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Biotransfer of heavy metals along the soil-plant-edible insect-human food chain in Africa

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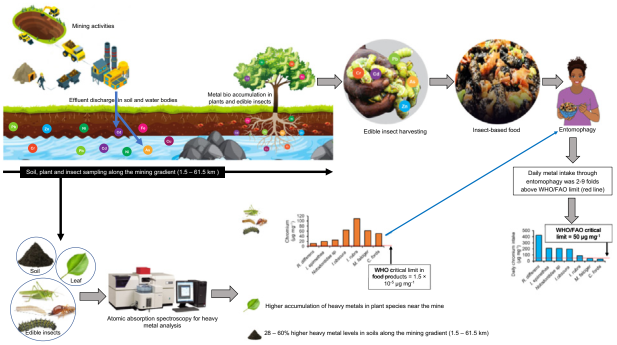
HIGHLIGHTS

- Heavy metals in soil decreased from 60 % to 28 % away from the mining zone (1.5–61.5 km).
- Soils accumulated significantly higher levels of heavy metals than plants except for Cd and Pb.
- Among the edible insects, *Cirina forda* showed higher values for Cd, Ni and Pb.
- Daily heavy metal intake through entomophagy was 2–9 folds above permissible levels.
- High dietary intakes of heavy metals remain a major safety concern for consumers in the mining zone.

GRAPHICAL ABSTRACT



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ABSTRACT

Although mining is Zambia's major economic activity, it is implicated in environmental contamination, particularly with heavy metals. This study investigated the accumulation and transfer of heavy metals along the soil-plant-edible insect-human food chain. Our results revealed the presence of eight heavy metals (Arsenic, Cadmium, Chromium, Copper, Iron, Nickel, Lead, and Zinc) with a 28–60 % increase in soil concentrations at the proximity of the mining facilities. There was a higher accumulation of Cd, Cu, Ni, Fe, Pb, and Zn than As and Cr in plant species near the mine. Among the insect species studied, *C. forda* accumulated nickel significantly higher (70–81 %), *I. obscura* had higher cadmium (2–84 %) and lead (10–79 %), while *I. rubra* and *M. falciger* accumulated higher iron (41–96 %) and zinc (1–67 %), respectively, than other insect species. The quantity of *I. obscura* consumed (248 g person^{-1} day^{-1}) was significantly higher (9–37 %) than other insect species. It was noted that the consumption of insects increased the daily intake of heavy metals, enhanced the target hazard quotient, and increased the associated health risks by up to 9 folds compared to the WHO permissible limits meaning that the daily intake of metals consumed depends on the daily quantity of insects consumed. Our findings suggest that the accumulation of heavy metals along the soil-plant-edible insect-human food chain could pose severe human and environmental health risks along the mining gradients. The potential consequences of heavy metal mobility in the consumer trophic levels and the ecotoxicological consequences are particularly concerning. Furthermore, physiological and biological studies are needed to investigate the abovementioned effects.

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1. Introduction

Globally, entomophagy has gained momentum because edible insects have better nutritional quality than conventional food sources (Magara et al., 2021; Tanga et al., 2021; Van Huis, 2013). Because of environmental, health, food security, and animal welfare concerns, alternative protein sources other than conventional meat are being considered, and insects present such an alternative as human food and animal feed (Yi et al., 2013). The consumption of insects in tropical and subtropical countries is extensively covered in the literature (Jongema, 2017; Costa-Neto, 2015; Van Huis, 2013; DeFoliart, 2005; Ramos-Elorduy et al., 1997). Over 2000 species of insects are consumed (Jongema, 2017), of which 31 % are beetles, 18 % are caterpillars, 15 % constitute wasps, bees, and ants, while 13 % are crickets, grasshoppers, and locusts, true bugs account for 11 %, while termites, dragonflies, flies, and others constitute 12 % of edible insects (Jongema, 2017).

The consumption of insects is threatened, particularly by anthropogenic pressure, including mining which may affect food safety due to the toxicity of insects due to contamination with heavy metals and allergies (Poma et al., 2017; Van Huis, 2013). Although mining provides several economic benefits (Nakayama et al., 2011; USEPA, 2009), mining activities have been implicated in environmental contamination in many parts of the world due to either their release of wastes or physical degradation of the terrain (Bian et al., 2012; Dudka and Adriano, 1997). Wastes from mining activities contain substantial, heavy metals and other toxic elements that contaminate the ecosystem's fauna and flora. For example, significant concentrations of xenobiotic elements such as lead (Pb) were found in the soil closer to the smelters in Zambia (Chungu et al., 2019; Mwaanga et al., 2019; Sikamo et al., 2016). Although other elements such as copper (Cu), zinc (Zn), and iron (Fe) are essential for plant growth and human and animal nutrition, mining activities have significantly increased their concentrations in the environment to levels beyond permissible limits (Sikamo et al., 2016; Ripin et al., 2014; Tembo et al., 2006). Because some of these elements are not degradable, their presence, especially in elevated concentrations, is a serious health issue. Their persistence has a long-term impact on the ecosystem. Their tenacity has a long-term effect on the ecosystem, threatening food safety.

Due to the long-term impact on the quality of the environment and public health, mining has enhanced strict government controls (Nakayama et al., 2011; USEPA, 2009). Considering the food chain, edible insects dwelling in contaminated ecosystems can accumulate heavy metals beyond permissible levels recommended by the World Health Organization (World Health Organization (WHO), 1996). Essential elements, including Zn, Cu, and Fe, play a significant role in the physiology of insects as long as they are within the recommended limits and have not been shown to accumulate in insect bodies. Still, metals such as Pb, Cd, chromium (Cr) and mercury (Hg) are toxic to the environment even in low concentrations (Dar et al., 2017; Diener et al., 2015) and pose health risks. Several studies have shown that high concentrations of Cd and Pb in the soil have led to higher concentrations of these metals in insects inhabiting these environments (Baiano, 2020; Murefu et al., 2019; Belluco et al., 2013).

Heavy metal contamination in insects is reasonably documented in the literature (Dobermann et al., 2017; Zhuang et al., 2009), but the impact of mining activities on edible insects, especially the wildly harvested insects in Zambia is not clear. Previous research has shown that metal concentration varies between with insect species and level of environmental contamination (Dar et al., 2017; Opaluwa et al., 2012). Heavy metals are transferred to phytophagous insects through food chains and impose toxicological effects on the growth and physiology of phytophagous insects. Recent studies have reported low concentrations of Cd and As in black soldier fly and yellow mealworm (Schrögel and Wätjen, 2019; Diener et al., 2015). However, the concentrations of heavy metals in insect species such as *Gynanisa maja*, *Gonimbrasia zambesina*, and *Macrotermes falciger* in Zambia are beyond the ‘country's regulatory limit (Kachapulula et al., 2018). Similarly, high levels of Cd and Zn were detected in insects fed on leaves exposed to different metals (Devkota and Schmidt, 2000; Jamil and Hussain, 1992). Furthermore, relatively high concentrations of heavy metals were detected in housefly (*Musca domestica* Diptera: Muscidae), dragonfly (*Libellula Luctosa* Odonata: Libellulidae), and phantom midge (*Chaoborus punctipennis* Diptera: Chaoboridae) (Butt et al., 2018; Zhang et al., 2009). Two heavy metals of greatest concern are Cd and As because of their potential to accumulate in some insects, including black soldier fly and yellow mealworm larvae, respectively, which are two main insect types that are of great interest for use as food and feed (Thrastardottir et al., 2021).

The accumulation of heavy metals in edible insects depends on many factors, including insect species, growth phase, and feed substrate (Schrögel and Wätjen, 2019; Zhuang et al., 2009). Some insect species may avoid contaminated sites, especially during oviposition. For example, females of *Drosophila melanogaster* (Diptera: Drosophilidae), *Plutella xylostella* (Lepidoptera: Plutellidae), and *Pieris rapae* (Lepidoptera: Pieridae) avoided ovipositing on heavy metal-rich plant material (Freeman et al., 2006). When ingested, an excess of metals can have a variety of impacts on insect fitness, including decreased immune response (Imathiu, 2020), extended development time (Behmer et al., 2005), and retarded growth (Noret et al., 2007; Behmer et al., 2005). However, many insect species have evolved metal tolerance or detoxification mechanisms (Janssens et al., 2009). Some species excrete metals in their faeces, limiting their metal uptake and decreasing metal transfer to higher trophic levels (Thrastardottir et al., 2021).

Consumption of insects contaminated with heavy metals may lead to serious health concerns. Retarded growth, impaired psychosocial behavior, and a weakened immune system have been reported in people who consumed insects contaminated with Cd and Pb (Alissa and Ferns, 2011). Furthermore, bone damage has been linked to Cd ingestion (USEPA, 2009), and this metal accumulates more easily in plants (Dar et al., 2017) than in insects. Although Zn, Cu, and Fe are essential for human nutrition, high concentrations above the limits set by WHO may be harmful (WHO, 1996). The increasing demand for food safety has motivated research regarding the risk associated with consuming food, including edible insects contaminated with xenobiotic elements (Belluco et al., 2013). The presence of high concentrations of heavy metals in the environment due to intensive mining activities in the Copperbelt province has raised concern about the safety of edible insects and food plants growing in Zambia (Nakayama et al., 2011).

Several studies have shown that the concentration of heavy metals in the soil is highest near emission sites (Chungu et al., 2019; Karadjova and Markova, 2014), suggesting that contamination of the environment with heavy metals decreases with the increase in distance from the emission source. However, whether environmental contamination in soils and plants is consistent with heavy metal accumulation in edible insects is not clear, and whether the consumption of these insects poses any health risk in Zambia. Therefore, this study aimed to determine the concentration of heavy metals in various edible insects, evaluate the relationship between metals in edible insects, soils, and host plants, and assess the potential health risk associated with consuming insects harvested from contaminated environments in the country.

2. Materials and methods

2.1. Study site

The study was conducted in the Copperbelt province (13° '01' S, 27° '32' E) located in Zambia (Fig. 1), between May 2021 and February 2022 during the insect collection season. The Copperbelt province is the largest mining province in Zambia, with commercial mining dating back to the 1920s (Sinkala et al., 2018; Mwaanga et al., 2019). The province is also highly industrialized and urbanized and has a total area of 31,328 km^2 (FAO, 2016; Nel et al., 2017). The Copperbelt province experiences a cold–dry season from May to June, a hot–dry season from August to October and a tropical summer rainy season from November to April with rainfall ranging from 1200 mm to 1500 mm per annum, and features seasonal differences with an annual temperature ranging from 7 °C to 35 °C.

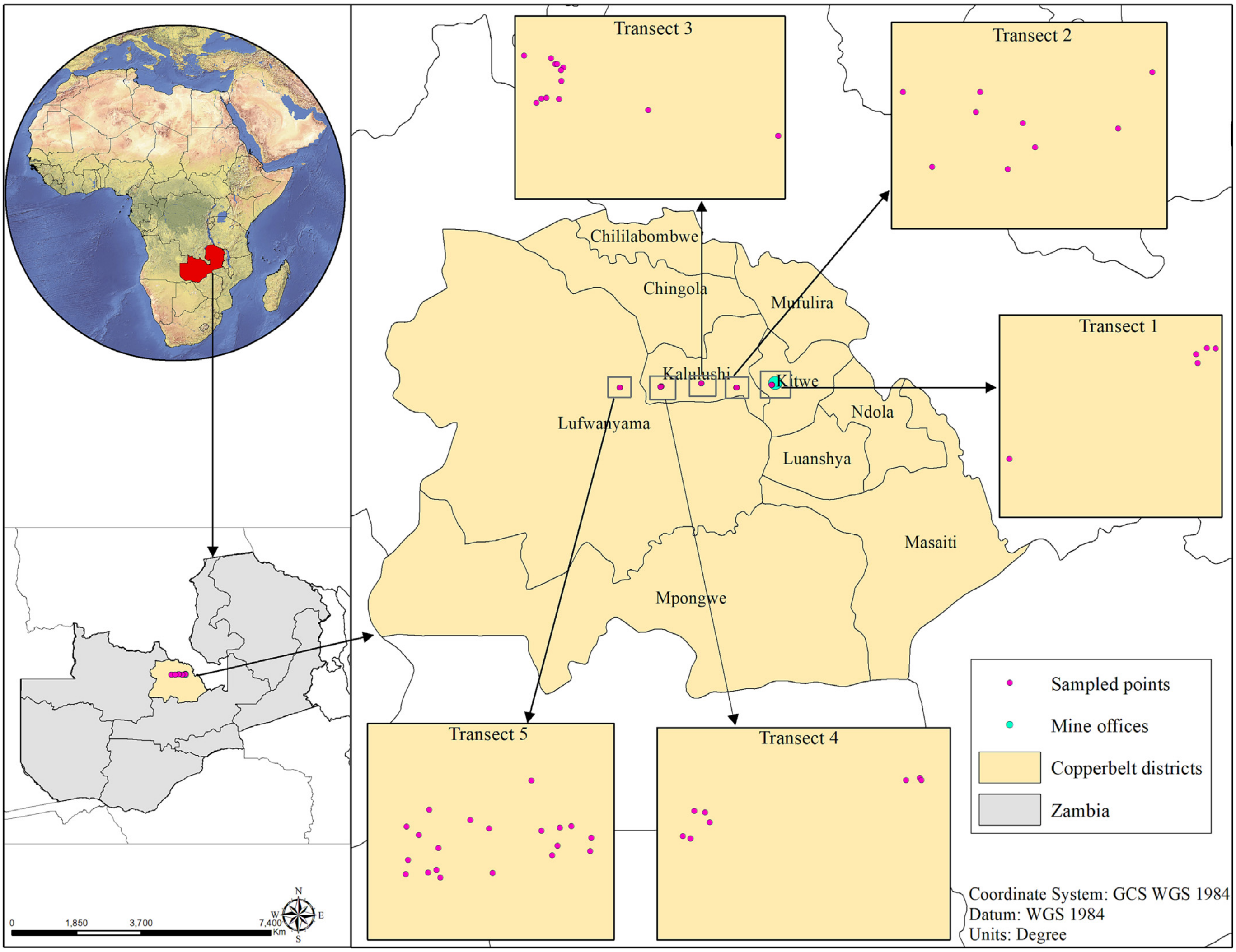

Fig. 1. Location of Copperbelt Province, mining site and transects considered during the study.

The province is dominated by strong south-easterly winds although light northeasterly winds are also common from November till February (Phiri et al., 2022). Soils are characterized as Oxisol subgroup Rhodic Haplustox (Soil Survey Staff, 2010) which is highly acidic and usually contaminated with several heavy metals with copper reported as a dominant soil contaminant in the area (Ettler et al., 2011).

2.2. Collection of edible insects, host plants, and soil samples

Edible insects were sampled randomly from the collection sites in the Copperbelt Province. This was done by collecting samples at distances of 1.5 km, 15, 16.5, 31.5, 46.5 and 61.5 km from the mine (Fig. 2).

At each site, an 800 m transect perpendicular to the contamination gradient (wind direction) was established, and along this transect, three study plots of 30 × 30 m each were set at an interval of 200 m. The edible caterpillars, termites, grasshoppers, and other edible insects were collected in each study plot. Sweep nets were used to catch flying edible insects while crawling insects, including caterpillars, were handpicked, put in plastic vials, and then transferred to the laboratory (Azam et al., 2015). The insects captured were identified by entomologists at the Copperbelt University in Kitwe using identification keys for Zambia (Séré et al., 2018), and their edibility status was determined on-site with the help of the local people.

Fifteen soil samples were collected from each sampling distance, at 0–10 cm depth from the same study plots where the insects were captured and analyzed for concentrations of heavy metals (arsenic, copper, zinc, cadmium. Chromium, nickel, iron, and lead) using standard laboratory methods. Furthermore, leaves were randomly collected from each host plant species in the study plot to determine heavy metal concentrations. This gave a total of 75 soil samples, 280 insect samples and 200 plant samples. The insect, plant, and soil samples were transported to the laboratory at Copperbelt University in Kitwe for chemical analyses. In the laboratory, insects and plant samples were cleaned of debris using deionized water, air-dried for seven days, and oven-dried for 24 h at 105 °C. After oven drying, insect and plant samples were separately ground into powder using a motor and pestle pending heavy metal analysis. Soil samples were air-dried for seven days, ground, and sieved through a 2 mm mesh sieve, pending laboratory analysis.

2.3. Determination of heavy metal concentration in edible insects, soil, and host plants

The insects, soil, and host plant samples were analyzed for the concentrations of eight heavy metals (As, Cu, Zn, Cd, Cr, Ni, Fe, and Pb). Copper, Zn, Ni, Fe, and Pb were determined using atomic absorption spectrophotometer (AAS; model Analyst 200, PerkinElmer Inc., Shelton, USA), with the limit of detection of 0.01 mg g^{-1}, while Cadmium, As, and Cr concentrations were, on the other hand, determined using ICP-OES (Turhan et al., 2010). Following this method, 1 g each of insect and plant samples

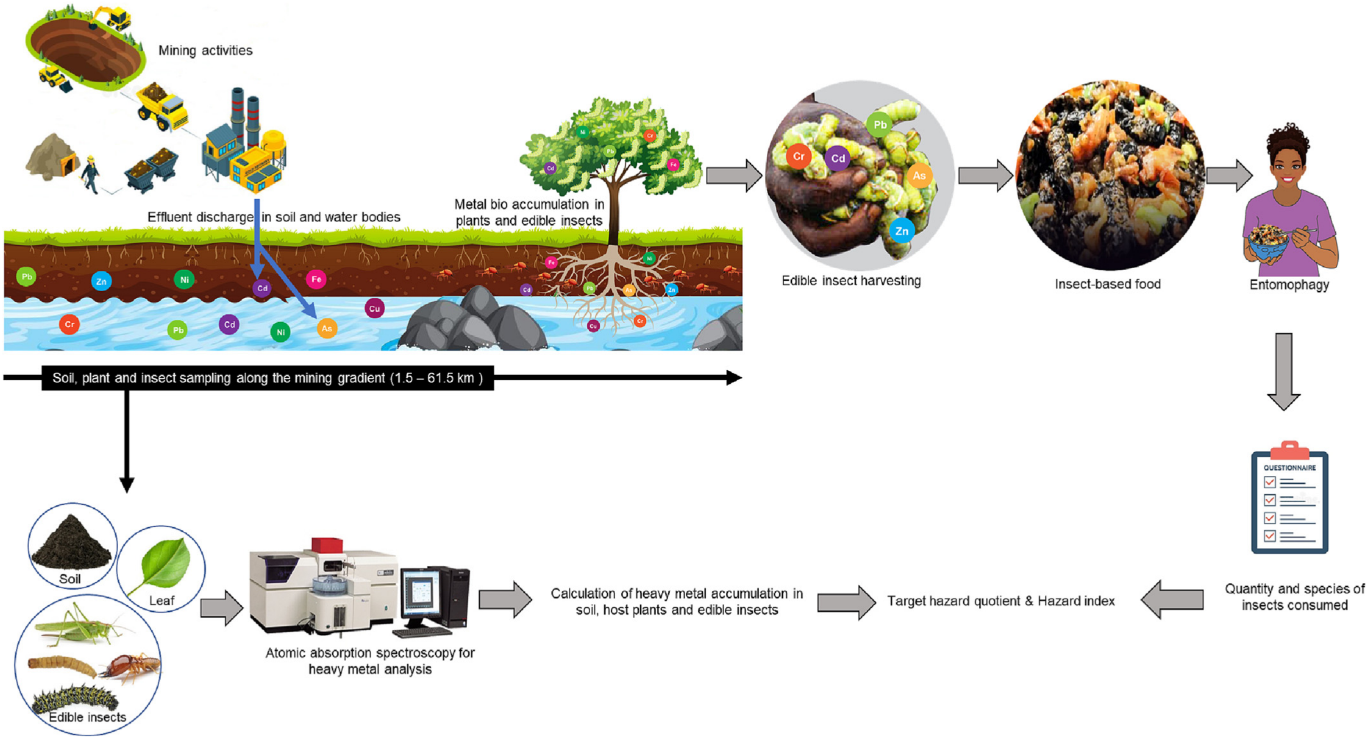

Fig. 2. Flow chart of the methodology utilized in the present study along the mining gradient.

was separately digested in 25 ml of concentrated nitric acid at 250 °C for 50 min. This was repeated with 10 ml of Perchloric acid. After cooling, 30 ml of deionized water was added, and the solution was reheated for 30 min. The solution was allowed to cool and diluted to 50 ml using deionized water pending reading on AAS. Regarding soil samples, 2 g of each soil sample was put in beakers, and then 4 drops of Hydrofluoric acid (H.F) were added to each sample. After that, 30 ml of Nitric acid was added to each sample and then heated on the hot plate for 30 min. After cooling, the samples were diluted up to 100 ml with distilled water using 100 ml conical flasks. The samples in the flasks were then thoroughly shaken and later filtered using filter papers. The filtrate was diluted up to 50 ml and aspirated on an AAS to determine concentrations of As, Cu, Zn, Cd, Cr, Ni, Fe, and Pb (Turhan et al., 2010). The wavelengths for the lamps used were 324.75, 283.31, 213.86, 248.33, and 232.00 for Cu, Pb, Zn, Fe, and Ni, respectively (Wasim et al., 2019).

2.4. Determination of heavy metal transfer factor

The uptake of heavy metals from soil to host plants and to edible insects was determined by calculating a factor for each species of edible insect and each host plant species. This factor is commonly referred to as the transfer factor (T.F.). It measures the ability and efficiency of edible insect species and host plant species to accumulate a particular heavy metal as a function of the concentration of that metal in the soil (Eq. (1)). This was done to assess the movement of heavy metals between soils, host plants, and edible insects (Chungu et al., 2019).

$$\mathrm{TF} = \frac{\mathrm{C_m}}{\mathrm{C_s}} \tag{1}$$

where,

C_m represents the concentration of heavy metal either in edible insects or host plants.

C_s represent the concentration of heavy metals in soils.

2.5. Estimated daily intake of heavy metals

The estimated daily intake (EDI) of each heavy metal was calculated as a product of daily insect consumption ($\mathrm{D_c}$) (g $\mathrm{d^{-1}}$) and mean metal concentration in each insect species ($\mathrm{C_m}$) (mg $\mathrm{kg^{-1}}$) and weighted by average adult body weight W (kg) (Eq. (2)).

$$\mathrm{EDI} = \frac{\mathrm{D_C} \times \mathrm{C_m}}{\mathrm{W}} \tag{2}$$

This procedure was repeated for every edible insect species. Dc was estimated by interviewing local people practicing entomophagy in the study site (250 adults, i.e., 56 males and 194 females) using a simple questionnaire. W, as weight, was taken to be 70.3 kg, the average weight for adults in Southern Africa, where Zambia is located (Chungu et al., 2019).

2.6. Target hazard quotient

The Target hazard quotient (THQ) is the ratio between the exposure and the reference dose of the heavy metal. To estimate THQ, the methodology described in USEPA (2001) was used. THQ was calculated using Eq. (3). If the THQ value is >1, the overall health risk of ingesting heavy metals through the consumption of food (such as insects, as in our case) is rated high and classified as harmful.

$$THQ = 0.001\,\frac{E_f \times E_d \times D_c \times C_m}{RfD \times W \times T} \tag{3}$$

where E_f is the exposure frequency (160 days $\mathrm{year^{-1}}$ was set in this study because the consumption of insects in this area increases when these insects are seasonally available. E_d is the exposure duration (which was set to be 50 years based on the average period the family has lived in the area at the time of the interview); D_c is the daily consumption of insects; C_m is the metal concentration in edible insects per species; RfD is the oral reference dose (mg $\mathrm{kg^{-1}\ d^{-1}}$), i.e. an estimated exposure per day of metal to the human body that has no hazardous effect during a lifetime, and RfD

values for Cu, Zn, Ni, Fe, Pb, Cd, Cr and As being 0.04, 0.30, 0.02, 0.0040.04, 0.001, 1.5 and 0.0003 (mg kg^{-1} d^{-1}), respectively (USEPA, 2001; Fairbrother et al., 2007). T was the average exposure time for non-carcinogens (365 days year^{-1} × E_d).

Considering that most heavy metals do not exist in isolation, the effect of pollution on the contamination of edible insects and, subsequently, health may not be attributed to one heavy metal alone. Still, a combination of all heavy metals present on the site, the pollution index for soils, host plants, and edible insects was, therefore, calculated by considering all heavy metals using Eq. (4).

$$HI = \sum \text{THQ} \tag{4}$$

where,

HI is the health index for each edible insect species, i.e., the risk posed by all the metals examined through the consumption of each examined insect.

THQ is the risk posed by a single metal.

2.7. Statistical analysis

Data normality was tested using the Shapiro-Wilk test. Analysis of variance test was performed to determine how insect metal contamination varied in soil along the mining gradient, and among host plants and edible insect species using the generalized linear model. Similarly, EDI, and THQ were separately expressed as a function of edible insect species and metals. This was done to detect the effect of edible insect species and metals on the level of exposure to contamination. The mean and standard error for the concentration of each metal (μg mg^{-1}) were calculated per site, host plant species, and edible insect species. We further computed Pearson's correlation coefficients between metal concentration in soil, in host plants, and contamination in edible insects per species to assess the strength of the relationship between metal concentration in the soil, host plants, and insect contamination. We examined the effect of mining using the hazard index as a proxy rather than each metal in the Copperbelt Province. THQ (the proxy for health risk) with generalized linear models (Quinn & Keough, 2002; Nieuwenhuis et al., 2012) using R version 4.1.0 (R Development Core Team, 2020).

Given that the current parameters used in health risk assessment are associated with the degree of uncertainty which may lead to over estimation or underestimation of the health risk, the Monte Carlo Simulation was conducted in this study. The Monte Carlo simulation combines the uncertainty assessment with the health risk, thereby reducing degree of uncertainty associated with the health risk assessment (Pirsaheb et al., 2021; Ramesh et al., 2021). The exposure parameters, heavy metal concentrations in edible insects and estimated daily metal intake via consumption could be the source of uncertainty in this study. The daily consumption of edible insect species and average body weight for adults in Southern Africa were taken as constants when calculating the estimated daily intake of metals using the random sample of metal concentrations in the studied insects with 10,000 iterations in R software version 4.2.2. The input values (metal concentration and target Hazard Quotient) were calculated multiple times for different random values giving a range of values from which the summary statistics where obtained. Principal component analysis was performed using the PAST (paleontological statistics) software version 3.26 to examine the relationship between heavy metals in plant species and insect species.

3. Results

3.1. Heavy metals in soils and host plants in the proximity of the mine

The concentrations of all heavy metals (As, Cd, Cr, Cu, Fe, Ni, Pb, and Zn) assessed significantly ($P < 0.01$) varied with distance from the mine (Table 1). For all heavy metals, it was noted that the concentrations decreased with an increase in distance from the mine, with the highest values observed in soils collected from points nearest to the mine and vice versa. The concentrations of As in soils were between 133 μg mg^{-1} and 333 μg mg^{-1}, whereby soil samples collected from distances of 1.5 km and 60 km from the mine had the highest and lowest values, respectively (Table 1). Increasing distance from the mine by 15 km, 30 km, 45 km, and 60 km significantly ($P < 0.01$) reduced the As concentrations in the soil by 28, 54, 48, and 60 %, respectively. The concentrations of Cd in soil samples collected at 1.5 km and 16.5 km from the mine were significantly ($P < 0.01$) higher than those of soils collected at 31.5 km, 46.5 km, and 61.5 km by 33–62 %, and 37–63 %, respectively. However, there was no significant difference in the concentration of Cd in soils collected at distances of 1.5 km and 16.5 km, and those collected at 46.5 km and 61.5 km from the mine.

The concentration of copper in the soil ranged between 711,524 and 424,960 μg mg^{-1}, whereby, the highest and lowest concentrations were recorded at distances of 1.5 km and 31. 5 km from the mine, respectively. Chromium concentrations in the soil were highest (56,470 μg mg^{-1}) at 1.5 km and lowest (56.7 μg mg^{-1}) at 61.5 km from the mine. While chromium concentration in the soil differed significantly at 1.5 km and 16.5 km from the mine, there were no statistically significant differences for soils collected at 31.5 km, 46.5 km, and 61.5 km (Table 1). Soil samples collected at 1.5 km from the mine had significantly ($P < 0.01$) higher Cu concentration than the rest. Likewise, the Cu concentration of soil samples collected at 16.5 km from the mine was significantly higher than those collected at other distances, except at 1.5 km. However, there were no significant differences in Cu concentrations of soil samples collected at distances beyond 16.5 km (Table 1).

The soil concentrations of Fe and Ni recorded at 1.5 km were significantly higher than those recorded at 16.5–61.5 km from the mine. The concentrations of Fe in soil collected at 16.5 km and 31.5 km were significantly higher than those recorded at 46.5 km and 61.5 km from the mine. The concentrations of Pb and Zn in the soil were statistically significantly different across distances ($P < 0.01$). The highest concentration of Pb was recorded at 16.5 km from the mine, significantly higher than the rest. The concentrations of Pb at 31.5 km and 61.5 km were significantly higher than the values recorded at 1.5 km and 46.5 km. The concentrations of Zn ranged between 31,006 μg mg^{-1} and 65,967 μg mg$^{-1,}$ with the lowest and highest values recorded at 46.5 km and 1.5 km, respectively.

Table 1

Heavy metal concentration (μg mg^{-1}) in soils collected at varying distances from the mine in Copperbelt province, Zambia.

Heavy metal	Distance from the mine (km)					χ2 value	df	P-value
	1.5	16.5	31.5	46.5	61.5			
Arsenic	333.3 ± 8.8a	240.0 ± 11.6b	153.3 ± 8.8d	173.3 ± 3.3c	133.3 ± 8.8e	2980	4	<0.01
Cadmium	6.0 ± 0.6a	6.3 ± 0.3a	4.0 ± 0.0b	3.3 ± 0.3c	2.3 ± 0.3c	716.8	4	<0.01
Chromium	56,470.0 ± 321.3a	83.3 ± 3.3b	63.3 ± 8.8c	73.3 ± 3.3c	56.7 ± 3.3c	367,589.0	4	<0.01
Copper	711,524.0 ± 197,260.8a	732,130.0 ± 280.0b	424,960.0 ± 357.3c	434,503.3 ± 199.4d	434,706.7 ± 91.2d	16,439,843.0	4	<0.01
Iron	317,774.3 ± 670.5a	240,856.7 ± 226.7b	239,796.7 ± 342.3b	68,326.7 ± 193.4c	63,260.0 ± 220.7c	952.9	4	<0.01
Nickel	31,833.3 ± 306.8a	21,193.3 ± 243.6c	21,846.7 ± 182.2b	13,833.3 ± 213.0d	13,740.0 ± 155.3d	38,257.0	4	<0.01
Lead	19,723.3 ± 197.8d	27,176.7 ± 433.8a	24,176.7 ± 289.0c	19,816.7 ± 196.5d	26,580.0 ± 310.5b	4888.0	4	<0.01
Zinc	65,966.7 ± 384.4a	54,880.0 ± 174.4c	5,797,333.0 ± 64.4b	31,006.7 ± 150.6e	36,650.0 ± 381.6d	106,530.0	4	<0.01

In the same row, means (± standard error) followed by the same letters are not significantly different at $P < 0.05$.

Table 2
Heavy metal concentrations in host plants (μg mg^{-1}) in the Copperbelt Province, Zambia.

Heavy metal	Host plant species					χ2value	df	P-value
	B. africana	*J. paniculata*	*U. kirkiana*	*I angolensis*	*B. glaberrima*			
Arsenic	5.527 ± 2.31a	13.847 ± 6.78a	2.167 ± 0.43a	6.280 ± 2.84a	8.353 ± 4.03a	4.859	5	0.433
Cadmium	100.093 ± 26.03a	127.253 ± 41.06a	38.933 ± 14.60a	108.227 ± 57.77a	83.893 ± 23.90a	3.751	5	0.586
Chromium	2.387 ± 0.65a	2.907 ± 0.89a	1.873 ± 0.34a	2.067 ± 0.38a	2.667 ± 0.44a	2.455	5	0.783
Copper	1926.200 ± 361.42a	1823.067 ± 352.55a	1717.467 ± 300.14a	1891.600 ± 354.40a	1797.933 ± 336.20a	0.34	5	0.99
Iron	2241.467 ± 212.93a	2216.933 ± 287.22a	2383.933 ± 346.84a	2114.133 ± 212.93a	2111.067 ± 262.96a	1.13	5	0.95
Nickel	1369.067 ± 157.33a	1554.867 ± 280.44a	1617.400 ± 299.17a	1452.000 ± 207.50a	1310.000 ± 140.53a	1.34	5	0.93
Lead	129.200 ± 23.01a	126.267 ± 26.79a	121.000 ± 20.48a	108.400 ± 17.08a	175.467 ± 37.23a	5.96	5	0.31
Zinc	883.267 ± 122.42a	879.200 ± 140.80a	1107.400 ± 244.77a	1914.267 ± 165.60a	1006.200 ± 164.33a	1.80	5	0.88

In the same row, means (± standard error) followed by the same letters are not significantly different at $P < 0.05$.

The concentrations As, Cd, Cr, Cu, Fe, Ni, Pb, and Zn did not differ significantly ($P > 0.05$) among host plants (Table 2). It was, however, noted that *J. paniculata* accumulated slightly higher concentrations of As, Cd, and Cr than other plant species, while *U. kirkiana* had the highest concentrations of Nickel and Iron. On the other hand, the concentrations of Zn in *I. angolensis* were 2-folds higher than those accumulated by *B. africana* and *J. paniculata*.

Regarding the relationship among metal concentrations in soil, host plant, and edible insect species, it was noted that there was a strong positive relationship between metal concentrations in the soil and host plant species ($r = 0.6$, $P < 0.01$). However, a weak positive correlation was found between metal concentration in edible insects and host plants ($r = 0.3$, $P < 0.01$), and metal concentration in edible insects and soil ($r = 0.2$, $P < 0.01$).

3.2. Heavy metal concentrations in edible insects

Average concentrations of heavy metals accumulated in seven edible insect species are presented in Table 3. The concentrations of Cd in the studied edible insects ranged between 10.5 μg mg^{-1} and 67.1 μg mg^{-1}. *Imbrasia obscura* accumulated significantly higher ($P < 0.01$) Cd and Pb concentrations than other insects. The concentrations of Fe in *C. forda* were 58 %, 41 %, 94 %, 89 %, 84 %, and 96 % higher than the concentrations recorded in *I. epimethea*, *I. rubra*, *I. obscura*, *M. falciger*, *Notodontidae* sp., *and R. differens* respectively.

The Fe concentrations recorded in *I. epimethea* were significantly lower than that recorded in *C. forda* and *I. rubra* but significantly higher than *I. obscura*, *M. falciger*, and *R. differens* by 88 %, 75 %, 63 %, and 90 % respectively. The concentrations of Pb recorded in *I. obscura* were significantly ($P < 0.01$) higher than those recorded in *M. falciger*, *I. epimethea*, and *C. forda* but not in other insects while *C. forda* accumulated significantly higher concentrations of Zn than *I. epimethea*, *Notonidae* sp., and *R. differens* but not *I. obscura*, *M. falciger*, and *I. rubra*.

3.3. Daily intake and target hazard quotient for edible insects

It was noted that consuming *I. obscura* and *M. falciger* would significantly result in the daily intake of As by 9 and 19 folds higher than *I. rubra*, respectively (Table 4). Consumption of *I. rubra* and *C. forda* would significantly increase the daily intake of Fe than other insects. Furthermore, consuming *I. epimethea* would lead to a higher daily Fe intake compared to *I. obscura*, *M. falciger*, and *R. differens.* However, the daily intake of Cd, Cr, Cu, Ni, Pb, and Zn did not vary significantly ($P \geq 0.05$) between insect species during the study (Table 4).

Overall, most edible insect species studied exceeded the recommended safe limits of Estimated Daily Intake (EDI) for heavy metals (Table 4). For example, a high daily intake of Ni (16,290 μg kg^{-1} d^{-1}) was estimated for the consumption of *C. forda* as the only species exceeding the WHO recommended daily limit for this heavy metal. The consumption of *I. obscura*, *Notodontidae* sp., and *R. differens* would lead to the daily intake of As two folds higher than the WHO permissible limits for food, while consuming *M. falciger* would raise As intake by 5 folds. It was noted that consumption of *C. forda* would cause a significantly lower target hazard quotient (THQ) for iron compared to *I. obscura* (Table 5). Likewise, all insect species had substantially lower Zinc THQ than *R. differens*. The THQ of other metals did not vary significantly among edible insects (Table 5).

The daily quantity of edible insects consumed also varied significantly between insect species ($P < 0.01$) (Fig. 3). The amount of *I. obscura* consumed was significantly higher than that of other insect species by 9–37 %, except *Notonidae* sp., *I. rubra*, and *I. epimethea*. *Notonidae* sp., *I. rubra*, and *I. epimethea* were consumed in significantly higher quantities than other species, except *I. obscura*. The daily intake of *M. falciger* was markedly lower than the values recorded for other insect species.

3.4. Monte Carlo simulation

The results of the Monte Carlo simulation indicate that *R. differens* consumption in the Copperbelt Province will lead to high daily intake of Pb, Ni and Zn than any other edible insects investigated in this study (Table 6). The consumption of *C. Forda* will lead to the highest intake of Cu, Cd and Fe while *M. falciger* and *Notonidae* consumption lead to high As intake (*C. forda* > *Notonidae* sp). The 1st and 3rd quartile, and the mean and median values are all above 1000 implying that insects in the Copperbelt Province pose a risk to human health. The hazard index posed by the studied insects is in the order *I. obscura* > *C. forda* > *I. rubra* > *I. epimethea* > Notonidae sp. > *M. falciger* > *R. differens* (Table 7).

Table 3
Heavy metal concentrations in edible insects (μg mg^{-1}) in the Copperbelt Province, Zambia.

Heavy metal	Edible insect species							χ2 value	df	P-value
	C. forda	*I. epimethea*	*I. rubra*	*I. obscura*	*M. falciger*	*Notodontidae* sp.	*R. differens*			
Arsenic	15.2 ± 4.2a	22.6 ± 5.9a	15.7 ± 4.0a	40.0 ± 0.0a	55.1 ± 32.4a	61.1 ± 20.5a	66.4 ± 17.1a	12.0	6	0.06
Cadmium	10.5 ± 2.8b	13.933 ± 4.4b	10.8 ± 5.3b	67.1 ± 19.9a	14.9 ± 4.1b	57.5 ± 20.6ab	49.7 ± 12.8ab	17.8	6	≤0.01
Chromium	11.7 ± 2.6a	19.5 ± 4.2a	25.5 ± 5.5a	64.2 ± 20.1a	109.5 ± 85.8a	61.6 ± 20.3a	50.5 ± 13.0a	12.6	6	0.083
Copper	1852.0 ± 220.9a	666.9 ± 172.2a	531.0 ± 137.1a	9733.3 ± 2347.4a	877.3 ± 181.5a	839.3 ± 134.4a	476.7 ± 123.1a	5.5	6	0.477
Iron	22,618.6 ± 2197.2a	9608.7 ± 1697.7b	13,171.3 ± 2597.7a	1136.0 ± 265.2c	2374.0 ± 188.3c	3526.7 ± 766.4c	968.7 ± 250.1c	223.0	6	≤0.01
Nickel	300.0 ± 97.9a	58.0 ± 7.1b	88.7 ± 15.3b	89.3 ± 16.7b	88.2 ± 21.9b	78.0 ± 21.3b	69.3 ± 17.9b	22.0	6	≤0.01
Lead	47.2 ± 9.2c	63.9 ± 12.4bc	82.7 ± 20.9abc	136.7 ± 20.6a	28.7 ± 4.1c	123.3 ± 17.6ab	124.0 ± 32.0ab	46.7	6	≤0.01
Zinc	1270.0 ± 252.6a	452.8 ± 119.1c	1063.3 ± 155.7abc	633.6 ± 120.9abc	1280.0 ± 246.2ab	421.3 ± 69.0c	516.7 ± 133.4bc	33.2	6	≤0.01

In the same row, means (± standard error) followed by the same letters are not significantly different at $P < 0.05$.

Table 4
Estimated daily intake of heavy metals (μg mg^{-1}) through consumption of edible insects in Copperbelt province, Zambia.

Heavy metal	Edible insect species							χ2 value	df	P-value
	C. forda	*I. epimethea*	*I. rubra*	*I. obscura*	*M. falciger*	*Notodontidae* sp.	*R. differens*			
Arsenic	56.0 ± 19.4ab	70.0 ± 35.8ab	26.0 ± 17.8b	234.0 ± 131.7a	488.0 ± 138.2a	200.0 ± 128.4ab	194.0 ± 102.5ab	16.0	6	≤0.01
Cadmium	32.0 ± 15.6a	46.0 ± 26.6a	44.0 ± 31.9a	220.0 ± 134.0a	32.0 ± 15.9a	192.0 ± 128.3a	138.0 ± 113.73a	5.5	6	0.48
Chromium	30.5 ± 13.6a	212.0 ± 151.5a	88.0 ± 33.7a	198.0 ± 137.6a	44.0 ± 14.4a	206.0 ± 126.2a	426.0 ± 270.8a	5.8	6	0.44
Copper	5968.0 ± 1255.9a	5522.0 ± 0.1265.9a	4434.0 ± 935.9	34,404.0 ± 32,579.2a	1988.0 ± 713.3a	2984.0 ± 772.3a	702.0 ± 359.1a	5.5	6	0.48
Iron	54,408.0 ± 18,015.4a	46,976.0 ± 8343.9ab	55,362.0 ± 12,885.9a	3992.0 ± 1754.8c	4388.0 ± 818.4c	11,858.0 ± 4816.0bc	2734.0 ± 780.8c	45.3	6	≤0.01
Nickel	16,290.0 ± 15,475.9a	152.0 ± 43.3a	158.0 ± 91.5a	314.0 ± 109.3a	220.0 ± 76.9a	258.0 ± 122.1a	196.0 ± 101.1a	6.5	6	0.37
Lead	108.0 ± 52.4a	194.0 ± 81.2a	8350.0 ± 8162.7a	1058.0 ± 311.1a	234.0 ± 179.6a	512.0 ± 120.8a	346.0 ± 65.2a	5.7	6	0.45
Zinc	3618.0 ± 2068.1a	298.0 ± 209.4a	2000.0 ± 921.8a	570.0 ± 202.5a	3102.0 ± 877.2a	1412.0 ± 431.8a	1458.0 ± 400.6a	10.0	6	0.12

FAO & WHO permissible levels for metals are 100 μg mg^{-1}, 50 μg mg^{-1}, 50 μg mg^{-1}, 10,000 μg mg^{-1}, 40,000 μg mg^{-1}, 500 μg mg^{-1}, 400 μg mg^{-1} and 1000 μg mg^{-1} for Arsenic, Cadmium, Chromium, Copper, Iron, Nickel, Lead and Zinc, respectively. In the same row, means (± standard error) followed by the same letters are not significantly different at $P < 0.05$.

Table 5
Target hazard quotient values through consumption of edible insects in Copperbelt province, Zambia.

Heavy metal	Edible insect species							χ2 value	df	P-value
	C. forda	*I. epimethea*	*I. rubra*	*I. obscura*	*M. falciger*	*Notodontidae* sp.	*R. differens*			
Arsenic	14.0 ± 4.0a	16.0 ± 8.120a	6.0 ± 4.0a	62.0 ± 33.5a	20.0 ± 15.5a	48.0 ± 31.2a	40.0 ± 20.7a	6.3	6	0.39
Cadmium	66.0 ± 24.2a	86.0 ± 49.2a	84.0 ± 59.8a	420.00 ± 255.1a	20.0 ± 15.5a	346.0 ± 230.9a	212.0 ± 173.2a	8.2	6	0.22
Chromium	58.0 ± 22.9a	396.0 ± 282.3a	166.0 ± 62.9a	378.0 ± 262.1a	50.0 ± 16.7a	372 ± 227.0a	650.0 ± 411.4a	5.2	6	0.51
Copper	10,334.0 ± 2174.9a	10,298.0 ± 2361.8a	8270.0 ± 1745.8a	65,450.0 ± 61,977.4a	2348.0 ± 843.1a	5390.0 ± 1395.4a	1068.0 ± 545.5a	5.6	6	0.47
Iron	4360.0 ± 1580.2b	5006.0 ± 889.9ab	5898.0 ± 1374.1ab	7596.0 ± 3338.5a	5186.0 ± 966.3ab	1222.0 ± 497.2ab	4158.0 ± 1185.6ab	18.1	6	≤0.01
Nickel	56,406.0 ± 53,586.5a	566.0 ± 161.3a	16.0 ± 4.00a	1170.0 ± 408.3a	5186.0 ± 966.3a	932.0 ± 441.4a	782.0 ± 372.0a	6.4	6	0.4
Lead	188.0 ± 90.2a	362.0 ± 151.2a	15,572.0 ± 1522.4a	1058.0 ± 311.1a	276.0 ± 211.7a	924.0 ± 217.3a	526.0 ± 99.8a	5.9	6	0.4
Zinc	836.0 ± 477.6b	298.0 ± 209.4b	494.0 ± 229.6b	570.0 ± 202.5b	488.0 ± 138.2b	342.0 ± 103.9b	4430.0 ± 1218.7a	49.8	6	≤0.01
Hazard Index	9032.8 ± 6886.1	2128.5 ± 1306.4	3813.3 ± 2024.9	9588 ± 8027	1696.8 ± 808.3	1197.0 ± 615.0	1483.3 ± 624.1			

Values of Hazard index above 1000 μg g^{-1} pose a risk to human health. In the same row, means (± standard error) followed by the same letters are not significantly different at $P < 0.05$.

3.5. Multivariate analysis of heavy metals in host plants and edible insects

The principal component analysis revealed that heavy metals significantly varied across host plant species and edible insects (Fig. 4). For the case of host plants, the first two components accounted for 97.4 % of the total variation, whereby PC 1 and PC 2 explained 93.3 % and 4.1 % of the total variation, respectively (Fig. 4a). In PC 1, iron, nickel, zinc arsenic and chromium were positively correlated, while copper, lead and cadmium were negatively correlated. On the other hand, the first two components accounted for 99.8 % of total variation in heavy metals among insect species (Fig. 4b). The first PC explained for 70 % of the variation while PC 2 accounted 29.8 %. It was noted that iron, copper and zinc were positively

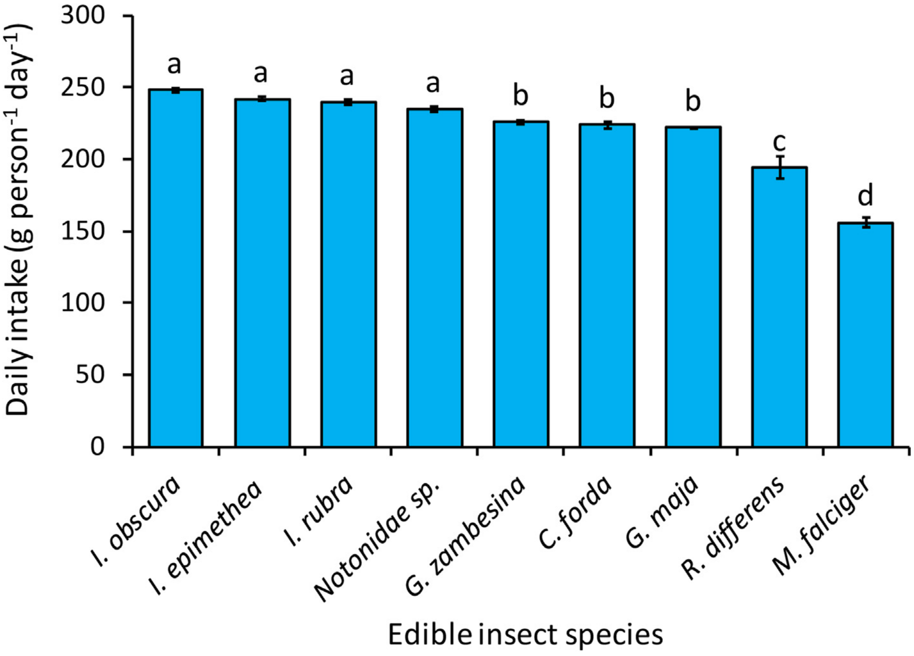

Fig. 3. Daily intake of edible insects in grams per day in the Copperbelt province, Zambia. Means (± standard error) followed by the same letters are not significantly different at $P < 0.05$.

Table 6

Monte Carlo Simulation summary estimated daily intake of metals via edible insect consumption in the Copperbelt Province, Zambia.

Edible insect species	Arsenic		Cadmium		Chromium		Copper		Iron		Lead		Nickel		Zinc	
	Mean	Min	Mean	Min	Mean	Min	Mean	Min	Mean	Min	Mean	Min	Mean	Min	Mean	Min
C. forda	0.08	0.08	7.07	7.07	0.02	0.02	8.26	8.26	90.74	90.74	0.09	0.09	0.07	0.07	2.17	2.17
I. epimethea	0.00	0.00	0.01	0.01	0.10	0.10	7.23	7.23	12.35	12.35	0.13	0.13	0.14	0.14	0.73	0.73
I. rubra	0.00	0.00	0.10	0.00	0.09	0.00	4.57	0.28	41.46	4.31	0.33	0.00	0.27	0.10	3.53	0.62
I. rbscura	0.44	0.14	0.46	0.18	4.01	1.18	2.82	0.75	5.36	0.75	0.69	0.64	0.46	0.18	2.69	0.75
M. falciger	0.44	0.44	0.02	0.02	0.07	0.07	4.85	4.85	5.90	5.90	0.08	0.08	0.27	0.27	0.73	0.73
Notonidae sp	0.12	0.12	0.60	0.60	0.61	0.61	3.09	3.09	3.31	3.31	0.69	0.69	0.67	0.67	2.54	2.54
R. differens	0.02	0.01	0.11	0.01	0.13	0.13	4.62	3.70	15.44	14.11	1.58	1.47	3.47	2.07	12.25	5.63

Table 7

Monte Carlo Simulation of hazard index associated with edible insects in the Copperbelt Province, Zambia.

Edible insect species	Statistics						
	Minimum	1st quartile	Median	Mean	3rd quartile	Maximum	Total health risk
C. forda	16.68	14,013.67	28,480.54	28,185.78	42,095.84	56,401.76	26,143.026
I. epimethea	16.16	2641.97	5156.42	5169.54	7697.80	10,297.33	7939.148
I. rubra	10.45	3924.09	7798.09	7774.62	11,648.61	15,568.97	11,735.975
I. rbscura	62.25	16,210.43	33,007.19	32,667.40	48,718.52	65,445.34	52,577.09
M. falciger	20.39	1323.99	2613.49	2609.51	3883.68	5185.32	2308.624
Notonidae sp	48.02	1359.44	2695.23	2711.86	4052.69	5389.53	1864.685
R. differens	40.00	1173.00	2264.00	2239.00	3317.00	4430.00	4006.2927

correlated in PC 1, while arsenic, chromium, cadmium and nickel were negatively correlated in the same PC.

4. Discussion

4.1. Heavy metal concentration in soil, host plants, and edible insects

Anthropogenic activities such as mining often contaminate soil and water with heavy metals that are transferred along food chains (Xing et al., 2020; Mishra et al., 2019; Schrögel and Wätjen, 2019; Butt et al., 2018). The high heavy metal concentrations observed in soil samples closest to the mine indicate that mining activities are mainly responsible for soil pollution with heavy metals (Chungu et al., 2019; Bian et al., 2012; Dudka and Adriano, 1997). Plants growing in soils contaminated with heavy metals accumulate higher concentrations of the metals present in their tissues and vice versa (Xing et al., 2020; Baghaie and Fereydoni, 2019; Kayika et al., 2017; Liu et al., 2013). This is a positive correlation between heavy metals in host plant species and metal concentrations in the soil obtained during the study. But the absence of significant differences in heavy mental concentration among plant species indicates uniform bioaccumulation capacity of metals by plant species assessed. Our findings contrast with previous studies that have reported considerable variation in heavy metals among plant species (Xing et al., 2020; Latif et al., 2018; Nagajyoti et al., 2010). This could be primarily attributed to the excessive concentration of heavy metals and triggered uniform uptake.

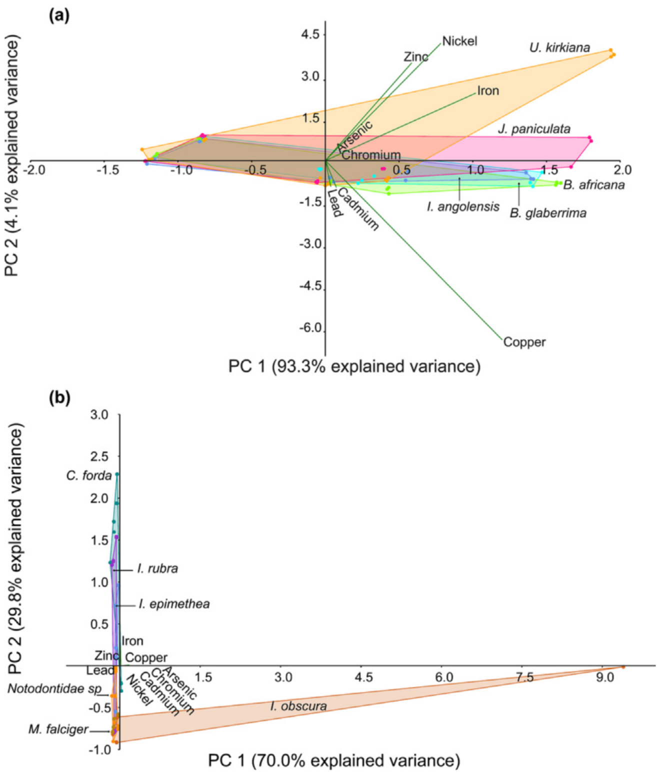

Fig. 4. Principal component of the relationship between heavy metal in host plants (a) and edible insect species (b) around Copperbelt Province.

Compared to soils, the higher concentrations of xenobiotic elements such as Cd and Pb in host plants indicate a higher accumulation of heavy metals in plant tissues around the mine. Cadmium and lead do not play any physiological or metabolic role in plants, but their presence affects the physiological and biochemical processes even at lower concentrations (Nagajyoti et al., 2010). The fact that concentrations of arsenic, cadmium, chromium, lead, and zinc were lower in edible insects than in host plants and soils was not surprising because insects have a more advanced detoxification mechanism for certain heavy metals (Janssens et al., 2009) than plants or soils. However, the levels of copper and iron in edible insects were substantially higher than those in host plants, implying an increase in concentrations of these heavy metals to higher level consumers along the soil-plant-edible insect food chain (Dar et al., 2017; Zhuang et al., 2009). Biomagnification of heavy metals seriously threatens insect diversity and reduces the safety of insects for human consumption. The significant variation in the concentrations of heavy metals among edible insects observed during the study has been previously reported and could be attributed mainly to differences in insect species, insect development stage, and heavy metal type (Schrögel and Wätjen, 2019; van der Fels-Klerx et al., 2018; Diener et al., 2015; Banjo et al., 2010).

4.2. Exposure to heavy metals through insect consumption

Although other elements such as copper, zinc, and iron are essential for plant growth and human and animal nutrition, mining activities have increased their concentrations in the environment to levels beyond permissible limits (Tembo et al., 2006). The presence of high concentrations of heavy metals in the environment due to intensive mining activities in the Copperbelt province has raised concerns about the safety of edible insects and food crops (Chungu et al., 2019; Karadjova and Markova, 2014; Nakayama et al., 2011). This study has demonstrated that the consumption of insects from areas close to mining areas may increase human exposure to heavy metals. The significant variation in the daily intake of different insect species suggests that the level of exposure to heavy metals largely depends on the species of insects consumed (Schrögel and Wätjen, 2019; Zhuang et al., 2009). For example, the consumption of *I. rubra* would result in 98 % higher Cu intake compared to *R. differens* while the estimated daily intake of Cu through the consumption of insects was within the limit recommended by WHO, except for *I. bouvier*.

Despite the low concentrations of Pb and Cd, it should be noted that the two elements are toxic even at low concentrations (Balali-Mood et al., 2021; Zhuang et al., 2014). In some insects, such as *I. rubra* the concentrations of Pb were 95 % higher than the WHO permissible limits suggesting that exposure to Pb through insect consumption in Zambia is alarming. Similarly, the mean concentration of Ni in *I. epimethea* was relatively higher than the values reported in the literature (Obodai et al., 2014; Belluco et al., 2013; WHO, 1996), suggesting that edible insects from Copperbelt province are among the most contaminated with Ni.

When ingested in amounts above WHO recommended limits, heavy metals can affect insect fitness, decrease immunity (Imathiu, 2020), extend development time (Burden et al., 2019; Schrögel and Wätjen, 2019; Behmer et al., 2005), and result in retarded growth (Noret et al., 2007). The results of the Monte Carlo simulation suggest that consumption of insects from mining regions poses serious health risks due to intake of metals at rates higher than WHO recommended thresholds. It was further noted than the daily number of edible insects consumed per day significantly influences the health risk posed. It was further noted that the daily amount of particular edible insects consumed per day significantly influences the health risk posed.

Findings from the study suggest that the health risk through the consumption of edible insects around the Copperbelt province is generally high, due to biomagnification along the soil-plant-edible insect-human food chain. Considering that significantly high concentrations of metals were observed in the soil and plants, the consumption insect from such contaminated environments poses a greater health risk. This highlights negative impacts of mining pollutants on ecosystem health, biodiversity, and safety of wildly harvested edible insects for human consumption.

5. Conclusion

The study revealed high bioaccumulation of heavy metal concentrations along the soil-plant-edible insect-human food chain and the high health risks posed by consuming edible insects around mining areas. Although the concentration of heavy metals did not vary among host plants, the accumulation of heavy metals varied significantly among insect species. The high estimated daily intakes recorded for *C. forda*, *I. rubra*, *Notodontidae* sp., and *I. obscura* suggest that consuming these insects would increase human exposure to heavy metals. Furthermore, the significantly higher values of daily heavy metal intake, target hazard quotients, and hazard indices recorded during the study suggest that the consumption of edible insects from the Copperbelt province could pose a high health risk through biomagnification. To conserve insect diversity, the government and regulatory bodies should regulate mining activities, especially the treatment of mining effluent, solid mine waste management, and establishment of buffer zones between mining areas and biodiversity conservation areas. To reduce risks associated with heavy metal ingestion in Zambia, insect consumption should be selective so that insects with high target hazard quotient and estimated daily metal intake values beyond WHO limits should be avoided. Furthermore, to reduce the risk associated with the consumption of wildly harvested insects, Zambia should invest in captive insect mass production to assure safety and increase the availability of edible insects as alternative food sources for improved food and nutrition security. Future research to study the metal fractionation and bioavailability in rhizosphere soil, and projection of future scenarios through modeling will be necessary for a comprehensive risk assessment.

CRediT authorship contribution statement

Susan Mwelwa: Conceptualization, methodology, investigation, data curation, formal analysis, validation, writing—original draft preparation, writing—original draft preparation, visualization. **Donald Chungu:** Conceptualization, methodology, investigation, project administration, resources, funding acquisition, supervision, validation, Writing - original draft, writing—review and editing, supervision. **Frank Tailoka:** Conceptualization, methodology, investigation, supervision, validation, project administration, writing—review and editing, supervision. **Dennis Beesigamukama:** Data curation, formal analysis, validation, visualization, Writing - original draft, writing—review and editing. **Chrysantus M. Tanga:** Conceptualization, methodology, investigation, formal analysis, validation, writing—original draft preparation, writing—original draft preparation, visualization, project administration, resources, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Data availability

Data will be made available on request.

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.

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