Bulk density was determined by using the cylinder method, while particle density was determined by pycnometers. Soil porosity was measured from bulk and particle density values. Soil pH was measured using method AS-02 with a soil-to-water suspension and a potentiometer model A211 (Orion Star model A211, Thermo Scientific USA). Electrical conductivity (EC) was measured in the saturation extract using the AS-18 method with a conductivity meter (Orion 160, Thermo Scientific USA).

Data analysis

Statistical analyses were conducted using R version 4.4.1 (R Core Team 2024). Descriptive statistics were calculated to evaluate the variability and distribution of HM concentrations. Relationships between HMs and soil physicochemical properties were analysed using Spearman's correlation coefficient with a 95% confidence level ($P < 0.05$).

Results

Physicochemical properties of the soil

The physicochemical properties of the studied soils are presented in Table 1. The soils showed variability in textural classification: sites 1, 5 and 6 exhibited clay textures; site 2 was classified as loam; site 4 as silty clay loam; and site 3 as clay loam. Bulk density varied amongst sites, with the lowest values recorded at sites 1 and 6, averaging 1.15 g cm^{-3} and 1.13 g cm^{-3}, respectively. The highest bulk density occurred at site 2 (average 1.42 g cm^{-3}). Particle density also varied across sites with the lowest value in site 1 (2.09 g cm^{-3}) and the highest in site 6 (2.23 g cm^{-3}). These differences in particle density were related to variations in mineralogical composition, compaction degree and soil porosity. Regarding pH, all sites were moderately alkaline, ranging from 7.3 to 8.3. Electrical conductivity (EC) was lowest in site 6, averaging 0.31 dS/m and highest in site 2, with an average of 0.61 dS/m.

Heavy metal bioavailability

The descriptive analysis of bioavailable HM concentrations (Table 2) showed variability amongst sites, following the order Cu > Cr > Pb > Cd. This variability and the elevated concentration at some sites reflect spatial heterogeneity across the study area. The coefficients of variation (CV) ranged from 3% to 63%, indicating moderate variability. The highest CV values were recorded for Cu at site 6 (63%) and site 3 (25%), for Pb at site 6 (28%), for Cr at site 2 (31%) and for Cd at site 1 (37%) and site 2 (34%). Comparative analysis of mean HM concentrations (Cu > Cr > Pb > Cd) revealed significant variability across locations (Fig. 2). The highest concentrations were found at site 3 for Cd (0.26 mg/kg) and Pb (0.44 mg/kg), and at site 5 for Cu (3.20 mg/kg) and Cr (0.83 mg/kg). In contrast, the lowest concentrations were observed at site 6 for Cu (1.16 mg/kg) and Pb (0.20 mg/kg), at site 2 for Cd (0.04 mg/kg) and at site 4 for Cr (0.15 mg/kg).

Correlation analysis

Spearman's correlation analysis ($p < 0.05$) indicated significant correlations between HM concentrations and soil physicochemical properties (Table 3). Soil porosity showed a negative correlation with Cu (r = -0.47) and Pb (r = -0.39) and a moderate positive correlation with Cr (r = 0.63). Bulk density exhibited a moderate positive correlation with Cu (r = 0.54) and a moderate negative correlation with Cr (r = -0.51). Particle density showed a moderate negative

Table 1. Physicochemical characterisation of the soils.

Site	Texture	Por	BD	PD	pH	EC
1	Clayey	44.96	1.15	2.09	8	0.50
2	Loam	35.14	1.42	2.19	7.9	0.61
3	Clay loam	38.42	1.34	2.17	8.3	0.38
4	Silty clay loam	39.45	1.30	2.15	8.2	0.38
5	Clayey	41.42	1.30	2.22	7.5	0.35
6	Clayey	49.24	1.13	2.23	8.3	0.31

‡ Por = Porosity (%), BD = Bulk density (g cm^{-3}), PD = Particle density (g cm^{-3}), EC = Electric conductivity (dS/m).

Table 2. Heavy metal contents in soils from orange orchards.

Element	Values	Site					
		1	2	3	4	5	6
	Mean	1.65	1.97	3.09	2.02	3.20	1.16
	Median	1.65	1.98	3.19	1.98	3.13	0.93
Cu	Minimum	1.52	1.84	0.95	1.67	2.85	0.87
	Maximum	1.82	2.06	3.74	2.61	3.82	3.25
	SD	0.11	0.06	0.79	0.32	0.27	0.73
	CV %	6.67	3.05	25.71	15.92	8.53	63.29
	Mean	0.07	0.04	0.26	0.20	0.18	0.19
	Median	0.07	0.04	0.26	0.24	0.19	0.19
Cd	Minimum	0.03	0.02	0.20	0.18	0.15	0.17
	Maximum	0.10	0.06	0.29	0.22	0.20	0.20
	SD	0.03	0.01	0.03	0.01	0.02	0.01
	CV %	37.14	34.21	10.67	6.68	11.78	5.37
	Mean	0.40	0.341	0.44	0.42	0.44	0.20
	Median	0.40	0.341	0.46	0.41	0.46	0.19
Pb	Minimum	0.38	0.312	0.27	0.38	0.27	0.16
	Maximum	0.44	0.374	0.51	0.46	0.51	0.36
	SD	0.02	0.020	0.07	0.03	0.07	0.06
	CV %	5.00	5.784	15.34	6.42	15.28	28.87
	Mean	0.38	0.22	0.26	0.15	0.83	0.77
	Median	0.39	0.21	0.27	0.14	0.84	0.76
Cr	Minimum	0.32	0.14	0.20	0.12	0.53	0.69
	Maximum	0.42	0.32	0.29	0.19	1.06	0.86
	SD	0.03	0.07	0.03	0.02	0.17	0.06
	CV %	8.68	31.09	10.71	15.24	20.74	7.51

‡SD = Standard deviation; CV = Coefficient of variation. Concentrations are reported in mg/kg.

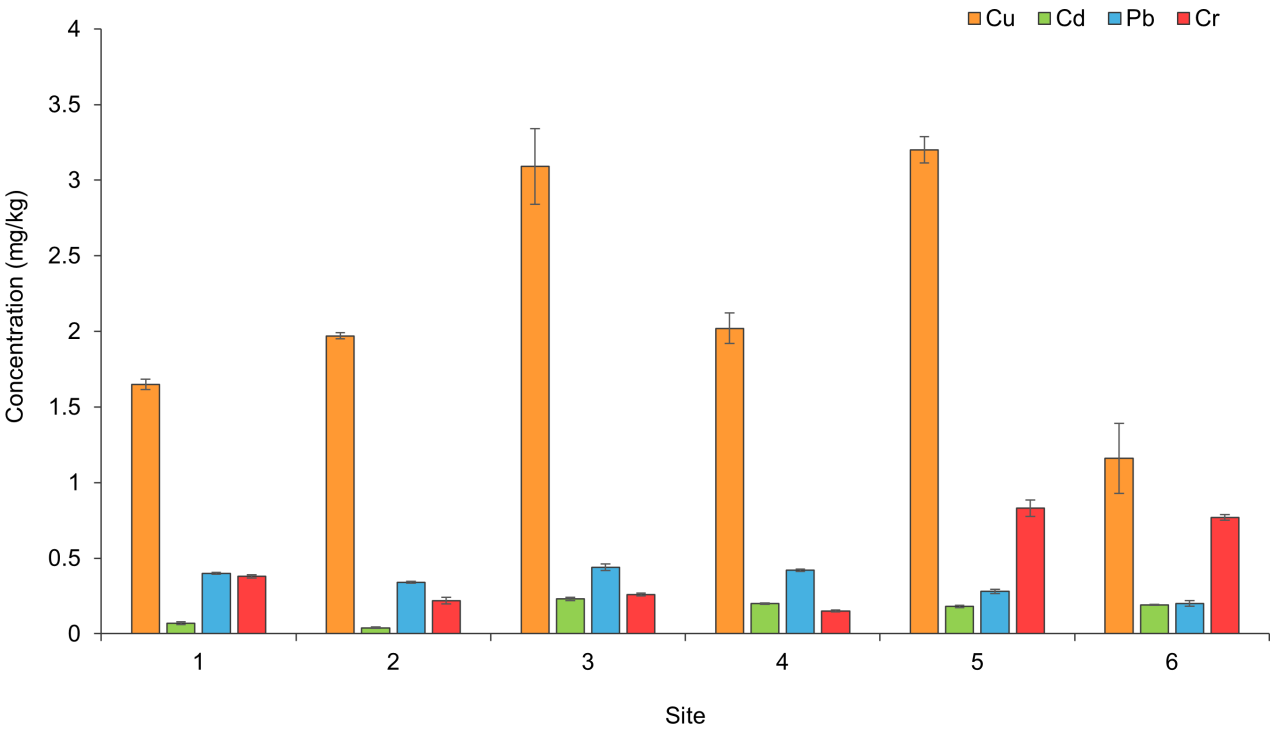

Figure 2. Average bioavailable concentrations of heavy metals by site.

Table 3. Correlation matrix of HMs and soil physicochemical properties.

	POR	BD	PD	pH	EC	Cu	Cd	Pb	Cr
POR	1	-0.9*	0.26*	-0.08	-0.45*	-0.47*	-0.006	-0.39*	0.63*
BD		1	0.11	-0.02	0.44*	0.54*	-0.03	0.23	-0.51*
PD			1	-0.14	-0.28*	0.11	0.06	-0.50*	0.32*
pH				1	-0.12	-0.27*	0.29*	0.27*	-0.3*
EC					1	0.07	-0.55*	0.25*	-0.37*
Cu						1	0.29*	0.2	-0.09
Cd							1	0.25	-0.079
Pb								1	-0.65*
Cr									1

‡POR = Porosity, BD = Bulk density, PD = Particle density, EC = Electric conductivity.

* Correlation is significant at the 0.05 level.

correlation with Pb (r = -0.50), but a positive correlation with Cr (r = 0.32). Soil pH was the only property that presented a significant correlation with all HMs, exhibiting weak negative correlations for the elements Cu (r = -0.27) and Cr (r = -0.3) and weak positive correlations with Cd (r = 0.45) and Pb (r = 0.27). EC was negatively correlated with Cd (r = -0.55) and Cr (r = -0.21). Additionally, positive correlations were observed between bioavailable concentrations of Cu and Cd (r = 0.29), while Cr and Pb showed a negative correlation (r = -0.65).

Discussion

Physicochemical characterisations of soil

Soil texture is a key property influencing the retention, mobility and bioavailability of HMs; it has been demonstrated that the accumulation of HMs in soils can significantly modify this property (Angon et al. 2024). In particular, clay soils exhibit a high retention capacity for HMs, mainly due to their high cation exchange capacity (CEC), which enables them to retain large amounts of cations (Pérez et al. 1995). Moreover, the fine soil fraction (clay) has a greater ionic absorption capacity than the coarse fraction (sand), enhancing HM retention and reducing their bioavailability (Rodríguez-Eugenio et al. 2019). Loamy soils contain a balanced mixture of sand, silt and clay and generally present relatively higher CEC values, which improves their ability to retain HMs (Rubio et al. 2024). According to the values established in Mexican standard NOM-021-RECNAT-2000 (SEMARNAT 2002), the bulk density (BD) values recorded at the study sites are consistent with those expected for soils of similar texture. The pH values at all study sites were classified as strongly alkaline. Previous studies have shown that soil pH plays a fundamental role in HM mobilisation and dissolution processes (Martínez et al. 2017; Zhang et al. 2018). Furthermore, the low electrical conductivity (EC) values observed across the study sites fall within the category of negligible salinity effects, indicating a low concentration of soluble salt. This condition favours the mobility and bioavailability of HMs by reducing ionic competition at absorption sites. Similar results were reported by Salem et al. (2020) for agricultural soils, where low EC values were associated with low salt content in irrigation water and higher leaching rates facilitated by sand-textured soil.

Bioavailable heavy metal concentrations

The coefficient of variation (CV) is an important statistical indicator for assessing spatial variability in HM concentrations and identifying potential anthropogenic influences. A CV ≤ 10% indicates low variability, whereas values between 10% and 100% suggest moderate variability (Adimalla et al. 2019). In the present study, CV values ranged from low to moderate across sites, indicating a heterogeneous spatial distribution influenced by multiple factors. The concentration of the four HMs varied considerably amongst sites. Ungureanu et al. (2016) reported that HMs under natural conditions generally exhibit low CV values, whereas higher CVs are often associated with anthropogenic inputs. In this context, the elevated CVs observed in the present study suggest that bioavailable HM concentrations at certain sites are influenced by anthropogenic activities. In particular, the high CV values for Cu recorded at site 6 are consistent with findings by Tun-Canto et al. (2017) in intensive agricultural systems, where HM concentration was linked to excessive fertilisers and agrochemical use. Copper exhibited the highest bioavailable concentration across all sites, suggesting a common contamination source. Previous studies have demonstrated that continuous agrochemical application promotes HM release and accumulation in agricultural soils (Tun-Canto et al. 2017; Anggraeni et al. 2024). A wide range of fungicides and insecticides currently in use contain significant levels of Cu in their formulations, including Cu-based fungicides such as copper oxychloride, copper hydroxide and copper sulphate, as well as some insecticides, such as cypermethrin, fipronil and methomyl which may contain Cu in trace amounts as impurities (Rashid et al. 2023). Although the intensive use of Cu-based fungicides has been crucial in controlling fungal pathogens (Alloway 2013), their prolonged use has resulted in Cu accumulation in soils, consistent with the management practices reported for the evaluated orchards. Although the bioavailable concentrations of HMs in this study did not exceed the maximum permissible limits (MPLs) established by NOM-147-SEMARNAT/SSA1-2004 for agricultural soils, it is important to note that current regulations are based solely on the evaluation of total HM concentrations and do not consider bioavailable fractions, which represent the fraction of metals that is potentially available for plant uptake (Violante et al. 2010). These fractions are, therefore, more relevant for assessing actual environmental and toxicological risks (Abuzaid and Bassouny 2019). As these fractions can be readily taken up by plants, they may enter the food chain through soil–plant transfer, representing a potential risk to both the environment and human health (Yang et al. 2018; Shehata et al. 2019). Therefore, risk assessments, based solely on total concentrations, may overestimate the actual hazards associated with these contaminants.

Relationship between soil physicochemical properties and bioavailable HM content

Soil porosity exhibited a negative correlation with Cu and Pb, indicating that increasing porosity reduces the bioavailability of these elements. In contrast, a positive correlation was observed with Cr, suggesting an association of high porosity and Cr bioavailability. These results highlight the influence of soil structure on HM mobility, generally due to effects on retention and transport processes

within the soil matrix. Bulk density was positively correlated with Cu, indicating that an increase in bulk density is associated with an increase in Cu bioavailability. Conversely, the negative correlation with Cr indicates that, in the study area, as bulk density decreases, Cr bioavailability tends to increase. This behaviour is attributed to greater soil compaction, which reduces pore space and affects HM mobility (Salamanca-Jiménez et al. 2004). These findings are consistent with those reported by Chaudhari et al. (2013), who observed a significant negative correlation between BD and metal bioavailability. A negative relationship was also found between PD and Pb, while a positive relationship was observed with Cr, indicating that PD influences changes in bioavailable concentrations of these elements and that higher PD increases Cr bioavailability.

The negative correlation between soil pH and the bioavailable concentration of Cu and Cr suggests that soil acidification reduces the absorption of these elements at clay cation exchange sites, increasing their mobility and bioavailability. These results are consistent with previous studies that reported similar patterns between soil pH and the bioavailable contents of these metals (Zhang et al. 2018; Fernandez-Ochoa et al. 2022), underscoring the critical role of pH in HM absorption-desorption processes. In contrast, positive correlations were observed between pH and the concentrations of Pb and Cd, indicating that under alkaline conditions, their bioavailability increases. This trend is consistent with findings by Albujar (2023) for Pb, where pH was shown to affect absorption strength, increasing solubility and bioavailability (Albujar et al. 2023). This behaviour is explained by the formation of Pb-organic and Pb-hydroxide complexes in alkaline soils (Steinnes 2013). For Cd, Arévalo-Gardini et al. (2016) reported that its mobility and bioavailability are mainly controlled by pH, supporting the findings of this study. Regarding EC, negative correlations were observed with Cd and Cr, indicating that higher EC is associated with lower bioavailability of these elements. According to Kabata-Pendias and Pendias (1985), increasing HM concentrations in surface soils are associated with salinity, which promotes complex formation that controls the solubility of elements. Conversely, a positive correlation was observed between EC and Pb, suggesting that increased soil salinity enhances lead mobility, possibly due to ionic competition and the formation of soluble complexes. However, this result differs from that reported by Yan et al. (2019), who found a negative correlation. This discrepancy may be attributed to the interaction of Pb with other physicochemical properties of the soil.

Significant correlations were also observed amongst the bioavailable concentrations of HMs. The positive correlation between Cu and Cd suggests a common contamination source (Chabukdhara et al. 2015; Adimalla et al. 2019), whereas the negative correlation between Cr and Pb indicates that these elements may derive from different sources or undergo competitive processes that affect their bioavailability in soil.

Conclusions

This study provided valuable information on the status and bioavailability of heavy metals in orange cultivation soils from agricultural lands in the Municipality of Hidalgo, Tamaulipas in north-eastern Mexico. The results indicated that, although the bioavailable concentrations of Cu, Cr, Cd and Pb in the study area did not exceed the maximum permissible limits (MPLs) established by

NOM-147-SEMARNAT/SSA1-2004 for agricultural soils, it is essential to assess the presence of these HMs based on their bioavailable fraction since an evaluation, based exclusively on total concentrations, may overestimate the real environmental risk. Statistical analyses revealed that soil physicochemical properties were significantly associated with bioavailability of the HMs. Factors such as bulk density, particle density, porosity, pH and EC were found to be associated with the bioavailability, with soil pH emerging as a critical factor influencing all four metals. These findings highlight the need for continuous soil monitoring in the area to assess changes over time. Furthermore, these results contribute to a better understanding of HM dynamics in perennial crop systems.

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Appendix 1

Table A1. Agronomic practices and agrochemical use in the study sites.

Site	Age (Years)	Fertilisation	Fungicide	Herbicide	Insecticide	Practice (Weeding)
1	3	Urea	Not applied	Glyphosate and 2,4-D	Malathion	No
2	40	Urea	Copper sulphate	Not applied	Malathion	Yes
3	50	Ammonium nitrates	Copper sulphate	Glyphosate	Not applied	No
4	25	Ammonium nitrates and Urea	Sulphur	Not applied	Malathion	No
5	70	Ammonium nitrates	Sulphur	Not applied	Bifenthrin	Yes
6	65	NPK	Sulphur and copper sulphate	Glyphosate	Cypermethrin and bifenthrin	No

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text.