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Effect of fertilization on the accumulation and health risk for heavy metals in native Andean potatoes in the highlands of Perú

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ABSTRACT

Soil infertility is a global problem, amendments such as organic fertilizers and mineral fertilizers are used to improve crop yields. However, these fertilizers contain heavy metals as well as essential mineral elements. The objective of the study was to determine the effect of organic and inorganic fertilizer on the accumulation and health risk of heavy metals in tubers. The plants were cultivated at an altitude of 3970 m using four treatments (poultry manure, alpaca manure, island guano and inorganic fertilizer) and a control group. Soil contamination levels and the degree of metal accumulation in the tubers were also determined. As a result, it was found that the use of inorganic fertilizer and poultry manure increased the values of Cu and Zn in soils, exceeding the recommended standards. The accumulation of heavy metals in potato tubers did not exceed the maximum recommended limits with the exception of Pb, which exceeded the limit allowed by the FAO/WHO (0.1 mg kg^{-1}). Poultry manure contributed to the highest accumulation of Zn, Cu and Pb in tubers with 11.62±1.30, 3.48±0.20 and 0.12 ±0.02 mg kg-1 respectively. The transfer of metals from the soil to the tubers was less than 1. Individual and total non-carcinogenic risk values were less than 1, indicating a safe level of consumption for children and adults. The cancer risk was found to be within an acceptable range. However, poultry manure and inorganic fertilizer treatments had the highest total cancer risk values in both age groups, suggesting a long-term carcinogenic risk.

1. Introduction

At a global level, the agricultural production of healthy foods largely depends on soil quality, climatic conditions, and genetic diversity [1]. However, various anthropogenic activities have been contaminating and degrading the physical, chemical, and biological properties of the soil [2], leading to the depletion of soil nutrients. This factor negatively impacts crop yields and affects global food security. Furthermore, when contaminants accumulate in plants and are transferred to consumers through biomagnification, they can pose serious health problems [1,3].

According to previous studies, heavy metals such as Cu, Zn, Cd and others are the most common contaminants in agricultural soils due to their toxicity, bioaccumulation, and stability in living organisms [4]. The content of heavy metals in soils is derived from natural and anthropogenic sources, heavy metals naturally exist in soils in small quantities, as a result of weathering processes of rocks. However, their concentrations increase due to human activities such as mining, smelting, the use of fertilizers and pesticides [5], atmospheric deposition, irrigation with wastewater, municipal wastes, the use of livestock and poultry manure [6–9].

On the other hand, crop yield depends on the levels of organic matter, as it makes macro and micronutrients in the soil available to plants. Organic matter is considered a soil conditioner, and therefore, organic fertilizers from livestock and poultry play a significant role in agriculture [10]. For instance, poultry manure aids in plant growth [11]. Additionally, other organic fertilizers improve the availability and

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retention of phosphorus [12] and increase the soil's nitrogen content [13]. However, although organic fertilizers provide essential nutrients, they can also introduce harmful amounts of heavy metals, affecting the soil-plant interaction and damaging crop quality [5,14]. Organic matter can retain certain metals like cadmium [15], which is why the application of compost to agricultural crops is one of the most relevant issues due to the potential accumulation of heavy metals in plant tissues and their subsequent bioavailability to humans and animals through consumption [16].

Metals in fertilizers are present in complex organic and inorganic forms and affect chemical reactions in the soil. Nitrogen fertilizers raise the level of Cd in the soil and phosphate fertilizers contain toxic elements such as Cd, Cr, Pb, Zn, As and Hg [17,18], accumulate in the soil and are easily available for plants [19], since added to the soil, these considerably increase the mobility of As, Cd, Zn and Pb [20]. Similarly, the use of organic fertilizers increases the concentrations of Zn, Cu, Cd, As and Hg in the soil [9] and the mobility of Cd and Zn [11].

Transfer of heavy metals from the soil to the plant is a very important route for the entry of toxic metals into the food chain. Crops absorb heavy metals through their roots and then transfer these elements to their edible parts [21]. Bioconcentration factor (BCF) or Bio-accumulation factor is an indicator that evaluates the ability of vegetables to absorb heavy metals in plant tissues [22–24]. Several studies determined the BCF in vegetables such as Khan et al. [25]; Liu et al. [26]; Jalali et al. [27] who reported BCF values greater than 1 for Cd, Cu, Zn, Ni and Pb in *Solanum tuberosum*; Ju et al. [28], Jalali and Meyari [29] reported high values in edible leafy and root vegetables; Eliku and Leta [30], Jolly et al. [31] in leafy and non-leafy vegetables reported low BCF values. BCF values greater than 1 indicate that plants are enriched with heavy metals, and it is a risk to human health because these metals can enter the food chain based on their mobility and bioavailability in the soil [32].

On the other hand, quantifying the degree of heavy metal contamination in soils and plants is essential to know the health of soils and the possible risk to the health of animals and humans. It is assessed by contamination indices such as the contamination factor (CF) and the enrichment factor (EF). The EF assesses the degree of influence of anthropogenic activity on soil, sediments and plant contamination [33–35]. For example, Wu et al. [36] reported contamination of agricultural soils ranging from slight to significant; Gogoi et al. [37] reported moderate contamination levels for Cd and Zn in tea growing soils; Mirzaei et al. [38] reported that all road dust was highly enriched with Cu. Ugulu et al. [10] reported high enrichment values for Cd in wheat plant samples, indicating a risk of excessive accumulation in cultivated plants.

In this study, we focused on Andean farmers in the central part of Peru who cultivate native potatoes in some of the highest regions in the world (above 3800 m above sea level). Peru boasts over 3500 varieties of native potatoes with diverse shapes, sizes, and colors [39,40]. These genetic resources are of great importance for global food security as they store genetic information that can be used to develop new varieties that are disease-resistant and can withstand abiotic stress such as water and salt stress. Additionally, potatoes are a commercially valuable crop in both the national and international markets [41] due to their high nutritional value as a source of carbohydrate storage. In 2021, the production of native potatoes achieved an average yield of 17.1 tons per hectare, covering a harvested area of 330,790 ha. This Andean tuber is grown by over 700,000 families in the Andean highlands, enabling local consumption of nearly 500 varieties and generating economic income through the national trade of just eight varieties [42].

The fertilizers used by native potato producers provide essential minerals for crop growth and development. However, excessive use of these fertilizers disrupts the balance of metals in the soil. For instance, phosphate fertilizers are a major source of contaminants such as Cd, Pb, Cr and other metals [18]. On the other hand, the use of animal manure as fertilizer is a limiting factor due to the concentrations of metals (Zn, Cu, Cd, Hg and As) in the manure [9,43]. The presence of heavy metals in animal manure could be closely related to the feed consumed by animals [44], due to the excessive application of additives (Cu, As, Zn, Cd) to feed to improve growth, increase weight, feeding efficiency, reproduction, pigmentation of animals [45–47].

Due to the growing demand for these Andean products, farmers may be repeatedly applying both organic and inorganic fertilizers, as well as pesticides, to increase the production of native potatoes. This could lead to the accumulation of metals in the soil and agricultural products, and their transfer into the food chain, posing potential health risks to the population. For this reason, the objective of this study was to (1) determine the effect of the application of organic fertilizer and chemical fertilizer on the accumulation of heavy metals in the soil and native potato tubers, (2) assess the enrichment factor (EF), bioconcentration factor (BCF), and non-carcinogenic and carcinogenic health risks for the high Andean population.

2. Materials and methodology

2.1. Experimental site

The research was conducted in the central region of the Peruvian Andes, specifically in the locality of San Antonio, Junín department, at an altitude of 3970 m above sea level (UTM coordinates 468514 E and 8692638 N) (Fig. 1). The experiment was carried out under field conditions for seven months from October to May. The cultivation of native potatoes was similar to agricultural practices in the high Andean zones. The experimental plot covered an area of 500 square meters (10 m wide by 50 m long) and had not been cultivated for a period of five years. The soils had a loamy sandy texture with an average effective depth of 25–30 cm and a terrain slope of 25%. Farmers grow the native potato in soils whose pH ranges from strongly acidic to slightly acidic (3.62 – 6.36) [48], in cold and humid climates, with an annual precipitation ranging from 700 to 1200 mm. The temperature is variable throughout the year, it has a mean annual temperature of 8.9 ∘C, the months of June to August have the lowest values. The annual rainfall is 929.3 mm, with the rainiest months being from December to March and the least rainy from May to July. During the rainy season, high precipitation values are associated with low minimum daily temperatures [49,50].

The experiment consisted of a control group (without the use of fertilizer) and four treatments (poultry manure, PM; alpaca manure, AM; island guano, IG and inorganic fertilizer NPK 20–20–20, IF). This allowed us to assess the effects on the accumulation of heavy metals in two varieties of native potatoes (*Solanum tuberosum* L.), "Peruanita" and "Huayro". We implemented a randomized complete block design with three replicates (Table S1). The number of tuber seeds per block per treatment was 20 (10 seeds per row), with four treatments plus the control, three replications, and two varieties of native potatoes, totaling 600 seeds (300 seeds of "Peruanita" and 300 seeds of "Huayro").

2.2. Planting, fertilization and sample collection

Planting took place in October 2021 with the participation of local farmers. The soil was tilled to a depth of 15 cm, and a seed was placed in each hole, which was then covered with the same block of soil that was removed. The spacing between rows was 60 cm, and there was a 20 cm distance between seeds. Two rows were planted for each treatment, with 10 seeds in each row. In November, the treatments (fertilization) were applied (Table S2). This involved applying 250 g of organic fertilizer and 100 g of inorganic fertilizer between each tuber, making sure that the fertilizer did not come into direct contact with the seed. After two months, the second round of fertilization with the same treatments and doses was applied. The fertilizer doses that farmers mostly practice in the cultivation of native potatoes were applied. The crop relied solely on rainwater for irrigation. In January and February, to prevent pest attacks, the insecticides Fipronil 200 g/L and Cypermethrin 300 mL were

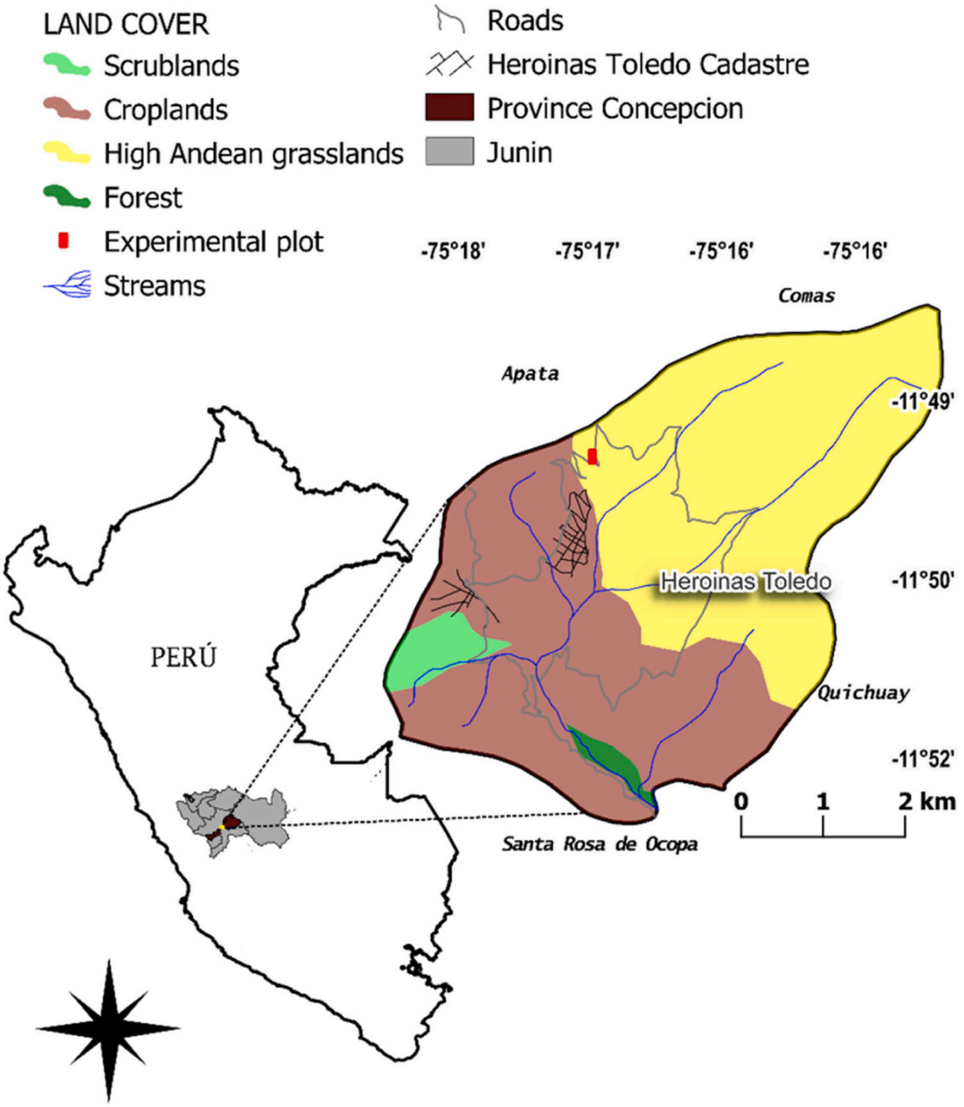

Fig. 1. Location of the experimental plot.

applied to the aerial part of the crop. After seven months from planting, soil and native potato tuber samples were collected (three replicates for each treatment).

For each treatment and crop, five composite samples of native potato, and soil samples were also collected from a depth of 0–20 cm from the topsoil, making a total of 30 samples of native potato (15 of "Peruanita" and 15 of "Huayro) and 30 soil samples. Composite samples (1.5 kg) were obtained after separately mixing six subsamples of native potato, and another six of soil taken at random from the two rows for each treatment. All samples were placed in hermetically sealed polyethylene bags, which were labeled and transferred to the laboratory.

2.3. Preparation and chemical analysis

Soil samples were dried at room temperature, crushed, and sieved with a 200-μm mesh sieve. Finally, 200 g of soil were sent to the Soil, Water and Foliar Laboratory of the National Institute of Agrarian Innovation (INIA) (12°04'35.1"S 76°56'42.8"W) for the determination of Cd, Cr, Cu, Fe, Pb y Zn. Soil samples (0.5 g) were accurately weighed, placed in a digestion vessel, and then digested with 10 mL of HNO_3 [51]. Concentrations of Cd, Cr, Cu, Fe, Pb and Zn were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Perkin Elmer, NexION 2000X, USA).

Native potato samples were washed three times with distilled water and dried in a drying oven at 70°C for 3 days. The material was crushed with a mechanical pulverizer, the edible portion (potato tubers) including the peel and pulp was used. Each sample (0.5 g) was weighed and digested in 2 mL of HNO_3 and 6 mL of HCl in a block digester at a temperature of 95°C for 30 minutes. Once the solution had cooled, its volume was adjusted to 50 mL with distilled water. The concentrations of heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) in the extracts obtained were determined by ICP-MS following the methods developed by INIA. To ensure the reliability of the research results, blank and duplicate samples were analyzed. The accuracy and precision of element determination was less than 10% (deviation and relative error). The values of the detection limits of the metals studied for soil and native potato are shown in Table S3.

Soil pH was measured using a pH meter with a 1:1 soil: water ratio according to the method suggested by USEPA 9045D [52]. Electrical conductivity was determined in an aqueous soil extract according to ISO 11265:1994 [53]. The soil texture was determined by the Bouyoucos method [54]. The organic matter content was quantified according to the Walkley and Black method [55]. Available phosphorus using the Bray and Kurtz procedure and available potassium in soils using the ammonium acetate saturation method [56].

2.4. Assessment of contamination and accumulation factors

To understand the effect of fertilizer incorporation into the soil, the following parameters were evaluated: Contamination Factor (CF), which assesses the degree of contamination of individual heavy metals in soils in relation to their reference values [57–59]. It was calculated using the Eq. (1):

$$CF = \frac{C_{soil}}{C_{ref-soil}} \tag{1}$$

where C_{soil} *and* $C_{ref\text{-}soil}$ represent the heavy metal contents in soil samples and their control reference value (control), the CF was classified as follows: no contamination ($CF < 1$); low contamination ($1 \leq CF < 2$); moderate contamination ($2 \leq CF < 3$); high contamination ($3 \leq CF < 5$) and extremely high contamination ($CF \geq 5$) [58].

To understand the origin of heavy metals accumulated in native potato tubers, this was assessed using the enrichment factor (EF), which compares the concentration of a metal accumulated in plants with that of soils as studied by Ugulu et al. (2020) [10]. The EF was determined using Eq. (2):

$$EF = \frac{C_{tuber}/C_{ref-tuber}}{C_{soil}/C_{ref-soil}} \tag{2}$$

where C_{tuber} and C_{soil} represent the metal concentrations in tuber and soil samples, and $C_{ref-tuber}$ and $C_{ref-soil}$ represent the reference concentrations of metals in tubers and soil, respectively [10]. Peru does not have a standard regulating the content of heavy metals in food, so limit values (standard concentrations of metals) for tubers were used as established by FAO/WHO (2015) [60] and those suggested by Romero-Crespo et al. [61] and Khan et al. [25] (Table S4). Likewise, the standard concentration values for Cd, Cr, Cu, Fe, Pb and Zn in the soil samples, as suggested by Taylor and McLennan [62] (Table S4) were used. To calculate the EF of each metal, Fe was used as a reference element [33]. If the EF approaches unity, it is likely that the parental material is the predominant source of the element; if the EF exceeds 10, the element has a significant contribution from anthropogenic sources [34].

The bioconcentration factor (BCF) was employed in this study to assess the potential migration of heavy metals from the soil to the tuber tissues [63]. The BCF was calculated using Eq. (3) [64]:

$$BCF = \frac{C_{tuber}}{C_{soil}} \tag{3}$$

where C_{tuber} and C_{soil} are the content of heavy metals in potato tubers and soils (mg kg^{-1}), respectively.

2.5. Health risk assessment

The estimated daily intake (EDI) of the risk elements was calculated using Eq. (4) [65,66]:

$$EDI = \frac{C_{tuber}xC_{f}xIR_{tuber}xEFxED}{BWxAT} \tag{4}$$

where: C_{tuber} is the concentration of metals in native potatoes (mg kg^{-1}); C_f is the conversion factor (0.085) for fresh vegetables to dry weight [66]; IR_{tuber} represents the average daily consumption rate of native potatoes in the highland areas (0.15 kg day^{-1} for children and 0.45 kg day^{-1} for adults) [67]; *EF* is the exposure frequency (365 days per year); *ED* is the duration of exposure (70 years for adults and 6 years for children) [68]; *BW* is the average body weight (18 kg for children and 64 kg for adults) [67]; *AT* is the average time for non-cancer risks (365 days × *ED*).

In the assessment of risks to human health, the target hazard quotient and target cancer risk were evaluated in association with heavy metals through dietary exposure pathways [57,68–70]. THQ (target hazard quotient), TTHQ (total target hazard quotient), TCR (target cancer risk), and TTCR (total target cancer risk) were calculated using Eqs. (5)–(8):

$$THQ = \frac{EDI}{RfD} \tag{5}$$

$$TTHQ = HI = \sum_{i=1}^{n} THQ \tag{6}$$

$$TCR = EDIxSF \tag{7}$$

$$TTCR = \sum_{i=1}^{n} TCR \tag{8}$$

where EDI refers to the estimated daily intake through ingestion in mg $kg^{-1}day^{-1}$. RfD is the reference dose for each metal in mg $kg^{-1}day^{-1}$. The RfD values for Cd, Cr, Cu, Fe, Pb, and Zn are 0.0001, 0.003, 0.04, 0.7, 0.004 and 0.3 mg $kg^{-1}day^{-1}$ respectively [71]. THQ represents the non-cancer risk of individual elements. TTHQ is the non-cancer risk of multiple elements, expressed as the sum of THQ values. TTHQ> 1 indicates an increasing non-cancer risk, and vice versa for TTHQ< 1. TCR refers to the cancer risk of toxic individual elements. SF is the cancer slope factor for each chemical element studied. The SF values for Cd, Cr, and Pb are 0.6, 0.5 and 0.0085 mg $kg^{-1}day^{-1}$ respectively [71]. TTCR is the cumulative carcinogenic risk. The acceptable range of cancer risk is between 10^{-6} and 10^{-4}. Values exceeding 10^{-4} indicate significant effects and risks of developing cancer, while values below 10^{-6} are considered non-significant for health effects.

2.6. Statistical analysis

To assess the variation in heavy metal content in soil, tubers, the bioconcentration factor (BCF), and the enrichment factor (EF) among different treatments (PM, IG, AM, and IF), a generalized linear mixed model (GLMM) with the Gamma family and a "log" link function was used. This choice was made because the frequency distribution of the data was positively skewed, it was necessary to include treatment as a random factor [72]. Additionally, Tukey's post hoc test ($p \leq 0.05$) was applied to compare the mean differences among the various treatments. All analyses and graphs were developed using the R platform (R Development Core Team 2015, version 4.3.3). The GLMM analysis utilized the lme4 package (including effects and lmerTest) [73], while packages investr and ggplot2 were employed for generating graphs [74]. The plyr package was used for time series analysis [75].

3. Results and discussion

3.1. Physical and chemical properties of soils

In all samples, the soil exhibited a sandy loam texture (PM, AM, IG, IF and the control treatment) (Table 1), providing good drainage, easy aeration, and high nutrient levels, making it ideal for agricultural purposes [76]. The pH of the soils studied varies from 4.4. to 4.8, classified as very strongly acidic according to the United States Department of Agriculture (USDA) [77]. However, the use of mineral fertilizers and the application of fresh organic matter could potentially modify the pH levels [78]. For instance, the application of organic fertilizers during cultivation slightly raised the soil pH. In the PM and IG treatments, the pH increased from 4.6 to 4.8. Nevertheless, Mendoza-Dávalos [79] reported different results when using island guano as an amendment in highland soils, where it reduced the pH from 4.64 to 4.56 at 4050 m above sea level (m.a.s.l) and from 4.26 to 4.04 at 4000 m.a.s.l altitudes.

Soils fertilized with AM and IF showed slight decreases in pH (4.5 and 4.4, respectively) compared to the pH of the control treatment (4.6), similar results were reported by Derakhshan et al. [80] using rice husk and maple leaves amendments. Electric conductivity (EC) values were

Table 1
Physical and chemical properties of soil with amendments. EC electrical conductivity, OM organic matter, AM alpaca manure, IG island guano, IF inorganic fertilizer, PM poultry manure.

Parameters	Units	Treatments				
		Control	PM	AM	IG	IF
Texture		Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Sand	(%)	62	62	60	68	60
Silt	(%)	31	29	35	25	37
Clay	(%)	7	9	5	7	3
pH		4.6	4.8	4.5	4.7	4.4
EC	dS m^{-1}	0.072	0.087	0.070	0.073	0.119
OM	(%)	9.7	10.7	9.7	9.8	8.3
N	(%)	0.49	0.54	0.49	0.49	0.42
P	(mg kg^{-1})	9.5	20.6	11.5	13.6	13.8
K	(mg kg^{-1})	190.0	422.9	211.6	294.2	193.0

relatively low, ranging from 0.07 to 0.12 dS $^{m-1}$, and did not exceed the regulatory limit of 2 dS $^{m-1}$. These findings align with the results reported by Jalali et al. [11]. However, Taeprayoon et al. [81] reported EC values exceeding 2 dS $^{m-1}$. This level of salinity does not adversely affect crop growth. Furthermore, the addition of PM slightly increased the organic matter (OM) content from 9.7% (control treatment) to 10.7%. The IG and AM fertilizers did not significantly alter the OM content, while the IF treatment recorded lower OM values than the control treatment. Regarding nutrient availability, soils amended with PM showed the highest availability of N, P, and K, followed by the availability of P in soils treated with IF and IG. Additionally, IG and AM treatments exhibited higher available K levels. In general, organic amendments increased the concentrations of essential nutrients such as available N, P, and K.

3.2. *Heavy metal concentration in soils*

The concentration of heavy metals in the soil varied among treatments. The treatments with IF and PM showed a higher average concentration of Cd (0.24 and 0.31 mg kg^{-1}), Cu (73.75 and 66.03 mg kg^{-1}), Pb (65.72 and 63.56 mg kg^{-1}), and Zn (212.05 and 155.75 mg kg^{-1}) respectively (Table S4). In contrast, the treatment with island guano showed the highest average content of Cr (30.60 mg kg^{-1}) and Fe (31558.67 mg kg^{-1}), while the control treatment had the lowest values. The trend in metal concentration across treatments was as follows: IF > PM > IG > AM > Control (Fig. 2). This indicates that the addition of inorganic fertilizer and organic manures (poultry and island guano) during tuber cultivation increased the levels of heavy metals in the soil. Originally, these soils, with a five-year resting period, contained moderate concentrations of these trace elements.

The average concentration values of Zn in the soil of the IF treatment exceeded the recommended standards set by Peruvian regulations [82] and the Canadian Council of Ministers of the Environment [83], with the exception of Cd, Cr, and Pb, for which values were lower than the national and international environmental quality standards. The concentrations of Cu in the soil with the application of IF and PM exceeded the

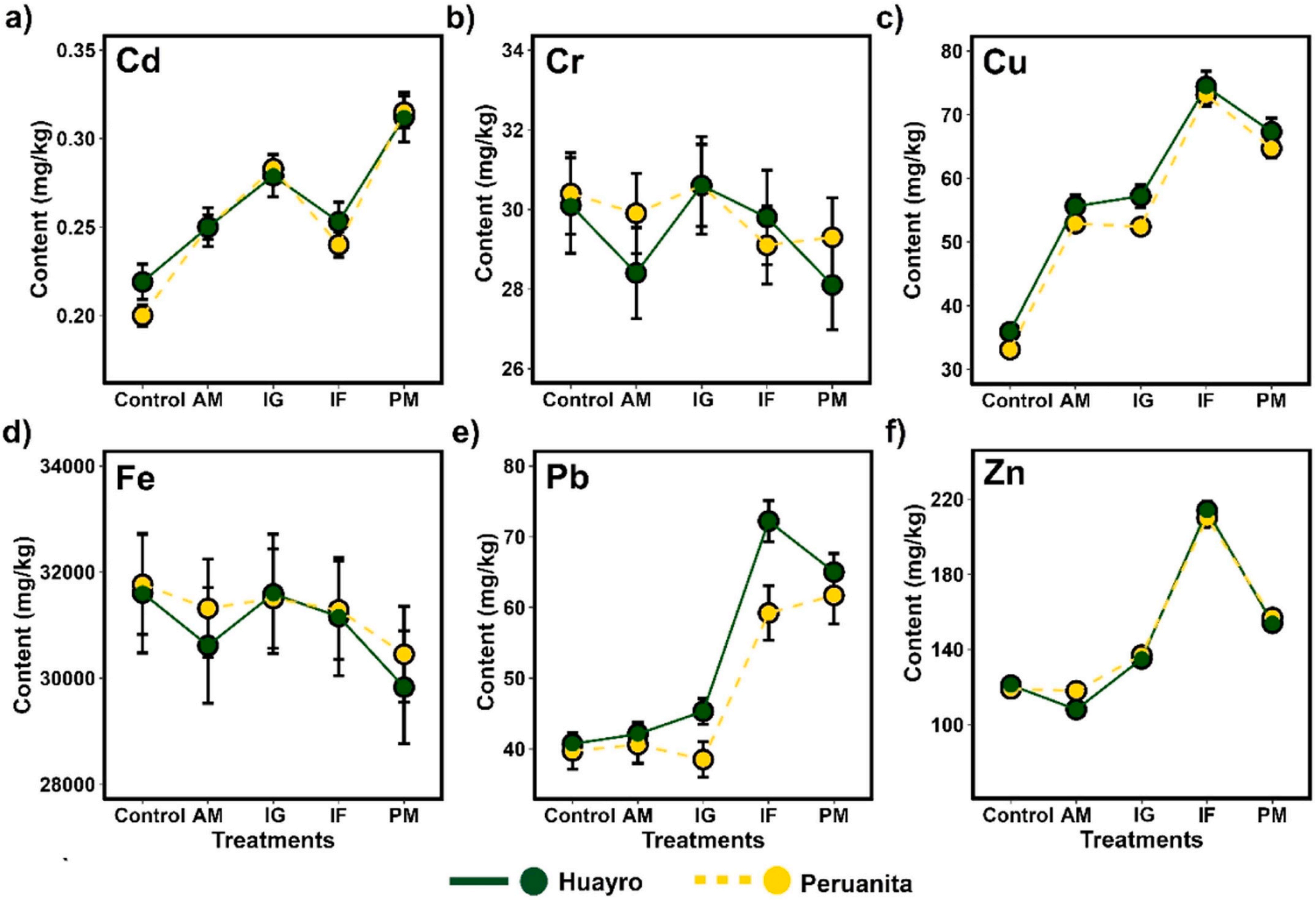

Fig. 2. Heavy metal concentration in soils under native potato cultivation according to fertilization treatments (AM alpaca manure, IG island guano, IF inorganic fertilizer, PM poultry manure).

Canadian standard. The trend in heavy metal concentrations in the soils across all treatments was Fe > Zn > Cu > Pb > Cr > Cd, which is consistent with other studies reported in the literature [10,67,84]. The presence of heavy metals (Cu, As, Zn, Cd) in animal manure could be related to the diet of the animals, due to the excessive application of additives to animal's feed to improve growth, increase weight, food efficiency, reproduction of the animals [44,45]. In China and several other countries, the application of livestock and poultry manure has been identified as the predominant source of heavy metals in agricultural soils [85]. The combination of inorganic fertilizers, poultry manure, and pesticides likely contributed to the accumulation of heavy metals in these soils [70,86]. On the other hand, organic matter is a crucial factor affecting the availability of heavy metals in soils [10,11,87]. This is because of the increased surface area of clay or organic colloids with negative charges, which attract cations like metals and metalloids. In contrast, sand particles are inert as they lack charges [88].

In this study, the organic manures used in sandy loam soils with highly acidic pH did not appear to reduce the bioavailability of metals in the soil, particularly for Cd. This might be attributed to the fact that sandy soils, rich in the sandy fraction, can lose trace elements at a relatively high rate, and these elements can also be absorbed by plants [89,90]. The changes in the heavy metal content could also be attributed to the soil properties (e.g., increased clay could bind more metals than sand/silt) [91,92].

3.3. *Concentration of heavy metals in native potatoes*

The accumulation of heavy metals exhibited a similar pattern in both varieties of native potatoes (Fig. 3). The results indicate that the metals evaluated in the tubers did not exceed the maximum allowed limits, except for Pb. Poultry manure exceeded the Pb content (0.1 mg kg^{-1}) in native potato samples as regulated by FAO/WHO [60]. The accumulation of heavy metals decreased in the order of Zn (2.12–12.37 mg kg^{-1}) > Cu (0.92–3.95 mg kg^{-1}) > Fe (0.31–0.41 mg kg^{-1}) > Pb (0.008–0.194 mg kg^{-1}) > Cr (0.043–0.058 mg kg^{-1}) > Cd (0.001–0.057 mg kg^{-1}) (Table S4). The trend of metal accumulation in the native potato samples according to fertilization treatments was PM > IF > IG > AM > Control. The types of fertilizers showed significant effects on the accumulation of Cd, Cu, Pb, and Zn in the "Peruanita" variety ($p<0.05$), except for Cr and Fe, while in the "Huayro" variety, the accumulation of Cd, Cr, Cu, Fe, Pb, and Zn was influenced by organic and inorganic fertilization. PM, IF and IG had a greater impact on metal accumulation compared to other treatments in both varieties. These results could be attributed to the fact that the organic matter in the fertilizers (PM, IG, and AM) used did not significantly increase soil pH, and, therefore, did not cause the immobilization of metals in the soil, as very acidic soil conditions are favorable for the solubilization and mobilization of trace metals [89,93]. Irfan et al. [94] reported that the reduction of Pb, Cd, and Cr in maize plants was not as efficient with the application of compost.

On the other hand, the concentrations of heavy metals in the crop depend on the type of manure and animal feeding practices, as well as

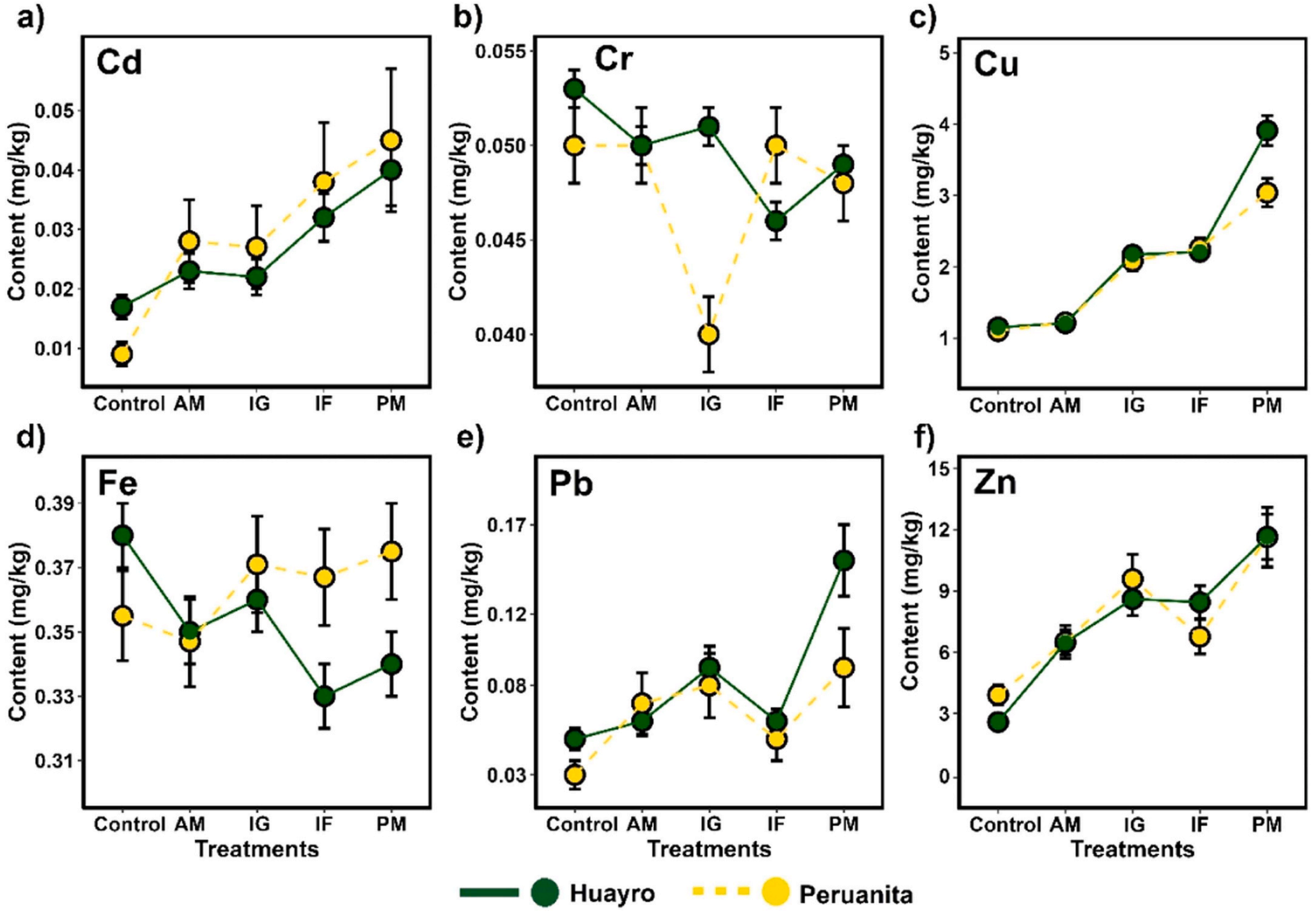

Fig. 3. Accumulation of heavy metals in native potatoes according to fertilization treatments (AM alpaca manure, IG island guano, IF inorganic fertilizer, PM poultry manure).

the physical and chemical properties of the soil, the chemical form of the metal, plant species, plant tissues, plant organs [6,95] and genetic patterns of accumulation [96]. The tubers in the control treatment recorded lower accumulation values compared to the other treatments; however, other studies have found higher values of accumulation of Cd, Zn, Cu, Pb, Fe, and Cr in non-native potato pulp [25,97]. Furthermore, potatoes grown at higher altitudes accumulate fewer metals than those grown at lower altitudes in cold and dry climate zones [98], because Andean farmers traditionally let their land rest for 3–5 years between harvesting one crop and planting the next. These results could indicate that the tubers absorbed these elements from the soil during their growth and development (seven months), influenced by the strongly acidic pH and high organic matter content in the soil.

3.4. *Assessment of pollution and accumulation factors*

The evaluation of soil contamination by heavy metals based on organic and inorganic fertilization was conducted using the contamination factor (CF). The measured CF values for Cr and Fe were below 1 in all four treatments and in the control soil, which could indicate that the soils in the experimental plot were not contaminated with these metals. On the other hand, soils amended with IG and AM showed low contamination levels for Zn, IG for Pb, and the control soil for Cu, Pb, and Zn. Soils with the application of AM, IG, and IF exhibited a moderate level of contamination for Cd, Cu, Pb, and Zn, while PM showed a high level of contamination for Cd and Pb (Table 2). The contribution of metals according to the type of amendment was as follows: IF > PM > IG > AM > Control, and the heavy metal contribution to the soil followed the trend: Cd > Pb > Cu > Zn > Fe > Cr. Therefore, the application of poultry manure, cattle manure, fertilizers, and pesticides contributes to soil contamination with elements like Cd, Cu, Pb, and Zn [6,99]. Hence, it is suggested to conduct studies on the content of potentially toxic elements based on the source, type of manure, degree of decomposition, composition, quantity, and frequency of application of organic and inorganic fertilizers used by native potato producers in the high Andean regions.

The degree of metal enrichment in the tubers was evaluated using the enrichment factor (EF) [10]. Pb (0.15–0.47), Zn (0.11–0.26) and Cd (0.08–0.15) exhibited higher enrichment values among the four treatments, while the lowest values were observed for Cr (0.024–0.026) and Fe (0.0008–0.0009) (Table 2). In the PM treatment, the highest EF values were seen for Pb and Zn, followed by the IG treatment. In the IF treatment, higher EF values were observed for Cd compared to the control treatment. The EF values were below 1 in all treatments and the

Table 2
Soil contamination factor (CF) and enrichment factor (EF) and bioconcentration factor (BCF) in tubers according to fertilization treatments. Difference of heavy metals between treatments (PM, AM, IG, IF and Control). Different letters are significantly different at $p \leq 0.05$ with Tukey's test after GLMM ($n = 3$ replicates for each treatment).

	Variety	Treatments	Cd	Cr	Cu	Fe	Pb	Zn
Bioconcentration factor (BCF)	Peruanita	PM	0.145 ± 0.04^{a}	$0.0016\pm7.19\text{E-}05^{a}$	0.047 ± 0.003^{a}	$1.24\text{E-}05\pm5.07\text{E-}07^{a}$	0.001 ± 0.0003^{ab}	0.073 ± 0.008^{a}
		AM	0.113 ± 0.03^{ab}	$0.0017\pm7.32\text{E-}05^{a}$	0.023 ± 0.001^{c}	$1.24\text{E-}05\pm4.55\text{E-}07^{a}$	0.002 ± 0.0004^{a}	0.055 ± 0.006^{a}
		IG	0.094 ± 0.02^{ab}	$0.0016\pm7.03\text{E-}05^{a}$	0.039 ± 0.003^{ab}	$1.24\text{E-}05\pm4.84\text{E-}07^{a}$	0.002 ± 0.0005^{a}	0.069 ± 0.008^{a}
		IF	0.162 ± 0.04^{a}	$0.0017\pm7.40\text{E-}05^{a}$	0.03 ± 0.002^{b}	$1.24\text{E-}05\pm4.84\text{E-}07^{a}$	0.001 ± 0.0002^{bc}	0.032 ± 0.003^{a}
		C	0.048 ± 0.01^{b}	$0.0016\pm7.12\text{E-}05^{a}$	0.033 ± 0.002^{b}	$1.24\text{E-}05\pm4.59\text{E-}07^{a}$	0.001 ± 0.0002^{c}	0.032 ± 0.003^{a}
	Huayro	PM	0.129 ± 0.01^{a}	$0.002\pm9.73\text{E-}05^{a}$	0.058 ± 0.001^{a}	$1.13\text{E-}05\pm5.70\text{E-}07^{a}$	0.002 ± 0.0003^{a}	0.075 ± 0.007^{a}
		AM	0.094 ± 0.01^{a}	$0.002\pm9.76\text{E-}05^{a}$	0.021 ± 0.0006^{e}	$1.15\text{E-}05\pm5.80\text{E-}07^{a}$	0.001 ± 0.0002^{ab}	0.059 ± 0.005^{a}
		IG	0.081 ± 0.01^{a}	$0.002\pm9.28\text{E-}05^{a}$	0.028 ± 0.001^{b}	$1.14\text{E-}05\pm5.73\text{E-}07^{a}$	0.002 ± 0.0002^{ab}	0.063 ± 0.006^{a}
		IF	0.129 ± 0.01^{a}	$0.001\pm8.69\text{E-}05^{a}$	0.029 ± 0.0009^{d}	$1.06\text{E-}05\pm5.34\text{E-}07^{a}$	0.001 ± 0.0001^{c}	0.039 ± 0.003^{b}
		C	0.08 ± 0.01^{a}	$0.0012\pm9.84\text{E-}05^{a}$	0.032 ± 0.001^{c}	$1.23\text{E-}05\pm6.16\text{E-}07^{a}$	0.001 ± 0.0001^{bc}	0.021 ± 0.002^{c}
Contamination factor (CF)	Peruanita	PM	3.21 ± 0.09^{a}	0.83 ± 0.028^{a}	2.59 ± 0.023^{b}	0.87 ± 0.026^{a}	3.09 ± 0.2^{a}	2.22 ± 0.04^{b}
		AM	2.55 ± 0.07^{c}	0.85 ± 0.028^{a}	2.12 ± 0.026^{c}	0.89 ± 0.026^{a}	2.03 ± 0.13^{b}	1.67 ± 0.03^{d}
		IG	2.89 ± 0.08^{b}	0.87 ± 0.029^{a}	2.1 ± 0.026^{c}	0.9 ± 0.027^{a}	1.92 ± 0.12^{b}	1.93 ± 0.04^{c}
		IF	2.45 ± 0.07^{c}	0.83 ± 0.028^{a}	2.93 ± 0.026^{a}	0.89 ± 0.026^{a}	2.96 ± 0.19^{a}	2.95 ± 0.06^{a}
		C	2.04 ± 0.06^{d}	0.86 ± 0.029^{a}	1.33 ± 0.026^{d}	0.9 ± 0.027^{a}	1.98 ± 0.12^{b}	1.68 ± 0.03^{d}
	Huayro	PM	3.19 ± 0.14^{a}	0.804 ± 0.032^{a}	2.69 ± 0.08^{b}	0.853 ± 0.02^{a}	3.25 ± 0.13^{a}	2.17 ± 0.04^{b}
		AM	2.55 ± 0.11^{c}	0.81 ± 0.032^{a}	2.23 ± 0.07^{c}	0.877 ± 0.03^{a}	2.11 ± 0.08^{b}	1.53 ± 0.03^{e}
		IG	2.85 ± 0.13^{b}	0.873 ± 0.035^{a}	2.29 ± 0.07^{c}	0.903 ± 0.03^{a}	2.27 ± 0.09^{b}	1.9 ± 0.04^{c}
		IF	2.58 ± 0.11^{c}	0.851 ± 0.034^{a}	2.98 ± 0.09^{a}	0.891 ± 0.03^{a}	3.61 ± 0.14^{a}	3.02 ± 0.06^{a}
		C	2.23 ± 0.10^{d}	0.856 ± 0.034^{a}	1.44 ± 0.04^{d}	0.905 ± 0.03^{a}	2.04 ± 0.08^{b}	1.71 ± 0.03^{d}
Enrichment factor (EF)	Peruanita	PM	0.14 ± 0.03^{a}	0.025 ± 0.001^{a}	0.029 ± 0.002^{a}	-	0.30 ± 0.07^{ab}	0.26 ± 0.03^{a}
		AM	0.11 ± 0.03^{ab}	0.025 ± 0.001^{a}	0.014 ± 0.001^{c}	-	0.36 ± 0.09^{a}	0.19 ± 0.02^{a}
		IG	0.09 ± 0.02^{ab}	0.024 ± 0.001^{a}	0.024 ± 0.002^{ab}	-	0.40 ± 0.10^{a}	0.24 ± 0.02^{a}
		IF	0.15 ± 0.04^{a}	0.026 ± 0.001^{a}	0.019 ± 0.001^{b}	-	0.17 ± 0.04^{bc}	0.11 ± 0.01^{b}
		C	0.05 ± 0.01^{b}	0.025 ± 0.001^{a}	0.020 ± 0.001^{b}	-	0.16 ± 0.04^{c}	0.11 ± 0.01^{b}
	Huayro	PM	0.12 ± 0.01^{a}	0.026 ± 0.001^{a}	0.036 ± 0.0008^{a}	-	0.47 ± 0.06^{a}	0.26 ± 0.02^{a}
		AM	0.09 ± 0.01^{a}	0.026 ± 0.001^{a}	0.013 ± 0.0004^{e}	-	0.31 ± 0.04^{ab}	0.21 ± 0.02^{a}
		IG	0.08 ± 0.01^{a}	0.025 ± 0.001^{a}	0.023 ± 0.0007^{b}	-	0.41 ± 0.05^{ab}	0.22 ± 0.02^{a}
		IF	0.12 ± 0.01^{a}	0.024 ± 0.001^{a}	0.018 ± 0.0005^{d}	-	0.15 ± 0.02^{c}	0.14 ± 0.01^{b}
		C	0.07 ± 0.01^{a}	0.027 ± 0.001^{a}	0.020 ± 0.0006^{c}	-	0.25 ± 0.03^{bc}	0.07 ± 0.007^{c}

control soil, indicating low absorption of metals by the tubers. This is in a soil categorized by CF as moderately to highly contaminated with elements like Cd, Pb, and Zn. Therefore, there is no risk of accumulation of toxic metals in native potatoes.

Furthermore, the bioconcentration factor (BCF) is one of the indicators that determine human exposure to toxic metals throughout the biomagnification process. In general, the BCF of the metals studied in the two potato varieties, according to fertilizer treatments, varied in decreasing order as follows: Cd > Zn > Cu > Pb > Cr > Fe, with averages ranging from 0.048±0.01–0.145±0.04, 0.021±0.002–0.075±0.007, 0.021±0.001–0.058±0.001, 0.001±8.69E-05–0.002±9.28E-05, 0.0010 ±8.69E-05–0.0017±7.32E-05 and 0.000010±5.34E-07–0.000012 ±4.84E-07 respectively. These results indicate that the tubers were slightly enriched with Cd, and that this element had the highest bioavailability, followed by Zn and Cu, while Fe had a lower value. The bioavailability of Cd and other metals is reduced in neutral to alkaline soils (pH > 6.5) [58], which did not occur in this study.

In the present study, all the BCF values determined were below 1, and this finding indicates that the transfer of metals from the soil to native potatoes is low in the experimental area. Shi et al. [64] found similar BCF values for Zn, Cu, Pb and Cr in potatoes, suggesting that the potato's ability to absorb metals is very low (BCF ≤ 1), and it does not accumulate them in its tissues [100]. The higher the BCF, the greater the transfer of heavy metals from the soil to plant tissues [66]. Therefore, metal absorption is influenced by soil characteristics (texture, pH, organic matter content), crop type, metal properties, and interactions of elements in the soil solution [90,99].

The BCF values for Cd, Cu, Pb, and Zn according to types of fertilizers were significant ($p< 0.05$). PM had higher BCF values for Cd, Cu and Zn, followed by IF, IG and AM (Table 2). Other studies using poultry manure and chemical fertilizers reported higher BCF values (BCF > 1) [86,101]. However, in the cultivation of *Beta vulgaris* treated with poultry manure, they found minimum BCF values [90] due to the low availability of metals in the soil as a result of reducing their forms available for uptake.

3.5. Assessment of human health risk

The estimated daily intake (EDI) through the ingestion route of the studied metals for children and adult populations revealed a decreasing trend of Zn > Cu > Fe > Pb > Cr > Cd. The intake of Zn, Cu, and Fe from the native potatoes "Peruanita" and "Huayro" was higher in children and adults, but the consumption of toxic elements Pb, Cr and Cd was lower (Fig. 4a, b, c, d and Table S5) indicating a low potential health risk.

According to the type of fertilizer, the EDI values for children and

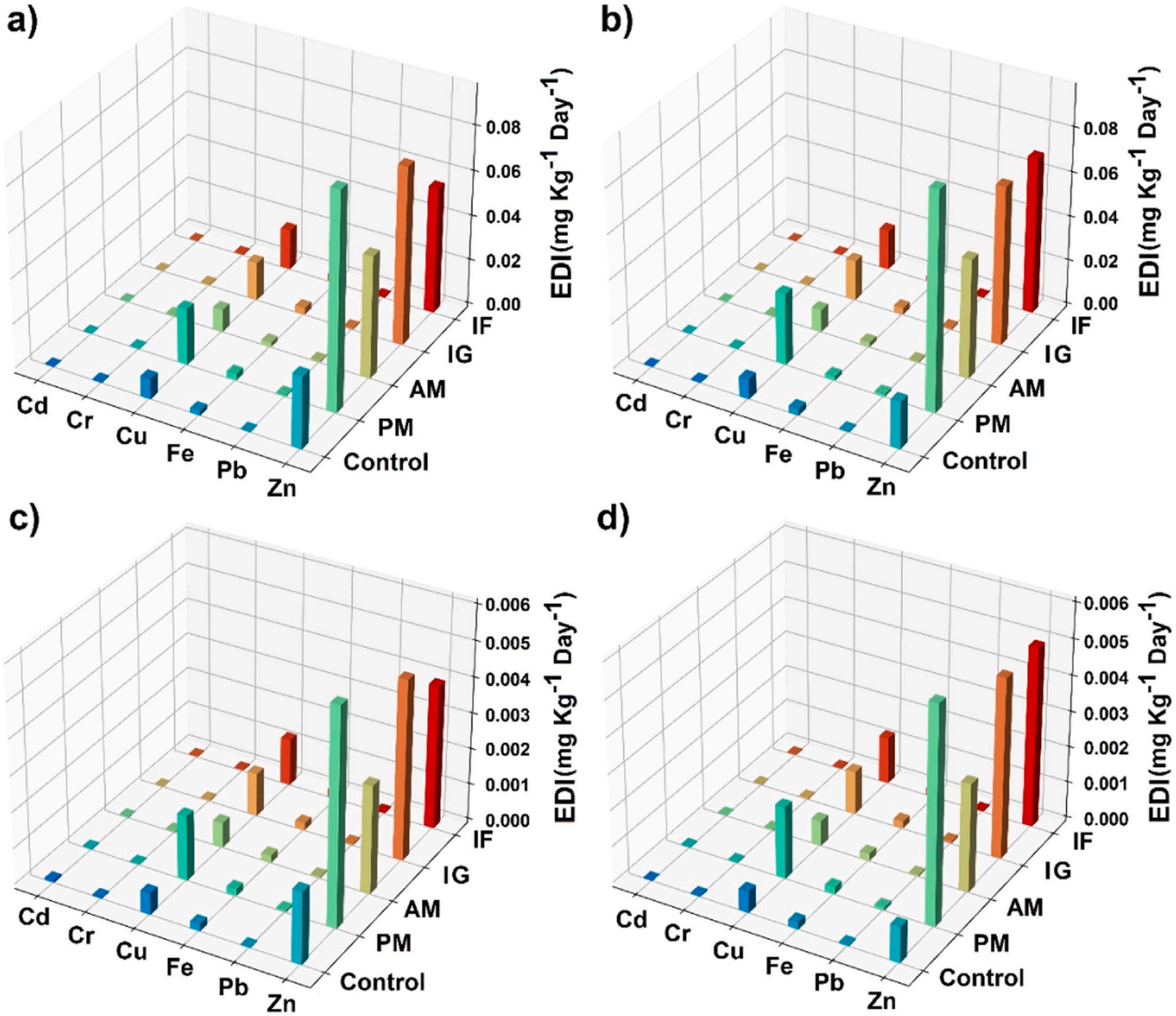

Fig. 4. Estimated daily intake (EDI) of heavy metals in children and adults according to fertilization treatments and native potato variety. a) EDI children "Peruanita," b) EDI children "Huayro," c) EDI adults "Peruanita," d) EDI adults "Huayro.".

adults were lower than the maximum tolerable daily intake (MTDI) for Cd (0.001 mg $kg^{-1}day^{-1}$), Cr (0.2 mg $kg^{-1}day^{-1}$), Cu (10 mg $kg^{-1}day^{-1}$), Fe (0.8 mg $kg^{-1}day^{-1}$), Pb (0.0035 mg $kg^{-1}day^{-1}$), and Zn (60 mg $kg^{-1}day^{-1}$) [102,103]. These results suggest that the application of organic and inorganic fertilizers during the cultivation of potatoes did not have negative effects on the health of local residents through the consumption of native potatoes. However, other studies [25,66] have reported much higher EDI values compared to this study. Additionally, it was found that the accumulation of metals in tubers, according to fertilization treatments, determined the variations in the daily intake of each metal.

3.5.1. Non-cancer health risk (THQ)

The use of poultry and cattle manure, inorganic fertilizers, and pesticides in cultivation contributes to the release of heavy metals, thus contaminating the soil [84]. However, for the conditions of our experiment, it was not demonstrated that there is a health risk for consumers due to exposure to heavy metals. This is because the values of THQ and TTHQ for all elements were below 1. This indicates that fertilization did not influence the THQ values in both children and adults, these results are similar to those reported in other studies [66,104,105].

The THQ values in children decreased in the order of Cd (0.068 – 0.325) > Cu (0.020 – 0.069) > Zn (0.006 – 0.028) > Pb (0.009 – 0.028) > Cr (0.011 – 0.013) > Fe (0.003 – 0.0004) respectively; in adults Cd (0.057 – 0.274) > Cu (0.017 – 0.059) > Zn (0.005 – 0.023) > Pb (0.008 – 0.023) > Cr (0.009 – 0.011) > Fe (0.002–0.003) respectively (Fig. 5). The TTHQ values according to fertilization treatments decreased in the order of PM > IF > IG > AM > Control in children and adults respectively (Table S6). The use of poultry manure and inorganic fertilizer contributed to TTHQ with 71% and 76% for Cd in both children and adults. Attention should be paid to the repeated use of poultry manure and compost, as it leads to the release and increase of chemical (heavy metals) and biological (bacteria, fungus) contaminants in agricultural soils. This is because heavy metals are non-degradable and persist in organic fertilizer [9]. The TTHQ was higher in children than in adults, indicating that children are more susceptible to adverse environmental effects, which is consistent with previous studies [57,58,70].

3.5.2. Cancer health risk (TCR)

The values of EDI were used to calculate the TCR values. The carcinogenic metals studied were Cd, Cr, and Pb, due to oral ingestion exposure to native potatoes. Previous studies consider this route as the primary route of cancer risk exposure [57,106,107]. The TCR values for children and adults through the consumption of native potatoes

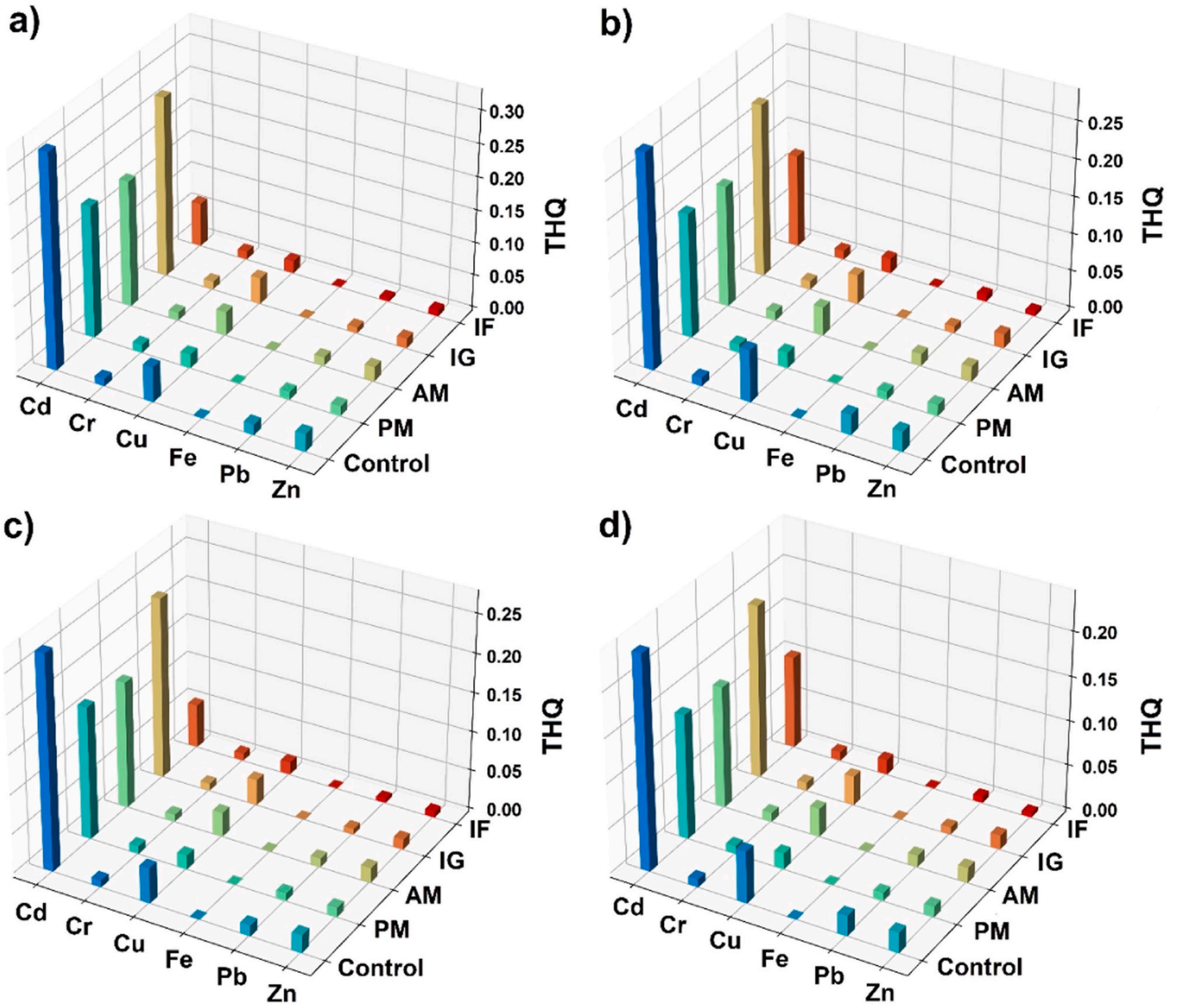

Fig. 5. Non-carcinogenic risk (THQ) associated with heavy metals in children and adults according to fertilization treatments and native potato variety. a) THQ children "Peruanita," b) THQ children "Huayro," c) THQ adults "Peruanita," d) THQ adults "Huayro".

according to fertilization treatments are presented in Table 3. There was variation in TCR values depending on the treatments, and they decreased in the order of Cr (1.66E-05 – 1.90E-05) > Cd (4.05E-06 – 1.95E-05) > Pb (2.01E-07 – 9.38E-07) in children, and in adults Cr (1.40E-05 – 1.54E-05) > Cd (3.41E-06 – 1.64E-05) > Pb (1.70E-07 – 7.92E-07).

These results revealed that the cancer risk caused by these toxic elements in the present experiment falls within an acceptable range (1×10^{-6} and 1×10^{-4}). This indicates that the risk is considered safe for the populations under investigation. However, the highest TCR values for Cr were observed in children and adults, and these corresponded to treatments with PM and IF, which could pose a carcinogenic risk for these toxic elements. Children are at a higher risk of cancer than adults and are more prone to heavy metal poisoning due to the development of their neurological systems [18]. Therefore, strategies for soil remediation should prioritize addressing the presence of Cd and Cr, as children are more vulnerable to heavy metal intoxication.

According to fertilization treatments, the TTCR values in children and adults followed the following trend PM > IF > AM > IG > control. PM and IF were the treatments that determined the high values of TTCR in both age groups. Therefore, attention should be paid to the use of poultry manure and chemical fertilizer in the cultivation of native potatoes. It is suggested to amend the soil with substances that raise the pH in order to minimize the risk of cancer in the high Andean populations that consume native potatoes daily.

4. Conclusions

The pH, organic matter content and NPK parameters of the soil were significantly affected by the application of organic and inorganic fertilizers. The trend of heavy metal concentration in the soils across all treatments was Fe > Zn > Cu > Pb > Cr > Cd. Poultry manure and inorganic fertilizer influenced higher concentrations of Cu and Zn, which exceeded recommended standards. Poultry manure showed a high level of contamination for Cd and Pb. The enrichment factor values for all the studied heavy metals were below 1, indicating poor enrichment in the soil and translocation to the tubers.

The accumulation of heavy metals in tubers decreased in the order Zn > Cu > Fe > Pb > Cr > Cd, they did not exceed the maximum recommended limits with the exception of Pb that exceeded the limit allowed by the FAO/WHO (0.1 mg kg^{-1}). The poultry manure contributed with the highest accumulation of Zn (11.62±1.30 mg kg^{-1}), Cu (3.48±0.20 mg kg^{-1}) and Pb (0.12±0.02 mg $kg^{-1)}$ in tubers. The transfer of metals from the soil to the tubers was low ($BCF < 1$), meaning that native potatoes do not accumulate heavy metals and are safe for human consumption.

Risk assessment indicates that the EDI values for children and adults according to the type of fertilizer were below the maximum tolerable daily intake. The THQ and TTHQ values for all elements were below 1, indicating a safe level for both children and adults. The use of poultry manure and inorganic fertilizer contributed to the hazard index in both children and adults. There was variation in TCR values according to treatments. The results revealed that the cancer risk caused by Cd, Cr and Pb falls within an acceptable range. However, poultry manure and inorganic fertilizer treatments had the highest TTCR values in both age groups, suggesting a long-term carcinogenic risk. It is recommended that soil properties be analyzed before applying fertilizers to the crop, and further research on soil amendments should be conducted to produce safe and healthy food.

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Author statement

The authors of the manuscript entitled: “Effect of fertilization on the accumulation and health risk for heavy metals in native Andean potatoes in the highlands of Perú”, declare that each of the members have contributed directly to the content of the work. We approve the content of the manuscript and agree that our name appears in the authorship of the manuscript. We also report that the manuscript has not been published or considered by any other journal or any other publisher.

CRediT authorship contribution statement

Violeta Quispe Coquil: Methodology, Investigation. **Roberto Carlos Cosme:** Methodology, Investigation. **Edith Pilar Orellana Mendoza:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Vladimir Camel:** Writing – review & editing, Formal analysis. **Luz Consuelo Yallico:** Methodology, Funding acquisition.

Table 3

Target cancer risk risk (TCR) and total target cancer risk (TTCR) in children and adults according to fertilization treatments and native potato variety. Difference of heavy metals between treatments (PM, AM, IG, IF and Control). Different letters are significantly different at $p \leq 0.05$ with Tukey’s test after GLMM (n= 3 replicates for each treatment).

	Variety	Treatments	Cd	Cr	Pb	TTCR
Adults	Peruanita	PM	1.64E-05±4.57E-06^{a}	1.45E-05±4.56E-07^{a}	4.81E-07±1.14E-07^{a}	3.15E-05
		AM	1.01E-05±4.57E-06^{a}	1.51E-05±5.77E-07^{a}	3.72E-07±8.84E-08^{a}	2.56E-05
		IG	9.73E-06±4.57E-06^{ab}	1.49E-05±5.71E-07^{a}	4.01E-07±9.51E-08^{a}	2.51E-05
		IF	1.39E-05±4.57E-06^{a}	1.49E-05±5.70E-07^{a}	2.71E-07±6.50E-08^{ab}	2.92E-05
		Control	3.41E-06±4.57E-06^{b}	1.50E-05±5.77E-07^{a}	1.70E-07±4.09E-08^{b}	1.86E-05
	Huayro	PM	1.44E-05±2.20E-06^{a}	1.48E-05±4.63E-07^{ab}	7.92E-07±1.03E-07^{a}	3.00E-05
		AM	8.38E-06±1.30E-06^{ac}	1.50E-05±4.68E-07^{ab}	3.38E-07±4.39E-08^{bc}	2.38E-05
		IG	8.19E-06±1.25E-06^{bc}	1.54E-05±4.81E-07^{ab}	4.72E-07±6.13E-08^{b}	2.41E-05
		IF	1.17E-05±1.79E-06^{ab}	1.40E-05±4.37E-07^{b}	2.84E-07±3.69E-08^{c}	2.60E-05
		Control	6.32E-06±9.64E-07^{c}	1.60E-05±5.01E-07^{a}	2.60E-07±3.37E-08^{c}	2.26E-05
Children	Peruanita	PM	1.95E-05±5.42E-06^{a}	1.72E-05±5.39E-06^{a}	5.70E-07±1.36E-07^{a}	3.73E-05
		AM	1.20E-05±3.34E-06^{a}	1.79E-05±6.83E-06^{a}	4.41E-07±1.05E-07^{a}	3.04E-05
		IG	1.15E-05±3.21E-06^{ab}	1.76E-05±6.76E-06^{a}	4.75E-07±1.13E-07^{a}	2.97E-05
		IF	1.65E-05±4.60E-06^{a}	1.77E-05±6.75E-06^{a}	3.21E-07±7.70E-08^{ab}	3.46E-05
		Control	4.05E-06±1.13E-06^{b}	1.78E-05±6.83E-06^{a}	2.01E-07±4.84E-08^{b}	2.21E-05
	Huayro	PM	1.71E-05±2.60E-05^{a}	1.75E-05±5.48E-07^{ab}	9.38E-07±1.22E-07^{a}	3.55E-05
		AM	9.94E-06±1.54E-06^{ac}	1.77E-05±5.54E-07^{ab}	4.01E-07±5.20E-08^{bc}	2.82E-05
		IG	9.71E-06±1.48E-06^{bc}	1.82E-05±5.70E-07^{ab}	5.60E-07±7.26E-08^{b}	2.85E-05
		IF	1.39E-05±2.12E-06^{ab}	1.66E-05±5.18E-07^{b}	3.37E-07±4.37E-08^{c}	3.09E-05
		Control	7.50E-06±1.14E-06^{c}	1.90E-05±5.94E-07^{a}	3.08E-07±4.00E-08^{c}	2.68E-05

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2024.05.006.

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