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Evaluation of Trace Metal Contamination in Soil and Native Maize From Tehuacán-Cuicatlán Biosphere **Reserve**, Mexico

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ABSTRACT: Trace metal enrichments in soil and native maize (Zea mays L.) in Tehuacán-Cuicatlán Biosphere Reserve was analyzed due to its direct relation with food security. pH and organic matter (OM) content were obtained in soil, concentrations trace metals were determined in agricultural soil and plant collected from 10 maize plots using ICP-OES. Soil contamination was evaluated using contamination factor (CF), enrichment factor (EF), and geoaccumulation index (Igeo), plant contamination was evaluated using bioconcentration factor (BCF). Soils characteristics indicates higher pH values (8-8.9), which favors metal's translocation from soil > plant. Mean concentrations (mg/kg) of Cr (soil: 0.02–0.04; plant: 0–0.01), Fe (soil: 0.77–1.17; plant: 0–0.40), and Zn (soil: 0–0.01; plant: 0–0.01) were within the FAO/WHO and Mexican government soil standards. CF values were classified as "low contamination," however, Cd, Mn, and Fe indicates "medium contamination" in maize crops. EF values of metals in farmlands were recorded as "lower enrichment values." BCF showed accumulation of trace metals (especially Fe and Mn) in roots, which acts as a binding element for other trace metals, where OM is low. Overall results in this study suggest that the selected trace metals in the agricultural soils and plant have no appreciable threat to food safety.

KEYWORDS: Soil contamination, maize, accumulation, natural protected areas

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Introduction

Soil contamination by metals is one of the main limitations of agriculture, affects food security (Rickson et al., 2015). The two main sources of trace metals in the soil are: (1) the unaltered parental material and (2) human activities such as mining, industry, and agriculture (Gao et al., 2023; Vasudhevan et al., 2022). Chemical fertilizers and pesticides in agriculture cause accumulation trace metals in soil, reaching a toxicity limit (Atta et al., 2023). For this reason, studies on contamination by trace metals in agricultural soils have currently more attention in recent years (Hoque et al., 2023; Sudarningsih et al., 2023).

With reference to the plant development, soil contributes to biochemical cycles and as a mean for its growth. However, when trace metals are concentrated in the edible parts of plants, they pose a serious problem for human health (Briffa et al., 2020), by remaining in food chains for long periods of time, causing a high risk of mutagenicity and carcinogenicity (Azizi et al., 2022). The accumulation of metals limits microbial activity, altering the physical and chemical properties of the soil (Angon et al., 2024). High levels of Cr, As, Ni, Pb, and Hg in soils can be absorbed by plants and crops, affecting food quality and safety and agricultural productivity (Rai et al., 2019).

Maize crop (Zea mays L.) is one of the most produced grains in world agriculture. Due to its high content of carbohydrates, CORRESPONDING AUTHOR: Pedro Francisco Rodríguez-Espinosa, Instituto Politecnico Nacional Centro Interdisciplinario de Investigaciones y Estudios sobre Medio Ambiente y Desarrollo, Calle 30 de junio S/N Barrio Laguna Ticoman, Ciudad de México 07340, México. Email: pedrof44@hotmail.com

proteins, iron, vitamin B, and minerals, it is mainly used as food for humans and livestock and as a source of biofuels (Arena et al., 2024).

Globally, research has been carried out to find the main sources of trace metal contamination in soils cultivated with maize (Sun et al., 2023; Tang et al., 2023a; Xiao et al., 2022; Xu et al., 2023), and pesticide residues in soil and maize (Hasnaki et al., 2022). In Mexico, metal contamination of soil grown with maize has been studied, and the patterns of distribution of heavy metals in agricultural soils is reported by Davila et al. (2012), Saha et al. (2023). Human health aspect studies in some of the matices indicate that elevated level of some metals (As, Cd, Pb, Cr, and Ni) are observed in soil samples of Feizabad city in Iran indicating a direct source from external inputs and it also poses potential threat to the human health and its surrounding ecosystem (Taghavi, Bakhshi et al., 2024; Taghavi, Zarei, et al., 2024). Likewise, metals accumulated in fruits are also considered to cause human health effects, where red grapes produced in Gonabad vineyards in Iran is higher in As and Zn than the permissible limit of FAO/WHO values (FAO/WHO 2011; Peirovi-Minaee et al., 2023). Saffron grown in Gonabad, Iran also indicates that the levels of Cd, Fe, and Zn were higher than the FAO/WHO limits and it also indicates possible carcinogenic effect in human health.

Tehuacán-Cuicatlán Biosphere Reserve (RBTC, for its acronym in Spanish), is an interesting area in southeastern



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Figure 1. Map of the study area.

Mexico, where the cultivation of native maize dates back 7,000 years (CONABIO, 2020; Smith et al., 2004). Soil monitoring contributes to the detection of contaminated areas and possibly related to the prevention of soil degradation in areas, where ancestral agricultural practices and the use of native seeds are conserved, and until now, there are no studies that determine the levels of metals in the soil cultivated with native maize (Song et al., 2022).

The main objectives of this research is: (1) to determine the levels of trace metals in soil and in native maize for self-consumption in this region, and (2) to estimate the changes in quality produced by the presence of 17 trace metals (As, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sb, Sr, Ti, Tl, V, and Zn) in the cultivated soils of the RBTC. These results can explain the dynamics of trace metals between the soil and the crop and provide a scenario on the risk of contamination for food security and human health.

Methods

Study area

The study area belongs to the community of Zapotitlán within the RBTC, taking the sampling points on the sub-basin of the Salado River, originating from the east flowing through the Sierra Negra and running as a permanent tributary through the Tehuacán valley, until joining the Río Grande (CONANP, 2013; Figure 1). The average annual temperature is 21°C, with average annual precipitation of 400 to 450 mm. From an ecological perspective, the region is surrounded by a mountain range where the Sierra Madre del Sur, the Trans-Mexican Volcanic Belt and the Sierra Madre Oriental converge. Geologically, the area has shallow soils, stony soils with different levels of alkalinity and salinity, which is influenced by the different geological substrates present at the site (CONANP, 2013; Dávila et al., 2002). The main soil units of the region are: lithosol, leptosol, calcium cambisols, calcisols, calcium xerosols, derived from evaporates from the Lower Middle Cretaceous, which is complemented by regosols. Likewise, calcareous fluvisols are formed from transported materials, derived from alluvial sediments (López et al., 2020; Oliveros-Galindo, 2000; Bolan et al., 2023).

Climatic changes in study area

The RBTC has a history of centuries-old vegetation with complete vegetation cover and is also a window for carbon production (CONANP, 2013).

Its importance lies between the geological, cultural, historical, and landscape richness, comprising 10% to 15% of the species of the Mexica flora, which also represents high biological diversity, of which 365 species (13.5%) are endemic (Dávila et al., 2002). In the last two decades, some of the factors that have affected the conservation of the RBTC is the deficiency in its Natural Protected Area (ANPs for its acronym in Spanish) management program and the distribution of land use (Dávila et al., 2002; Hernández Ortega et al., 2008). The use of land for agriculture, in the case of maize cultivation, is distributed in three different zones, preserving traditional values and focusing on sustainable ecosystem values (CONANP, 2013; Smith et al., 2004). Furthermore, ecological conditions, agricultural activities and soil quality in the region have deteriorated in recent years due to climate change and other external forces such as change in land use (Montesillo-Cedillo, 2016). However, the use and management of resources in the Tehuacán valley is a practice that has allowed human survival in the area. Knowledge and experience in resource management in the region have

added various development activities to flourish in the region. It has also allowed the conservation of biological and cultural diversity among the indigenous community, turning the region into one of the important areas with richness and ethnobotanical knowledge in Mesoamerica (Casas et al., 2014).

Sampling of soil and maize plants

Soil samples were collected from 10 maize growing sites, chosen randomly within the Salado River sub-basin, with similar climatic conditions and soil classification, during October 2022. In each plot, a zig-zag path was made, collecting five samples at 0 to 10 cm depth and a total of 50 samples was collected (Figure 1). All collected samples were mixed and prepared according to the laboratory's standard operating procedures. Soil samples was air-dried in room temperature after removing roots and rocks from the sample. Powdering of soil sample was selected after homogenization (core and quartering method), where 2 mm of pieces was removed to present a composite blend of sample (Glavič-Cindro et al., 2023). The collected samples belong to a Leptosol with clay loam soil, which can be compared based on International Soil Classification System (FAO/WRB, 2015) and INEGI edafological letter E14B75 (INEGI, 2013).

Maize plants were also collected from the same soil sampling sites and a similar zig-zag path was followed for the collection. Three plants were collected from each of the five points to ultimately have a representative sample of each crop plot. The sections of the plant were divided into root (r), stem (s), leaf (l), ear (g), and seedling (w), depending on the growth state of the plant in each plot. They were air dried, under shade to lose moisture until constant weight and pulverized using a mechanical mill prepared for digestion and subsequent analysis.

Analysis of soil and plants

Standard protocols were used to determine the physicochemical parameters of soil such as pH and OM along with macronutrients (Ca and Mg). The pH analysis was measured using distilled water and electrometric evaluation of pH was done. It was measured potentiometrically in the remaining suspension of a 2:1 water/soil ratio mixture (pH, H₂O). The determination of soil OM was carried out through the AS-07 method, by Walkley and Black (SEMARNAT, 2000).

Trace metals As, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sb, Sr, Ti, Tl, V, and Zn was determined using 1.5g of dry and sieved soil sample (Lindsay & Norvell, 1978). Likewise, each section of maize plant was placed inside a sterile conical centrifuge tube and mixed with $HClO_4$ – HNO_3 –HCl–HF acids (all analytical grade) which was digested at 260°C for 3 min. These two distinct samples (soil & plant) were filtered and diluted using HCl (0.1 M) for 50 mL. Concentration of the above trace metals was measured using Inductively Coupled Optic Spectrometry (ICP-OES optima 8300) for both soil and plant samples. Precision of the analysis was done using the certified high-purity standard set with 21 components at $100 \,\mu g \,m L^{-1}$ in 4% HNO₃+Tr HF (NIST SRM 3100) after every eighth sample. The limit of detection covered a range from 163 to 782 nm with a resolution of 0.006 at 200 nm. The accuracy of trace metal analysis was of the range 97% to 99% for the above-mentioned metals. Blank samples were also used in all analytical process.

Evaluating contamination using different methods

Contamination/ determination of metals in agricultural soil and parts of de maize plant (r, s, l, g, and w) was analyzed for geochemical elements (As, Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sb, Sr, Ti, V, and Zn) and was evaluated using contamination factor (CF), enrichment factor (EF), geoaccumulation index (Igeo), and bioconcentration factor (BCF; Lindsay & Norvell, 1978).

Contamination factor (CF). CF indices is used to evaluate the contamination for trace metals reflecting largely anthropogenic input of metals (Ahmed et al., 2016), where the grade of soil contamination is determined using the following equation (Hakanson, 1980; Shaheen et al., 2020):

$$CF = HM_s / HM_b \tag{1}$$

Where, HM_s and HM_b , are concentration of metals (in mg/kg) for soils and several parts of maize was determined, respectively.

CF is categorized as: low contamination (CF < 1); moderate contamination ($1 \le CF < 3$); considerable and significant contamination ($3 \le CF < 6$) and very high contamination (CF ≥ 6 ; Shaheen et al., 2020).

Enrichment factor (EF). EF is used to determine the contamination of trace metals which are enriched due to anthropogenic activities and natural sources such as crustal materials (Chen et al., 2016). EF values of 0.5 to 1.5 indicates trace metals process as well as weathering of upper continental crustal values (Zhang & Liu, 2002). EF values of more than 1.5 indicates that it is an external influence and the following when EF is <1, No enrichment; <3 enrichment is minimum; 3 to 5 enrichment is moderate; 5 to 10 enrichment is severe; 10 to 25 enrichment is more severe; 25 to 30 very severe enrichment; and finally >50 is extremely severe enrichment. In the present study, Fe is used as a reference value, and it is calculated using the following formula:

$$EF = (HM_s / HM_b) / (Fe_s / Fe_b)$$
⁽²⁾

where, HM_s and HM_b are concentration of trace metals in soils and the reference value for the reference value is "*n*," respectively. Fe_s and Fe_b are concentration of analyzed soils and as reference values, respectively (Kowalska et al., 2018). *Geoaccumulation index (Igeo).* A common approach (Igeo index) proposed by Müller (1981), was used to measure the enrichment in metals, the factor 1.5 is introduced to minimize the effect of possible variation in the background values. The following equation was applied to calculate the contamination level of sediments:

$$I_{geo} = \log_2 \left(\frac{(C_n)_{Sample}}{1.5(B_n)_{Background}} \right)$$
(3)

Where, C_n is the measured concentration of element n in sediment, Bn is the background (NASC) concentration of element n, and 1.5 is the factor introduced to rectify the lithogenic variations in the sediments. Based on the intensity of pollution, the Igeo index was classified into seven classes from class 0 to 6 such as: uncontaminated or unpolluted (Igeo < 0), unpolluted to moderately polluted ($0 \le Igeo < 1$), moderately polluted ($1 \le Igeo < 3$), strongly polluted ($3 \le Igeo < 4$), strongly to very strongly polluted ($Igeo \ge 5$) (Angulo, 1996; Adimalla et al., 2019).

Bioconcentration factor (BCF). The BCF is an indicator on the grade of cultivation for trace metals (Mingorance et al., 2007; Yoon et al., 2006). This grade of contamination depends on each cultivation and the grade of accumulation also depends on the accumulation of trace metals in the maize plants (Chen et al., 2021). The BCF is estimated for all the materials are calculated based on the following formula:

Bioconcentration Factor (BCF) =
$$M_{plant} / M_{soil}$$
 (4)

Where, (M) represents plants and (M) soil represents the trace metal concentration in cultivated soil in the sampling zone (Hellen & Othman, 2016). Likewise, when BCF of >1 indicates absorption of trace metals in maize from soils whereas, if the values are <1 it signifies a major concentration of trace metals in soils apart from plants.

Statistical analysis

Descriptive statistics, such as mean, minimum, maximum, standard deviation (SD), and standard error (SE) and to analyze macronutrients and trace metals in soil and maize respectively. Principal component analysis was used to determine possible sources of contamination and was carried out in the STATISTICA software, version 8.0 (StatSoft, 2007).

Results and Discussion

Physico-chemical parameters

Descriptive statistic of heavy metal concentrations and soil physicochemical characteristics in RBTC soils and native maize are presented in Supplementary Tables S1 and S2.

pH of soil in the ranged between 8 and 8.9, this indicates that basic conditions predominate in these soils, mainly due to the high content of carbonates from the parent rock, causing the soil to be more susceptible to erosion, releasing calcium and, in dry conditions, this is responsible for cementation on the soil surface, creating an impermeable crust (Figure 2(a); Amjadian et al., 2016; Li et al., 2013).

In highly alkaline soils, characteristic of arid regions such as this study site, most heavy metals are likely to be found in a less mobile form (Muñoz-Rojas et al., 2016; Škrbic & Durisic-Mladenovic, 2002), because the mobility and retention of heavy metals are strongly affected by soil pH (Esmaeili et al., 2014).

Agricultural soils of the study area had low OM content and the mean value in the agricultural soils was 2.04% (Figure 2b), Supplementary Table S1). Organic matter has also been found to influence heavy metal absorption in soils (Martin & Kaplan 1998; Romic & Romic, 2003). The influence of organic matter on the total metal content is low since the percentage of OM is greater than 2% and overall only 40% of the soil samples fall in this category. Moreover, this low content in the present study is related to the soil management system (López et al., 2018), effects stability of the structure, availability of nutrients, water retention capacity, aeration, and due to the high-capacity cation exchange (CEC) relationship with OM (Carter, 2002). Higher values of Ca (3.64–16.79 mg/kg; Figure 2(c), Supplemental Table S1) in the soils is mainly due to the secondary calcium and its accumulation in the soil, where the low value of OM induces to the formation of low humus calcite (Franzluebbers et al., 2007).

Fe and Mn in plants often indicate uptake of metals (Gayomba et al., 2015). The interplay of Fe-Mn homeostasis which is critical in remobilizing vascular Fe/Mn in leaves and roots highlighting the interaction and accumulation of metals between both nutrients (Lanquar et al., 2010). Fe and Mn exist in dissolved and particulate forms as they have similar chemical properties and they coexist in the redox process (Stumm & Morgan, 1996). The binding property of Fe and Mn in relation to other metals is the thermal stratification in the soils, where low dissolved oxygen in the bottom sediments often accumulate the metals along with the Fe-Mn oxides and they are described as the elemental scavengers when the soils are oxidized with presence or excessive presence of water in the study area (He et al., 2023; van Zelm et al., 2020).

In general, the mean levels of the trace metals in the soil samples and plant varied according to the following trend in mg/kg: Soil: As = 0.0004; Cd = 0.0001; Co = 0.0006; Cr = 0.0027; Cu = 0.0015; Fe = 0.8922; Li = 0.0040; Mn = 0.0245; Mo = 0; Ni = 0.0014; Pb = 0; Sb = 0, Sr = 0; Ti = 0.0011; V = 0.0033; Zn = 0.0047, and plant: As = 0; Ca = 0.5211; 0, Cd = 0, Co = 0; Cr = 0.0004; Cu = 0.0004; Fe = 0.0158; Li = 0.0003; Mg = 0.1369; Mn = 0.0070; Mo = 0.0001; Ni = 0; Pb = 0; Sb = 0; Sr = 0.0039; Ti = 0; V = 0, and Zn = 0.0028 respectively. The comparison on the concentrations of trace metals in this study with FAO/



Figure 2. Physico-chemical parameters distributions; (a) pH; (b) OM and geochemical elements; (c) As; (d) Ca; (e) Cd; (f) Co; (g) Cr; (h) Cu; (i) Fe; (j) Li; (k) Mg; (l) Mn; (m) Mo; (n) Ni; (o) Pb; (p) Sb; (q) Sr; (r) Ti; (s) V; and (t) Zn.

WHO and Mexican government soil standards for remediation of soil for agricultural/residential use in NOM147-SEMARNAT/SSA1-2004 (SEMARNAT, 2007) the levels didn't exceed the reference values, indicating that the metals doesn't represents a potential danger (Kabata-Pendias, 2010).

Analysis of contamination indices

Geoaccumulation index (Igeo). The Igeo values were <0 in 15 in the elements analyzed in soil, which indicates that there is no contamination according to the Igeo classification of Müller (1981). Zero tillage and not using agrochemicals and fertilizers



Figure 3. Evaluation of contamination (a) Geoaccumulation Index (Igeo); (b) Contamination Factor; (c) Enrichment Factor; and (d) Bioconcentration Index.

are the answer as they are not present geoaccumulation of contaminants. However, the presence of Ca was classified between 0 and 1, being a naturally present element, which in excess can be challenging if its concentration exceeds the adsorption capacity in the soil, forming insoluble complexes with other elements that are difficult to be assimilated by plants, promoting their accumulation (Figure 3a).

Contamination factor (CF). CF indicates the degree of impact about anthropogenic influence of trace metals in the agricultural soils (Ahmed et al., 2016). Median and average values of CF in mg/kg for Cu $(2.96 \times 10^{-5}; 1.35 \times 10^{-5}-5.33 \times 10^{-5})$, Zn $(7.30 \times 10^{-5};$ $4.79 \times 10^{-5} - 1.04 \times 10^{-4}$), Fe $(1.92 \times 10^{-1};$ 1.51×10^{-1} - 2.32×10^{-1}), Mn (2.46×10^{-1} ; 1.76×10^{-1} - 3.26×10^{-1}) 10⁻¹), Cd (1.02×10⁻³; 0–1.70×10⁻³), Pb (2.18×10⁻⁶; 0–1.55 \times 10⁻⁵), and Ni (8.13 \times 10⁻⁵; 8.13 \times 10⁻⁵) were observed on superficial soils on maize crop (Figure 3b). The order of trace metals based on the average values for CF values were Mn > F e>Cd>Ni>Zn>Cu>Pb. According to CF values of <1 by Shaheen et al. (2020), all trace metals studied are classified as "low contamination." However, Cd, Mn, and Fe indicates "medium contamination" in maize crops as per the

classification which is mainly due to increased concentration of calcite in the region (Figure 3b).

Enrichment factor (EF). EF indicates highest values of Ca was in sites 3 to 5 and Mn in sites 1, 4, 7, and 10, were classified as highly enriched (EF = 10–20) that elements were higher than the other studied elements, thereby showing a higher degree of dispersion mainly due to parental rock geological composition (Figure 3c), compared to the others sampling sites that were classified with a lower enrichment value (<1; EF = 1–3; Marrugo-Negrete et al., 2017). The results indicate that the enrichment source of these trace metals in the study area is of natural weathering processes and soil erosion.

Plant bioconcentration factor (BCF). BCF in the different parts of the maize plant (Figure 3d) show a trend of enrichment of trace metal content following the order: roots>stems or leaves>grains (Chen et al., 2023; Rosas-Castor et al, 2014), which can be strongly related to the accumulation capacity and the transport process of trace metals depending on the management. The concentration ratio of the main elements was Ti>Zn>Cr>Cu>Mg, showing a greater probability of

accumulating in the root, indicates that the absorption and migration is from root to stems, where lower values are observed (0.5 mg/kg) in grains (Fan et al., 2018).

The results obtained in the contamination evaluation indicate very significant aspects about the genesis and current situation of the soils in the studied region of RBTC. This is mainly due to the traditional agricultural practices and the use of native seeds are safe to consumption since they do not represent a danger in the concentration and bioaccumulation of trace metals according to WHO/FAO.

Statistical analysis

Four factor series (Factor 1, Factor 2, Factor 3, and Factor 4) was generated with an overall cumulative percentage of 88.29% and a high eigen value of 12.67 for factor 1; 2.41 for factor 2; 1.45 for factor 3; and 1.13 for factor 4.

In the first factor (F1), strong negative values are observed for physico-chemical parameters and some of the trace metals (except Pb, Sb, Sr, Ti, and Zn). The reverse correlation of these elements clearly infers that these elements are associated among each other, which is due to the absorption capacity and recycling of soils in the study area. The second factor (F2) shows strong negative values for Sr indicating that it is from the leaching of calcareous minerals in the region (Smičiklas et al., 2015). In the third factor, Ti has a special relation with the sediments in the study area, which is directly related to the titanium mineral with low pH, which is mobilized and high concentration prevails (Liu et al., 2019). In the fourth factor the strong positive value of Sb indicates that it is being mobilized due to the direct bulk molar mass presence of plants, which is present in these type of agricultural land areas forming humid acids (Tang et al., 2023b; Vleek et al., 2011; Supplementary Table S4).

Comparative analysis

The concentrations of metals in the soil and the maize plant were compared with other studies of contamination in the maize crop, in the plant and in the soil in research around the world (Asia, Middle East, USA, and South America) indicating the extraction methods to compare the contamination status in different regions.

The average dissolved concentration (values in mg/kg) of Cr (soil, 0.02–0.04; plant, 0–0.01), Fe (soil, 0.77– 1.17; plant, 0–0.40), and Zn (soil 0); plant 0–0.01 mg/kg) in the Salado River sub-basin area is lower than that of other studies. However, Ca (soil, 3.65–16.79; plant, 0.01–2.32) levels were present in both plant and soil, due to the dissolution of CaCO₃ in calcareous soils in the study area (Supplementary Table S3). On the other hand, the average values were not higher than the limits allowed for human consumption established by the Mexican government (SEMARNAT, 2007).

Conclusions

Trace metal levels in native maize soils and crop plants were estimated to evaluate contamination risks in RBTC. By comparing the results of this study with the FAO/WHO soil standards and the Mexican standard, it can be concluded that the soils for agricultural purposes and the consumption of native maize were safe in terms of trace metals.

An important factor is the effect of soil pH on nutrient availability and soil chemical reactions for the fixation of contaminants such as metals and its relationship with organic matter content. Mobilization/ transfer of metals to plant indicates are embedded directly in the soil and subsequently absorbed by roots > stems > grains in stages, deployed due during each plant cycle of maize. The results also help us to understand the enrichment of trace metals.

From soil indices perspective, CF values of 16 (except Ca) elements in farmlands were <1, showing low contamination. EF values were <2, showing no enrichment (except Ca with enrichment is moderate). Furthermore, it can be concluded that all soils were uncontaminated based on Igeo. The results of the indices and BCF in maize did not show contamination conditions throughout the area.

In general, the findings of this work could be valuable as basic information on the state of contamination by trace metals within the RBTC, where the use of native species is preserved and the population has a safe consumption of this basic grain.

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