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Assessment of heavy metal accumulation and microbial contamination and potential health risks in fruits and vegetables cultivated in the vicinity of a landfill in the central region of Brazil

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ABSTRACT. The effects of growing vegetables and fruits near landfills pose a food security problem due to the accumulation of heavy metals in soils and food crops, causing potential risks to human health through the consumption of these crops. Thus, this study aimed to evaluate the content of heavy metals at four different points in the soil and food crops produced near a sanitary landfill in Porto Nacional, Brazil (central plains). The average concentrations of metals in the soil were in the order of iron > manganese > copper > nickel > zinc > cadmium, and for fruits and vegetables, in the following order: iron > manganese > zinc > copper. Notably, nickel, cadmium, chromium, and lead were not detected in any of the ten samples of vegetables (cassava and pepper) and fruits (pequi, papaya, cajá fruit, acerola, mango, guava, jackfruit, and lemon) analyzed in the present study. Regardless of the different types of vegetables, age, and gender, the EDI values ranged from Manganese (1.08×10^{-02} to 7.10×10^{-05}) > Iron (2.30×10^{-02} to 7.81×10^{-04}) > Zinc (1.42×10^{-03} to 7.10×10^{-04}) > Copper (1.55×10^{-03} to 7.10×10^{-04}). Furthermore, the results showed that children are at greater risk than adults of ingesting heavy metals according to the responses obtained by the EDI, THQ, and HRI indexes. However, the potential health risks from residual metals are considered insignificant based on the results. On the other hand, papaya, acerola, and jackfruit are not recommended for consumption due to their high microbiological contamination, mainly by *E. coli* and *Staphylococcus*.

Keywords: Bioaccumulation; Soil nutrient management; Target hazard quotient; Health risk index; Vegetable contamination; Microbial contamination.

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Introduction

The world's waste generation is growing rapidly in volume and toxicity (Morais et al., 2024). The amount of urban waste generated per capita has increased by 70% in the countries of the European Union during the last decades. Landfills remain a predominantly used management method for waste disposal worldwide despite being the ecologically incorrect option (Eurostat 2020; Mohanty et al., 2023). Sanitary landfills accumulate large amounts of waste that decompose into toxic compounds, releasing gases and particulate matter called leachate into the environment. Thus, the quality and quantity of compounds released into the environment will depend on the composition of the waste in the landfill, drastically affecting the quality of air, soil, and rainwater (Siddiqua et al., 2022; Yaashikaa et al., 2022).

Recent global attention has shown severe concern about the health risks associated with the food chain resulting from the environmental release of toxic chemicals. Due to contaminated soil and wastewater, metal contamination poses a significant threat to the quality and food safety of consumer populations. High concentrations of metals such as copper (Cu), cadmium (Cd), and lead (Pb) in fruits and vegetables were related to the high prevalence of upper gastrointestinal cancer. These elements can migrate from the soil to the roots of vegetables and fruits and accumulate in the body through direct consumption (He et al., 2019; Rajendran et al., 2022). The absorption of heavy metals in vegetables and fruits is directly influenced by some factors such as climate, atmospheric deposition, concentrations of metals in the soil, the nature of the soil in which the vegetables are grown, and the degree of maturity of the plants at the time of harvest (Gori et al., 2019; Weber et al., 2019). Thus, consuming such contaminated food can lead to serious health risks, including promoting carcinogenesis-induced tumors. For this reason, safe agricultural products and stable soil fertility have become the main focus of sustainable agriculture (Pandey et al., 2012).

Prominent among the wide variety of contaminants reported in the environment, heavy metals are of concern due to their non-degradability, high toxicity, and adverse effects on humans. Heavy metals can accumulate in human adipose tissues and internal organs through direct inhalation, ingestion, and absorption through contact, posing risks to human health, especially for young children (Rehman et al., 2018; Shi and Wang, 2021). Therefore, effective assessment of risks to consumers' health is imperative so that any adverse effects can be avoided as much as possible. Although the health risks of heavy metals have been extensively studied, most researchers have only considered the potential health risks of ingesting them through a single route. However, little attention has been given to the possible full health risks of eating food produced in contaminated soil and wastewater (Yang et al., 2022). Several studies calculated the health risk values at specific points or averaged the health risk values for different collection points in other countries. However, a more in-depth analysis of matrices widely marketed in Tocantins and Brazil has yet to be reported. In this sense, the central objective of this work was to quantify the concentration of heavy metals in soil, vegetable, and fruit samples, as well as the calculation of bioaccumulation, estimated daily intake, Target Health Quotient (THQ), and Health Risk Index (IRH) of the consumption of contaminated fruits and vegetables, in addition to microbiological analyses.

Materials and methods

Characterization of the study area and sampling

The collection zone is called the watershed of the Água Dirty River, one of the tributaries of the Tocantins River, and is considered one of the main watersheds in Brazil. It is located directly from the artificial lake of the Luís Eduardo Magalhães hydroelectric plant, with a total flooded area of 630 km^2, acting as a control center for regulating and allocating water resources. It has several functions, such as flood protection, water supply, navigation, aquaculture, tourism, and crop irrigation. The study area is located in the rural area of Porto Nacional, a municipality of the State of Tocantins, in the central plains of Brazil (10°42'28" S, 48°25'1" W), close to the TO 050 highway and the margins of the Tocantins River watershed (Figure 1). The average annual temperature in the study region is 27.4 °C, the annual precipitation is 1664 mm, and humidity can vary from 30 to 80%. The tested soil was classified as clayey or of medium texture, well-drained, little susceptible to erosion, and with low natural fertility (Guimarães et al., 2013).

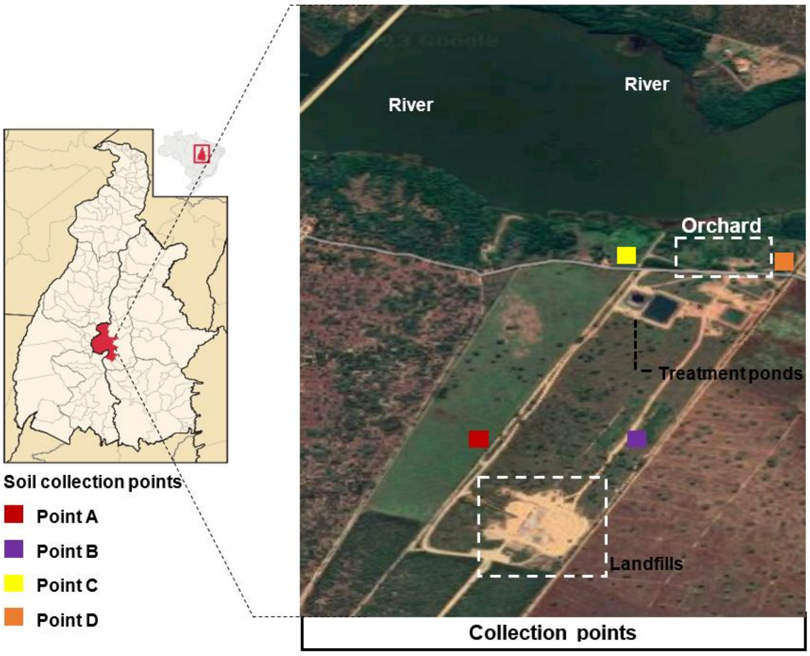

Figure1. Location and layout of the study area and sampling sites.

All experiments were conducted at the biochemical analysis laboratory at the Federal University of Goiás, Goiânia, Brazil. Samples of some of the vegetables and fruits commonly grown around the landfill were randomly selected in the middle row (to avoid the edge effect) (Figure 1), including cassava (*Manihot esculenta*), papaya (*Carica papaya*), cajá fruit (*Spondias mombin*), acerola (*Malphigia emarginate*), mango (*Mangifera indica*), guava (*Psidium guajava*), jackfruit (*Artocarpus heterophyllus*), lemon (*Citrus latifólia*), pepper (*Capsicum baccatum*) and pequi (*Caryocar brasiliense*). These crops, in particular, are inserted in the vegetable market (irrigation source unknown) and agricultural fields irrigated with fresh water and wastewater. Only the edible parts of the plant samples were used to analyze metals. The samples were harvested at the optimum maturation state and ready to be consumed. The orchard is in the landfill area at coordinates 10°32'31.9 "S 48°22'05.0" W (Figure 1).

For the collection of soil samples, the collection points were determined according to the specific and strategic control site already determined by the company that operates the sanitary landfill, where there may be possible contamination by the passage of underground waste located: point A (S 10 ° 32' 56", W 48° 22' 28"); point B (S 10° 33' 0", W 48° 22' 16"); point C (S 10° 32' 32", W 48° 22' 14"); and point D (S 10° 32' 31", W 48° 22' 0"), as shown in (Figure 1). Soils were collected at depths between 10 and 20 cm at these points. Soil samples were dried at 35°C and ground to pass through a 2 mm nylon sieve to remove stones and plant roots. The samples were stored in low-density polyethylene bags and a freezer at −20°C until further analysis (Li et al., 2012).

On the other hand, fruit and vegetable samples were collected and stored individually and packed in high-density polyethylene packages to avoid possible contamination. After sample collection, vegetables, and fruits were immediately transported to the Kinetics and Process Modeling Laboratory at the Federal University of Tocantins and cleaned with deionized water. All harvested samples' fresh weights (FW) were recorded before being dried in an oven at 60 °C until they reached a constant weight. Dried samples were ground to a 250 μm standard in a knife mill and stored in a freezer at −20°C until further analysis.

Analytical methods

Heavy metals in the soil

The total concentrations of heavy metals in the soils at four different points in the landfill (Figure 1) were prepared and determined according to the standard extraction method using hydrochloric acid (HCl), nitric acid (HNO_3), hydrofluoric acid (HF), and perchloric acid ($HClO_4$). For the digestion of soil samples, 100 mg of soil was digested with 3 mL of 37% HCl, 1 mL of 65% HNO_3, 6 mL of 65% HF, and 0.5 mL of 65% $HClO_4$. The digestion was divided into two steps: the first (10 min to reach 200 °C) and the second (15 min at 200 °C). After cooling, the digestion solutions were evaporated under a nitrogen atmosphere and dissolved in 1.0 mL of 65% HNO_3. After the drying and resuspension, 20 mL of deionized water was added to each sample. The solutions were stored in 25 mL amber bottles at 4 °C until further analysis (Hu et al., 2011). The resulting solutions were used for mineral determination by optical emission spectrometry (ICP-OES). The calibration curve was constructed using iron, zinc, copper, Manganese, nickel, chromium, cadmium, and lead standards. Analyses were performed in triplicate, and results will be expressed as mean ± standard deviation in mg per kilogram of the sample (mg kg^{-1}).

Determination of soil pH and zero load point

The samples' hydrogen ion potential was measured using an electrode with a microprocessor benchtop meter from the MS Tecnopon brand. For the point of zero charge (PZC), potentiometric soil titration was performed in different ionic strengths of the medium. First, 4.0 g of soil was added to a beaker with KCl solution for three days with stirring at 12-hour intervals, after which titration was performed with acid (HCl) and base (NaOH) solutions for electrolyte concentrations (Teixeira et al. al., 2017).

Analysis of heavy metals in vegetables and fruits

To analyze plant samples (fruits and vegetables) collected in the orchard near the landfill, 0.5g of the sample was weighed and placed in a digestion tube, where 4 mL of concentrated nitric acid was added. Then, the sample was taken to the digester block for 2 hours at 110°C. After cooling, another 2 mL of nitric acid and 2 mL of concentrated hydrogen peroxide were added, and the sample returned to the digester block for another 2 hours at 130°C. Then, the volume of the samples was completed with ultrapure water to 25 mL in a volumetric flask, and then the samples were filtered through filter paper. The same process was performed for the control sample containing the reagents without the plant sample. The resulting solution was used for mineral determination by optical emission spectrometry (ICP-OES). Standards of iron, zinc, copper, magnesium, manganese, sodium, potassium, selenium, aluminum, and chromium were used to obtain calibration curves of the tested compounds. The analysis was performed in triplicate, and the results will be expressed as mean ± standard deviation in mg per kilogram of the sample (mg kg^{-1}).

Data analysis

Bioaccumulation factor

The ability to translocate heavy metals from the soil to the edible parts of cultivated plants can be described by calculating the bioaccumulation factor (BAF). The concentrations of metals in the soil and the studied vegetables and fruits were calculated based on their dry weight. The BAFs of Iron, zinc, copper, magnesium, manganese, sodium, potassium, selenium, aluminum, and chromium were calculated according to Equation 1, proposed by Cai et al. (2015):

$$\text{Bioaccumulation factor} = \frac{C_{plant}}{C_{soil}} \quad (1)$$

C_{plant} is the individual total concentration of a given metal in a given plant, and C_{soil} is the corresponding metal concentration in the same soil environment of that specific plant.

Estimated daily intake (EDI) of heavy metals for children and adults

The estimated daily intake (EDI) of heavy metals was determined based on the concentrations of metals in the crops and the consumption of the respective food crop. The estimated daily intake (EDI) of trace elements (Iron, zinc, copper, magnesium, manganese, sodium, potassium, selenium, aluminum, and chromium) was calculated according to Equation 2, proposed by several studies (Santos et al., 2004; Ji et al., 2013; Wang et al., 2014; Qureshi et al., 2016):

$$EDI = \frac{(C_{metal} \times W_{food})}{B_w} \quad (2)$$

C_{metal} is the concentration of metals found in the respective cultures analyzed (mg kg^{-1}), W_{food} represents the average daily intake of vegetables and fruits in general, and B_w is the estimated body weight for adults and children. The mean body weights adopted were 70 kg for adults and 32.7 kg for children. The average daily intake of vegetables and fruits for adults and children was 0.345 and 0.232 kg, respectively (Arora et al., 2008; Qureshi et al., 2016). In response to this index, values lower than 1.0 are expected since values greater than 1.0 are associated with adverse health effects and various pathologies.

Health risk index

Heavy metal contamination of food is one of the most critical aspects of ensuring food safety. Therefore, the health risk index (HRI) was determined according to Equation 3, proposed by Qureshi et al. (2016) and Hussain et al. (2020).

$$HRI = \Sigma n \frac{(C_n \times D_n)}{(RfD \times B_w)} \quad (3)$$

C_n represents the average concentrations of a given metal found in a fruit or vegetable targeted for this study, based on fresh weight (mg kg^{-1}) (applied conversion calculation); D_n indicates the average daily intake rate of a specific vegetable in a year; R*f* D represents the safe lifetime oral exposure level; B_w is the average body weight (considered as 70 kg for adults). Dietary Reference Intakes (DRI) of elements were considered R*f* D (Food and Nutrition Board, 2004).

Target hazard quotient

The target hazard quotient (THQ) is a ratio between the determined dose of heavy metal and a reference level of this same compound. The risks to human health associated with consuming these vegetables and fruits were assessed according to Equation 4 (Yang et al., 2011). The USEPA Region III Risk-Based Concentration Table (USEPA, 2006) was used to estimate the target hazard quotient's value.

$$THQ = \frac{(C \times I \times 10^{-3} \times EF_r \times E_{tot})}{RfD \times BW_a \times AT_n)} \quad (4)$$

C is the average content of metals in the vegetable (mg kg^{-1}, fresh weight); I is the per capita intake rate (255 g day^{-1}); EF_r is the exposure frequency (350 days of the year); ED_{tot} is the total duration of exposure for an adult (70 years); BW_a is the average adult body weight (70 kg); and AT_n is the meantime, non-carcinogenic (ED_{tot} × 365 days of the year).

Statistical analysis

Data were analyzed for normality and through analysis of variance (ANOVA). When differences between treatment means were verified, Tukey's test was applied at 95% reliability ($p < 0.05$) using Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA). Linear models, in general, were used for data analysis using SPSS software (version 19.00) for Windows (SPSS Inc., Chicago, IL, USA). OriginPro 2022 software (OriginLab Corporation, Northampton, MA, USA) was used to analyze and plot the graphs.

Results and discussion

Heavy metal contamination in soil

Many heavy metals were observed in soils throughout the study area. According to (Figure 2), the concentrations of heavy metals in the soils of each experimental field plot showed significant variation, and samples were collected from depths between 10 and 20 cm.

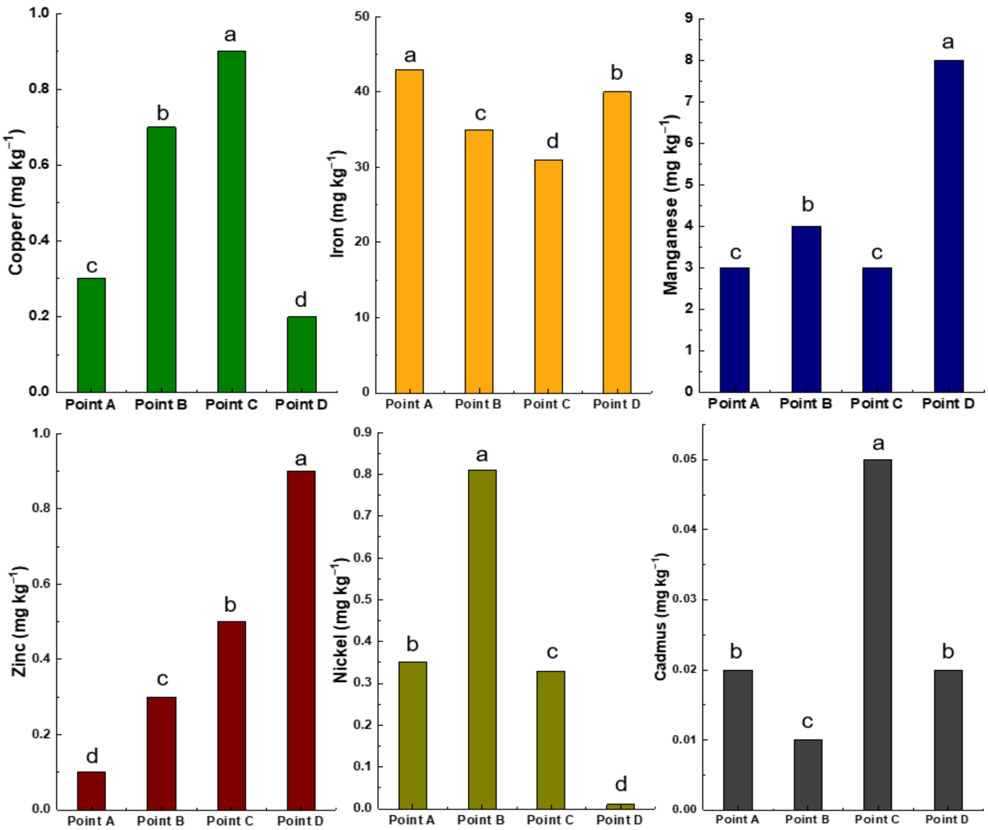

Figure 2. Concentrations of heavy metals at different collection points at the Porto-Nacional landfill. Different lowercase letters within the same mineral show statistical differences by the Tukey test ($p < 0.05$).

The concentrations of heavy metals in soils are of the same order as iron > Manganese > Copper > Nickel > Zinc > Cadmium. The highest concentration ranges were reported for iron, which ranged from 31 to 43 mg kg^{-1}. This was followed by Manganese, whose average concentration was 3.0 to 8.0 mg kg^{-1}, and copper, from 0.20 to 0.90 mg kg^{-1}. The other elements, such as nickel, zinc, and cadmium, showed low concentrations in the four analyzed soil points. These results demonstrate prominent accumulations of heavy metals in these soils, among which iron, Manganese, and copper accumulations were the most significant. High amounts of these three heavy metals were added to the soils in the study area from external sources, mainly due to the sanitary landfill present there. Despite being detected in small amounts in the analyzed soil samples, nickel, zinc, and cadmium, when detected on a large scale, can denote a threat to agricultural production and human health. Notably, significant concentrations of lead and chromium were not found in the analyzed soil samples. It is also important to mention that all samples of heavy metals are within the threshold current Brazilian legislation allows (Brasil, 2005).

Heavy metals remain in upper soil layers and can be metabolized in plant tissues, especially as organic complexes absorb metals. According to the US Environmental Protection Agency (EPA), the lead standard in soils for non-residential areas should not exceed 1200 mg kg^{-1}. In the present study, the Point C sample revealed an amount within the established standard compared with the US agency. Lead, being in a high concentration in the soil, can be absorbed by plant tissues, including aerial parts, and can thus enter the human food chain, accumulating in the body and causing various dysfunctions and pathologies such as cancer (Edelstein et al., 2018; Singh et al., 2018; Uddin et al., 2021). The fact that the landfill works with its reduced capacity may have corroborated the low concentrations of metals found in the four different points. Furthermore, no leachate was observed in the soil, which is one of the significant causes of soil and water contamination around sanitary landfills. Therefore, landfill facilities must always use adequate leak detection systems to ensure the proper containment of these leachates, guaranteeing the soil quality and the vegetables produced.

The values of pH and zero charge potential found in the soil points analyzed were points A (5.27 and 3.28), B (5.19 and 3.17), C (4.98 and 3.00), and D (5.17 and 3.17), respectively, ranging from acidic to slightly acidic. Characteristics such as pH, organic matter content, the interaction between metals, amount of clay, and zero charge potential are responsible for the translocation behavior of heavy metals in soil to plants. Soil pH is an essential factor as it controls the geochemical behavior of heavy metals. It also governs the processes of sorption/desorption and chemical speciation of heavy metals in soils (Ashraf et al., 2017). The results of the zero-charge potential of the present study indicate that pH values lower than the one at the zero charge point of the soil tend to have positive charges. In contrast, above the zero-charge potential, the costs will be negative. Decreased soil pH (< 7) feeds heavy metals into the soil, while at higher pH values (> 8), metals precipitate within the soil matrix (Shahid et al., 2012). Metals generally have high solubility, mobility, and bioavailability at lower pH values and vice versa. In addition, heavy metals are present in their cation form, which would facilitate their absorption by plants if the soil was contaminated due to the negative charges available in the pH range encountered.

Evaluation of metals in different vegetables and fruits

The vegetables and fruits cultivated in the landfill orchard exhibited varied concentrations for the analyzed metals (mg kg^{-1}) with significant differences between almost all variables ($p < 0.05$) (Figure 3). Overall, the highest concentration was detected for the element iron in 50% of the evaluated samples (acerola, jackfruit, lemon, papaya, and pepper), followed by substantial levels of manganese and zinc. Iron was considerably higher than other heavy metals, such as zinc and copper, which can act through different physiological mechanisms in plants. In general, pepper was the vegetable with the highest concentrations of metals in this study, especially iron (3.60 mg 100 g^{-1}) and zinc (0.51 mg 100 g^{-1}). Pequi exhibited the highest iron levels (0.68 mg kg^{-1}), followed by substantially higher metal zinc and manganese (1.02 and 1.50 mg kg^{-1}, respectively). However, significantly higher levels of copper were observed in cassava and mango compared to all other vegetables. In general, acerola, lemon, papaya, and guava had the lowest concentrations of the analyzed metals. It is worth mentioning that the metals nickel, cadmium, chromium, and lead were not detected in any of the ten samples of vegetables and fruits analyzed in the present study. Other researchers have reported that vegetables and fruits exhibit different mechanisms for the uptake, absorption, and accumulation of metals in various organs. Several biochemical factors, such as soil physical condition, movement of trace elements in the soil, soil structure, plant species, and genotypes within the same plant species, play a significant role in the uptake and uptake of heavy metals in plants (Hu et al., 2014; Hussain et al., 2020).

According to the standards established by ANVISA (Brazil, 1965), which set limits for residual or migrated substances present in food as a result of the production, processing, storage, and transport of food or raw materials, all vegetables and fruits analyzed in the orchard of the sanitary landfill of Porto Nacional are in accordance with the legislation. In the present study, copper concentrations were present in all vegetables, with higher concentrations in cassava and mango (0.60 mg 100 g^{-1}), below the limits determined by legislation, 3.0 mg 100 g^{-1}. This was also the element that had the slightest variation in the composition of the vegetables. In large amounts, copper is very harmful to human health, causing damage to the reproductive system, neurological capacity, immune system, and gastrointestinal distress (Toth et al., 2016; Sousa et al., 2023). Manganese was the element that statistically varied the most among the studied vegetables ($p < 0.05$), and papaya and cassava were the only ones that presented similar means of 0.01 and 0.03 mg 100 g^{-1}, respectively. Brazilian legislation does not determine a limit for this metal, but according to the World Health Organization, the maximum allowed is 50 mg 100 g^{-1} (World Health Organization [WHO]., 1998).

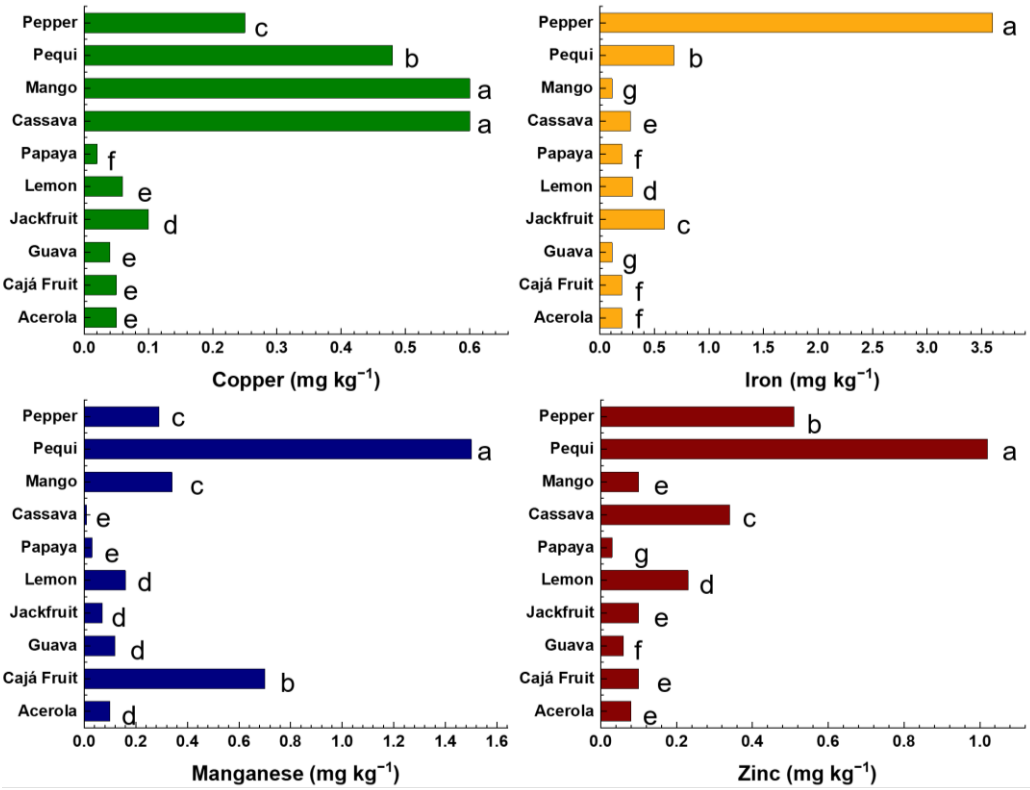

Figure 3. Concentrations of heavy metals in different fruits and vegetables cultivated around the Porto-Nacional landfill. Different lowercase letters within the same mineral show statistical differences by Tukey's test ($p < 0.05$).

Plant diversity can adequately indicate the environmental effects of landfill activities. Bear in mind that the proximity of the points under study is shown with various plants, native trees, and an orchard, which may indicate low concentrations of metals. Soils with low plant diversity, with a predominance of creeping plants such as mosses, are bioindicators of contamination by heavy metals. Mosses are widely used as bioindicators of metal pollution in the atmosphere and soil, as they allow the simultaneous monitoring of a large number of contaminants (Lazo et al., 2018; Pająk et al., 2021). Due to their morphological and physiological characteristics, these plants can accumulate large amounts of metals, much larger than those in the air, along their entire surface (Shahid et al., 2017; Hozhabralsadat et al., 2022).

On the other hand, as it is an area with little time for depositing waste, the impacts on the soil are not yet perceptible in the studied sanitary landfill. In addition, heavy metals showed variations in their concentrations, and these characteristics may be related to natural sources of soil formation or even to the plant's physiological aspects. Therefore, the interaction between different heavy metals occurs on the surface of the roots and inside the plant, affecting the metals' translocation. However, in this study conducted at the landfill in Porto Nacional, metals were found in the soil, which can be considered non-contaminated soil according to the legislation. Therefore, these metals found are not capable of contaminating the plants, indicating that until the present study, the area can be used for planting vegetable crops.

Evaluation of contamination by the bioaccumulation factor in the soil-plant system

The bioaccumulation factors (BAF) of metals are used to assess the potential of plants to transfer metals from the soil to edible tissues such as roots, leaves, stems, and fruits. Metals with high bioaccumulation factors are more easily transferred from soil to plant parts than those with low factors (Cai et al., 2015). Thus, bioaccumulation factors were calculated for each heavy metal and the fruits and vegetables under analysis to better understand the movement process (absorption and translocation) of heavy metals in the soil-plant system (Figure 4). Trends and variations of bioaccumulation factors for heavy metals in different vegetables and fruits were in descending order of Zinc > Copper > Manganese > Iron. These results are strongly corroborated by the findings of Qureshi et al. (2015) when assessing the accumulation of heavy metals and potential health risks in vegetables irrigated with treated wastewater in Dubai.

Among the analyzed fruits and vegetables, there were significant differences in bioaccumulation values among all vegetables ($p < 0.05$), and the highest bioaccumulation factor was observed for zinc with (2.27) in the pequi samples. On the other hand, the lowest bioaccumulation level was obtained for manganese in cassava (0.002) (Figure 4). Zinc bioaccumulation values ranged from 0.67 to 2.27, with an average of 0.571, the highest among the analyzed metals. Therefore, the high values of soil zinc bioaccumulation for plants indicate a substantial accumulation of this metal by food crops, mainly vegetables, as reported in the literature for food crops (Cai et al., 2015). On the other hand, when analyzing the bioaccumulation factors for iron, this metal was the lowest, ranging from 0.003 to 0.097, with an average of 0.017 in the area affected by the landfill. The results indicated that iron bioavailability was relatively low compared to other studies (Cai et al., 2015; Hussain et al., 2020). The bioaccumulation factors were higher for pepper, pequi, and mango when evaluating the fruits and vegetables studied in general.

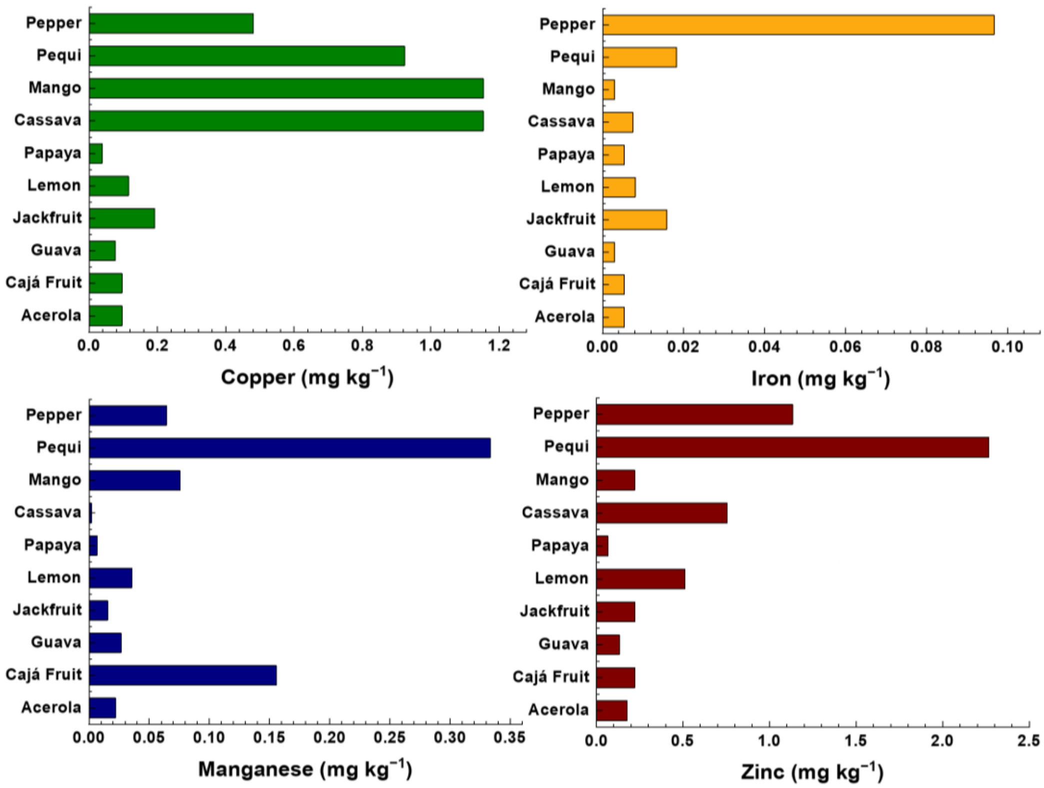

Figure 4. Bioaccumulation factors ratio between the concentrations of heavy metals in the edible parts of vegetables and fruits and those in the corresponding soil.

Except for iron and manganese, relatively low bioaccumulation factor values were observed for other metals. However, the present results indicate that the average values of bioaccumulation for vegetables were significantly higher compared to the tested fruits. Thus, there were significant differences in bioaccumulation values between all vegetables and fruits because plants' uptake of heavy metals depends on the plant type, its physiological character, and the distribution and ecophysiological attributes of the plant (Leblebici et al., 2020). As a general contribution, the bioaccumulation factor is widely used, mainly in environments with high levels of contamination by metals. This justifies values much lower than those found in the present study since the soil studied has adequate levels of metals according to legislation. This means that the amount of a given metal in the soil is sometimes lower than that found in vegetables.

Estimated dietary intake of metals for adults and children

It is essential to obtain the exposure level by detecting exposure routes to target organisms to estimate the health risk of any heavy metal. Thus, the assessment of the potential health risk due to the accumulation of heavy metals in the edible parts of vegetables and fruits cultivated in the vicinity of the Porto-Nacional landfill was conducted. The health risk of heavy metals was assessed for the four metallic components identified and quantified in the studied samples. Table 1 presents the results of the estimated dietary intake (EDI) of heavy metals in adults and children calculated based on the average daily values of vegetables and fruits consumed. Like the bioaccumulation factor, estimating dietary intake of metals is crucial to understanding health risks. Although exposure to heavy metals occurs in different ways (e.g., dermal contact, dust inhalation, consumption of metal-contaminated soils, and through food) (Khan et al., 2013; Guadie et al., 2021).

Table 1. Estimated daily intake of heavy metals from fruits and vegetables by adults and children.

Samples		Copper	Iron	Manganese	Zinc
Acerola	Adults	3.09×10^{-04}	1.24×10^{-03}	6.18×10^{-04}	4.94×10^{-04}
	Children	3.55×10^{-04}	1.42×10^{-03}	7.10×10^{-04}	5.68×10^{-04}
Cajá fruit	Adults	3.09×10^{-04}	1.24×10^{-03}	4.35×10^{-03}	6.18×10^{-04}
	Children	3.55×10^{-04}	1.42×10^{-03}	5.00×10^{-03}	7.10×10^{-04}
Guava	Adults	2.47×10^{-04}	6.80×10^{-04}	7.41×10^{-04}	3.71×10^{-04}
	Children	2.84×10^{-04}	7.81×10^{-04}	8.52×10^{-04}	4.26×10^{-04}
Jackfruit	Adults	6.18×10^{-04}	3.66×10^{-03}	4.32×10^{-04}	6.18×10^{-04}
	Children	7.10×10^{-04}	4.21×10^{-03}	4.97×10^{-04}	7.10×10^{-04}
Lemon	Adults	3.71×10^{-04}	1.86×10^{-03}	9.89×10^{-04}	1.42×10^{-03}
	Children	4.26×10^{-04}	2.13×10^{-03}	1.14×10^{-03}	1.64×10^{-03}
Papaya	Adults	1.23×10^{-04}	1.24×10^{-03}	1.85×10^{-04}	1.85×10^{-04}
	Children	1.42×10^{-04}	1.42×10^{-03}	2.13×10^{-04}	2.13×10^{-04}
Cassava	Adults	3.73×10^{-03}	1.73×10^{-03}	6.17×10^{-05}	2.11×10^{-03}
	Children	4.28×10^{-03}	1.99×10^{-03}	7.10×10^{-05}	2.42×10^{-03}
Mango	Adults	3.73×10^{-03}	6.80×10^{-04}	2.11×10^{-03}	6.18×10^{-04}
	Children	4.28×10^{-03}	7.81×10^{-04}	2.42×10^{-03}	7.10×10^{-04}
Pequi	Adults	2.98×10^{-03}	4.23×10^{-03}	9.40×10^{-03}	6.36×10^{-03}
	Children	3.42×10^{-03}	4.86×10^{-03}	1.08×10^{-02}	7.31×10^{-03}
Pepper	Adults	1.55×10^{-03}	2.30×10^{-02}	1.80×10^{-03}	3.16×10^{-03}
	Children	1.78×10^{-03}	2.65×10^{-02}	2.06×10^{-03}	3.64×10^{-03}

The highest intake of heavy metals resulted from the consumption of vegetables and fruits cultivated around the Porto-Nacional landfill. For example, the maximum daily intake of copper, iron, Manganese, and zinc for both ages resulted from consuming papaya > pepper > cajá fruit. Values of estimated dietary intake of metals were higher for children than adults, resulting from the difference in body weight used to calculate the EDI (Table 1). Regardless of the different types of vegetables, age, and gender, the EDI values ranged from Manganese (1.08×10^{-02} after children consumed pequi to 7.10×10^{-05} after children consumed cassava) > Iron (2.30×10^{-02} after consumption of chili pepper by adults up to 7.81×10^{-04} after consumption of guava and mango by children) > Zinc (1.42×10^{-03} after consumption of lemon by adults up to 7.10×10^{-04} after consumption of mango, cajá fruit, and jackfruit by children) > Copper (1.55×10^{-03} after consumption of pepper by adults to 7.10×10^{-04} after consumption of jackfruit by children) (Table 1).

The results also demonstrate that children are at greater risk than adults of ingesting heavy metals by consuming vegetables and fruits near the Porto-Nacional landfill. The highest EDIs for iron and manganese in children were found in all vegetables and fruits, followed by copper. The risk of iron intake by adults and children was lower. Similar results were reported by Arora et al. (2008), Qureshi et al. (2016), Hussain et al. (2020), and Guadie et al. (2021), who demonstrated that children are at greater risk than adults of intake of metals cultivated in areas with landfills, or irrigated with sewage wastewater. The results reported here on the intake of heavy metals were much lower than the recommendation of the international standard. Therefore, there was no risk in consuming these vegetables and fruits in the local population, mainly cassava, guava, and jackfruit. However, pepper should be consumed in smaller amounts due to potential risks associated with increased absorption and translocation in leafy vegetables. Thus, the occasional consumption of these vegetables is not harmful to human health.

Human Health Risk Assessment of heavy metals

To assess the health risk associated with heavy metal contamination of vegetables and fruits grown in the orchard in the vicinity of the Porto Nacional landfill, health risk assessment (HRI) values and target hazard quotients (THQ) were calculated, and the results are presented in Table 2. The THQ model is a more direct approach that helps study the impact on human health due to heavy metals in leafy vegetables, roots, and fruits. According to USEPA (2006) reports, THQ and HRI should be less than 1. Thus, the results indicated that the THQ and HRI values of each plant analyzed for humans are within safe limits (< 1). However, according to the HRI, the metals were determined to be zinc > manganese > copper > iron. On the other hand, according to THQ, metals were classified in the following order: iron > copper > manganese > zinc. THQ and HRI values for copper, zinc, and manganese were significantly less than 1.0 (Table 2). The THQ values for iron were also lower than the USEPA standard of 1.0 in all vegetables and fruits tested, except for pepper, which was greater than 1.0 (1.79647). The THQ values for iron in all vegetables were the highest for all tested samples, even though they were within the safe limit. Other vegetables that showed results in levels of attention to iron were cassava (0.63874), pequi (0.83835), jackfruit (0.29442), and lemon (0.14970).

Table 2. Estimated daily intake target health quotient (THQ) and health risk index (HRI) of consuming contaminated vegetables and fruits.

Samples	Copper		Iron		Manganese		Zinc	
	THQ	HRI	THQ	HRI	THQ	HRI	THQ	HRI
Acerola	0.00436	0.00328	0.09980	0.00066	0.00249	0.00478	0.00093	0.00843
Cajá fruit	0.00436	0.00328	0.09980	0.00066	0.01746	0.03352	0.00116	0.01054
Guava	0.00349	0.00262	0.05489	0.00036	0.00299	0.00574	0.00069	0.00630
Jackfruit	0.00873	0.00656	0.29442	0.00196	0.00174	0.00335	0.00116	0.01054
Lemon	0.00524	0.00394	0.14970	0.00099	0.00399	0.00766	0.00267	0.02424
Papaya	0.00174	0.00131	0.09980	0.00066	0.00074	0.00143	0.00034	0.00316
Cassava	0.05239	0.03936	0.63874	0.000935	0.0002	0.00047	0.00395	0.03583
Mango	0.05239	0.03936	0.05489	0.00036	0.00848	0.01628	0.00116	0.01054
Pequi	0.04191	0.03149	0.83835	0.00226	0.03742	0.07184	0.01187	0.10750
Pepper	0.02183	0.01640	1.79647	0.01199	0.00723	0.01389	0.00593	0.05375

* Health Risk Assessment (HRI) values and Target Hazard Quotients (THQ).

Although some heavy metals taken into human tissues can be removed from the body, long-term accumulation of metals due to their properties can harm human health (Briffa et al., 2020; Collin et al., 2022; Cui et al., 2023). The results indicated that, except for pepper, each plant's THQ and HRI values calculated for humans are within safe limits (< 1). However, THQ and HRI values greater than 1 may have dangerous consequences for public health. Differences in total metal THQ for various vegetables are mainly attributable to significantly different iron contributions. The THQs and HRIs of zinc, copper, and manganese for all vegetables were less than 1, suggesting that locals consuming these vegetables and fruits will not be exposed to a potential health risk. However, in the case of pepper, due to THQ values greater than 1, perennial ingestion should be viewed with caution, as it may not immediately cause problems. Still, excessive consumption will induce adverse health effects. Similar results were reported by Qureshi et al. (2016) when evaluating the accumulation of heavy metals and potential health risks in vegetables irrigated with wastewater, and they observed that the vegetables were suitable for consumption. Above all, to alleviate anxiety about the harmful effects of consuming products cultivated in danger zones such as the Porto-Nacional landfill, knowledge of potential health risks, proper environment and soil selection, and irrigation management are needed to guarantee food in the food safety guidelines.

Microbiological contamination

All fruit and vegetable samples collected in the landfill orchard showed contamination by different microorganisms, as seen in Table 3. All samples obtained the presence of mesophiles, molds and yeasts, *Staphylococcus*, and *Pseudomonas*. *Escherichia coli* contaminated 20% of the vegetables, and none of the samples had the presence of *Salmonella*. Aerobic mesophilic microorganisms showed contamination levels in all evaluated species, ranging from 2.0×10^3 to 1.9×10^7 CFU g^{-1}. Considering more generally, 60% of the samples had counts above 10^4 CFU g^{-1}. Acerola obtained the highest contamination, followed by papaya, guava, jackfruit, and cajá fruit. As for mold and yeast counts, they were also found in all samples. Only 40% of the vegetables meet the levels allowed by law, and a large percentage of the samples showed contamination more significant than 10^4 CFU g^{-1} (50%). The highest results for molds and yeasts were reported for guava, acerola, and papaya (5.2, 3.4, 2.9×10^4 CFU g^{-1}, respectively), and cassava had the lowest concentration of 1.2×10^2 CFU g^{-1}. These results indicate that the analyzed vegetable and fruit samples are more susceptible to deterioration.

Table 3. Microorganisms present in fruit and vegetable samples cultivated near the Porto Nacional – Brazil landfill.

Samples	Concentration (CFU g⁻¹)					
	* Mesophiles	* Molds	*Staphylococcus*	*E.Coli*	*Pseudomonas*	*Salmonella*
Acerola	$1.9 \times 10^{7\,a}$	$3.4 \times 10^{4\,b}$	$1.5 \times 10^{5\,b}$	Positive	$2.0 \times 10^{2\,cde}$	Absence
Cajá fruit	$4.2 \times 10^{6\,d}$	$2.0 \times 10^{4\,c}$	$9.2 \times 10^{4\,c}$	Absence	$4.2 \times 10^{2\,cd}$	Absence
Guava	$6.3 \times 10^{6\,c}$	$5.2 \times 10^{4\,a}$	$1.02 \times 10^{5\,c}$	Absence	$1.5 \times 10^{3\,a}$	Absence
Jackfruit	$5.2 \times 10^{6\,cd}$	$2.3 \times 10^{4\,c}$	$8.0 \times 10^{3\,d}$	Absence	$3.0 \times 10^{2\,cde}$	Absence
Lemon	$1.6 \times 10^{2\,e}$	$7.0 \times 10^{2\,d}$	$3.0 \times 10^{2\,d}$	Absence	$5.2 \times 10^{2\,bc}$	Absence
Papaya	$1.2 \times 10^{7\,b}$	$2.9 \times 10^{4\,bc}$	$3.0 \times 10^{3\,d}$	Positive	$4.2 \times 10^{2\,cd}$	Absence
Cassava	$7.0 \times 10^{3\,e}$	$1.2 \times 10^{2\,d}$	$3.2 \times 10^{5\,a}$	Absence	$3.2 \times 10^{2\,e}$	Absence
Mango	$6.0 \times 10^{3\,e}$	$7.0 \times 10^{3\,d}$	$5.0 \times 10^{1\,d}$	Absence	$8.0 \times 10^{2\,b}$	Absence
Pequi	$2.0 \times 10^{3\,e}$	$3.2 \times 10^{3\,d}$	$2.3 \times 10^{3\,d}$	Absence	$6.2 \times 10^{2\,de}$	Absence
Pepper	$1.1 \times 10^{4\,e}$	$2.5 \times 10^{3\,d}$	$1.5 \times 10^{3\,d}$	Absence	$8.2 \times 10^{2\,de}$	Absence

Means followed by the same lowercase letter in the same column do not differ statistically by Tukey at 5% probability ($p \leq 0.05$).

Mean count levels for *Staphylococcus* ranged from 5.0×10^1 to 1.5×10^5 CFU g⁻¹. Evaluating (Table 3) more concisely, we can see that only the mango (5.0 × 10 CFU g⁻¹) was within the standards established by several international bodies for this microorganism. The other 90% of the samples analyzed were unfit for human consumption. However, this type of contamination can be a public health problem since food contaminated with *Staphylococcus* is directly related to outbreaks of foodborne illnesses (Mahros et al., 2021). On the other hand, *Pseudomonas* is related to the rotting of vegetables and fruits, being allocated on the surfaces of vegetables, and can develop even at refrigeration temperature, their presence serves as an indication of deterioration by bacteria (Shahidi; Hossain, 2022). In the vegetables analyzed, all had the presence of Pseudomonas. Guava had the highest concentration of 1.5×10^3 CFU g⁻¹; cassava, pequi, and pepper showed the lowest results.

E. coli was detected in 20% of the analyzed vegetables, only papaya and acerola were contaminated, which could indicate fecal contamination. The two vegetables and the jackfruit had dark spots on their external structures throughout the ripening cycle. Thus, it can be said that the two contaminated vegetables are unfit for consumption. The results found for *Salmonella* were satisfactory, that is, they were free of the microorganism, in accordance with international food control and safety bodies. The absence of these microorganisms in vegetables is essential, as it demonstrates that the consumer is not exposed to the risk of foodborne infection. The microbial quality of fresh fruit depends on the variety of fruit, minimal harvesting operations, storage, transport, and packaging conditions. Several intrinsic and extrinsic factors affect the survival and growth of microorganisms in fruit. On the other hand, the survival and growth of microorganisms in fruit also depend on their physiological activity (viability, specific growth rate) and adaptability to stress conditions, as well as their behavior in mixed populations (competition, antagonism, and synergism) (Alegbeleye et al., 2018; Rolfe & Daryaei, 2020). Although contamination by various microorganisms was found in all the vegetables studied, it is recommended that the vegetable be appropriately cleaned before consuming them to eliminate or minimize the contaminating microbiological load. However, papaya, acerola, and jackfruit are not recommended for consumption due to their high microbial load, mainly *E. coli* and *Staphylococcus*.

Conclusion

This study investigated the accumulation of heavy metals in soil and vegetables and fruits cultivated in the vicinity of a sanitary landfill in Porto Nacional, Brazil. The average concentrations of metals in the soil were in the order of iron > manganese > copper > nickel > zinc > cadmium, and for fruits and vegetables, in the following order iron > manganese > zinc > copper. Notably, nickel, cadmium, chromium, and lead were not detected in the ten samples of vegetables and fruits analyzed in this study. Therefore, several indicators such as EDI, THQ, and HRI help assess heavy metals' absorption, bioaccumulation, and translocation and their possible risks by consuming leafy vegetables and fruits. Furthermore, the results showed that children are at greater risk than adults of ingesting heavy metals when consuming vegetables and fruits near the Porto-Nacional landfill, according to the responses obtained by the EDI, THQ, and HRI indexes. The potential health risks associated with residual metals were considered insignificant based on the results presented, suggesting that the consumption of vegetables cultivated in the region is safe for the inhabitants. On the other hand, papaya, acerola, and jackfruit are not recommended for consumption due to their high microbiological contamination, particularly by *E. coli* and *Staphylococcus*.

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Availability data

The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

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