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Probabilistic health risk assessment of heavy metals in honey, *Manihot esculenta*, and *Vernonia amygdalina* consumed in Enugu State, Nigeria

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Abstract Honey is a nutritive fluid product of bees with an array of pharmacological/medicinal effects. As a natural product from honeybees, honey is regarded to be safe and free from any contaminants. Cassava tuber (*Manihot esculenta*)–based meal, *Vernonia amygdalina* (bitter leaf soup), and honey are common local cuisine in Nigeria. This is a human health risk assessment of heavy metals associated with the consumption of honey (*Apis mellifera scutellata*), cassava (*Manihot esculenta*), and bitter leaf (*Vernonia amygdalina*) in Enugu State, Nigeria. Concentrations of lead (Pb), Cd, As, Cu, Ni, Mn, Fe, Cr, and Fe in honey, soil, *Manihot esculenta*, and *Vernonia amygdalina* were determined using an atomic absorption spectrophotometer (AAS). The principal component analysis (PCA) was performed as a factor reduction technique among 12 factors (3 locations against 4 sources of heavy metals). The mean concentration of metals in honey, soil, *Manihot esculenta*, and *Vernonia amygdalina* ranged from 0.001 to 4.28 mg kg^{-1}, from 0.0026 to 170.52 mg kg^{-1}, from 0.012 to 80.63 mg kg^{-1}, and from 0.0016 to 126.48 mg kg^{-1}, respectively. Pb showed the highest carcinogenic risk with values of 3.18E−04–1.43E−01 in *Vernonia amygdalina* and 1.69E−04–3.47E−04 in *Manihot esculenta* for adults and children, respectively. In honey, Cd showed the highest carcinogenic risk with values of 1.26E−02–1.07E−01. About 51.5% of total cancer risk (TCR) was below 1E−04. Consumption of honey, *Vernonia amygdalina*, and *Manihot esculenta* in some urban parts of Enugu may pose some adverse health effects.

Keywords Heavy metal risk assessment · Honey · *Manihot esculenta* · *Vernonia amygdalina* · Public health · Nigeria

Introduction

Honey is a fermented sweet fluidly product of bees (*Apis mellifera* and *Apis dorsata* Fabricius) with a wide range of both nutritional and medicinal benefits. It is produced from plants' nectar and flower pollen, as well as from insects' secretions and is stored in honeycombs for ripening and maturation (Ioannidou et al. 2005). Honey contains carbohydrate, proteins, minerals, trace elements, vitamins, and polyphenols. The composition of honey depends greatly on the botanical origin, climate, environment, soil, manner of harvest, and storage conditions, which are manifested in its color and taste (Sitarz-Palczak et al. 2015). Honey serves as a probiotic, antimicrobial, antioxidant, antiviral, antiparasitic, anti-inflammatory, antimutagenic, anti-anemic, anticancer, and immunosuppressive ((Gallmann et al. 2009);

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Komosinska-Vassev et al., 2015). During forage, bees travel over an area of 7 km^2; hence, they are exposed frequently to various environmental contaminants and consequently accumulate these in honey they produce (Ioannidou et al. 2005). The concentration of heavy metals in honey reflects their amount in the whole region; thus, honey has been recognized as a biological indicator of environmental pollution (Costanza et al. 1997).

Soil serves as a fundamental background for several agricultural practices due to the constituent minerals that support crop and major reservoir where most heavy metals from pesticides, fertilizer, effluents, fossils, and other emissions are deposited (Adelekan 2010). Cassava (*Manihot esculenta*) is not only the staple food in Nigeria, but the milled cassava flour is used in pharmaceutical and other industries. Bitter leaf (*Vernonia amygdalina*) is a commonly consumed vegetable in Nigeria. In fact, the combination of the hard porridge of cassava and bitter leaf soup is considered an important delicacy in Nigeria.

Heavy metals (HMs) that occur extensively in diverse environments are among the factors responsible for inducing mutagenesis, carcinogenesis, and teratogenesis (Zhan et al. 2013) and have been placed on the list of pollutants with priority by the USEPA as far back as the 1970s, and this has been identified as a public health concern worldwide (Orisakwe et al., 2015). Risk assessment evaluates the potential hazards of contaminated sites or products and is based on considering linkages between pollution sources, pathways, and receptors. These linkages can be dissociated by either source removal or pathway management or modifying exposure of the receptors. Information about HM concentrations in foodstuffs and their dietary intake is very important for assessing their risk to human health (Hague et al., 2008).

Since honey (*Apis mellifera scutellata*), *Vernonia amygdalina*, and *Manihot esculenta* are commonly consumed in Enugu State, the present study has evaluated the human health risk assessment of heavy metal contaminations in the consumption of honey (*Apis mellifera scutellata*), *Vernonia amygdalina*, and *Manihot esculenta* from Enugu State of Nigeria. For proper guidance for both public health and policy makers, it is imperative to identify the extent and severity of heavy metal contamination in soils and crops from different places in the intensively farmed areas in Enugu State, Nigeria. This study has also employed the principal component analysis (PCA) of honey, *Vernonia amygdalina*, and *Manihot esculenta* soil samples and heavy metal concentrations.

Materials and methods

Sample collection and preparation

Honey, cassava (*Manihot esculenta*), bitter leaf (*Vernonia amygdalina*), and soil samples were collected from two rural locations (Enugu-Ezike and Uzo-Uwani) and three urban locations (Opi-Nsukka, Obollo, and 9th Mile) in Enugu State, Nigeria, within the months of February and March 2016. The honey samples were extracted directly by squeezing the honeycombs. Ten honey samples were from rural communities while fifteen samples were from urban communities. The honey samples were collected in amber-colored glass bottles and stored at 4 °C prior to analysis.

Digestion and ashing of honey, *Manihot esculenta*, *Vernonia amygdalina*, and soil samples

Two milliliters (2 mL) of honey, 3 g each of cassava (*Manihot esculenta*) and bitter leaf (*Vernonia amygdalina*), and 5 g of soil samples were measured separately. After that, approximately 9 mL of 65% (*v*/*v*) concentrated nitric acid and 3 mL of perchloric acid were added in a ratio of 3:1 prior to heating. The solution was then transferred to a hot plate and heated to a temperature of 120 °C for about 5 h. Afterwards, the sample was introduced into an oven at a temperature that was gradually increased by 10 °C every 60 min until the final temperature of 450 °C was reached 18 h after and white ashes were obtained. Following this, samples were left to cool, and the white ash was dissolved in 5 mL of 1.5% nitric acid, and a final volume of 25 mL was made up with deionized water. The resulting solution was filtered using a Whatman filter paper (no. 42) fitted into a Buchner funnel to avoid residues from getting into the beaker before transferring it into a tightly sealed plastic container (Orisakwe et al. 2019). Pb, Cd, As, Cu, Ni, Mn, Cr, Cu, Fe, and Zn concentrations were assayed with an atomic absorption spectrophotometer (model 205; Buck Scientific, East Norwalk, CT, USA).

Quality control

The instrument was recalibrated after every ten runs. The analytical procedure was checked using the spike-recovery method (SRM). A known standard of each metal (Pb, Cd, As, Cu, Ni, Mn, Cr, Cu, Fe, and Zn) was introduced into already analyzed samples and was reanalyzed. The results of the recovery studies for Pb, Cd, As, Cu, Ni, Mn, Cr, Cu, Fe, and Zn were more than 95%. The relative standard deviation between replicate analyses was less than 4%. The limit of detection (LOD) for Pb, Cd, Cr, Ni, and Zn was 0.005 ppm, with blank values reading as 0.00 ppm for all the metals in deionized water with an electrical conductivity value of less than 5 μS cm^{-1}. The limit of quantification (LOQ) for Pb, Cd, As, Cu, Ni, Mn, Cr, Cu, and Fe was 0.04 ppm, and that for Zn was 0.06 ppm.

Statistical analysis

Statistical analysis was carried out using the GraphPad Prism (version 6.5). All results were expressed as mean value ± standard deviation. The data were analyzed using one-way analysis of variance and the Tukey post hoc test at 95% confidence level. A p value < 0.05 was considered statistically significant. The PCA was performed as a factor reduction technique among 12 factors, that is, 3 locations against 4 sources of heavy metals. After varimax rotation, the identified principal factors were adopted in developing multiple regression models of which a parabolic model came out best with an R^2 value of 0.994 and a mean square error (MSE) of 0.008.

Human health risk assessment

Determination of non-cancer risk assessment

This study investigated the non-carcinogenic human health risk following the chronic exposure of these metals through the consumption of these foods.

Calculation of daily intake of metals

The daily intake of metal (DIM) was calculated as follows (Li et al. 2009; USEPA 2000):

$$\text{DIM}\ \left(\text{mg}\,\text{kg}^{-1}\,\text{day}^{-1}\right) = \frac{C\ \left(\text{mg}\,\text{kg}^{-1}\right) \times \text{IR (kg)}}{\text{BW (kg)}} \quad (1)$$

where IR is the ingestion rate, C is the concentration of metal, and BW is the average adult and child body weight which were considered to be 60 kg and 24 kg, respectively (Igbiri et al. 2017).

Target hazard quotient of metals

The target hazard quotient (THQ) was calculated using the method of Singh et al. (Singh et al. 2010)

$$\text{THQ} = (\text{Efr} \times \text{ED} \times \text{IR} \times C/\text{RfDo} \times \text{BW} \times \text{ATn}) \times 10^{-3} \quad (2)$$

where Efr is the exposure frequency (365 days $year^{-1}$); ED is the exposure duration (55 years), equivalent to the average lifetime of a Nigerian adult; IR is the ingestion rate; C is the concentration of metal; RfDo is the oral reference dose; BW is the average body weight of adult (60 kg); Atn is the average exposure time for a non-carcinogen in days (Efr × ED); and 10^{-3} is the unit of conversion.

The hazard index (HI) was used to estimate total chronic toxicity risks of multiple metals on the assumption of dose additivity (US EPA, 1999)

$$\text{HI} = \text{HQ1} + \text{HQ2} + \ldots + \text{HQ}n \quad (3)$$

The average daily ingestion of honey, cassava (*Manihot esculenta*), and bitter leaf (*Vernonia amygdalina*) among adults and children as obtained from food consumption survey was 8.4 g day^{-1} and 4.2 g day^{-1} for honey and 350.5 g day^{-1} and 140.25 g day^{-1} for cassava. For *Manihot esculenta* and bitter leaf (*Vernonia amygdalina*), the values were 345 g day^{-1} and 232 g day^{-1} in adults and children, respectively (Ozoani 2018).

Cancer risks

Incremental lifetime cancer risk (ILCR) is obtained using the cancer slope factor (CSF), which is the risk produced by a lifetime average dose of 1 mg kg^{-1} body weight (BW) day^{-1} and is contaminant specific.

$$\mathrm{ILCR} = \mathrm{CDI} \times \mathrm{CSF} \quad (4)$$

where CDI (chronic daily intake of chemical, mg kg^{-1} BW day^{-1}) represents the lifetime average daily dose exposure to the chemical.

$$\mathrm{CDI} = (\mathrm{EDI} \times \mathrm{EFr} \times \mathrm{ED})/\mathrm{Atn} \quad (5)$$

where EDI is the estimated daily intake of metal via consumption of honey, EFr is the exposure frequency (365 days $year^{-1}$), ED is the exposure duration (55 years), and ATn is the average exposure time for a non-carcinogen in days (Efr × ED) (Orisakwe et al. 2015).

The total cancer risk as a result of exposure to multiple contaminants due to consumption of a particular honey sample will be assumed to be the sum of the individual metal incremental lifetime cancer risk (Σ ILCR, n = 1 to n).

Result and discussion

Heavy metal concentrations

Table 1 shows the concentrations of Pb, Cd, As, Cu, Ni, Mn, Cr, Cu, Fe, and Zn in honey from two rural sites and three urban sites in Enugu State, Nigeria. All the metals assayed were detected at varying concentrations in the honey (range 0.001–4.28 mg kg^{-1}). The highest level of Pb (0.081 mg kg^{-1}), Cd (0.098 mg kg^{-1}), and As (0.06 mg kg^{-1}) contaminations were detected in honey sampled from Obollo. Honey samples from Obollo had the highest concentrations of Cu, Ni, Mn, Cr, and Zn whereas honey samples from Opi-Nsukka had the highest concentrations of Fe. Ni contamination in all honey samples ranged from 0.10 to 0.27 mg kg^{-1}.

Table 2 shows the concentrations of Pb, Cd, As, Cu, Ni, Mn, Cr, Cu, Fe, and Zn in *Vernonia amygdalina*, *Manihot esculenta*, and soil from one rural site (Uzo-Uwani) and two urban sites (9th Mile and Obollo) in Enugu. Soil lead concentrations ranged from 6.92 (rural) to 16.78 mg kg^{-1} (urban). *Vernonia amygdalina* and *Manihot esculenta* lead concentrations ranged from 7.60 (rural) to 11.78 mg kg^{-1} (urban) and from 3.97 (rural) to 8.14 mg kg^{-1} (urban), respectively. Other metals such as Cd, As, Cu, Ni, Mn, Cr, Cu, Fe, and Zn were all detected in the *Vernonia amygdalina* and *Manihot esculenta*.

Human health risk assessment

The EDI of heavy metals for adult and child population via consumption of honey, *Manihot esculenta*, and *Vernonia amygdalina* harvested in Enugu-Ezike, Uzo-Uwani, Opi-Nsukka, Obollo, and 9th Mile is shown in Table 3. The EDI of Pb for adults and children ranged from 5.88*E*−07 to 9.72*E*−06 mg kg^{-1} BW day^{-1} and from 8.58*E*−07 to 1.42*E*−05 mg kg^{-1} BW day^{-1}, respectively.

The THQ and HI of heavy metals in honey, *Manihot esculenta*, and *Vernonia amygdalina* samples in both adults and children are shown in Table 4. The THQs of Pb, Cd, As, Cu, Ni, Mn, Cr, and Zn are in the ranges of 8.0*E*−07–1.2*E*−02 and 2.33*E*−07–1.2*E*−02 for adults and children, respectively. Hazard indices of heavy metals in honey were less than 1 for both adults and children and ranged from 9.80*E*−03 to 1.88*E*−02 and from 6.97*E*−03 to 1.39*E*−02, respectively. The THQ of Pb in *Vernonia amygdalina*, for adults and children, ranged from 9.364 to 14.515 mg kg^{-1} BW day^{-1} and from 18.367 to 28.468 mg kg^{-1} BW day^{-1}, respectively. The HIs for all the metals in the three sampled sites were above the threshold value of 1, ranging from 13.09841 to 21.62436 and from 25.06832 to 41.81089 for adults and children, respectively. The THQ values for Cd, As, Cu, Ni, Cr, Cu, Fe, and Zn in *Manihot esculenta* in the rural site were all below 1 except Pb and Mn which were above 1 with the highest THQ in Pb. The HIs for all the metals in Uzo-Uwani, 9th Mile, and Obollo were above 1.

Table 5 shows the ILCR and Σ ILCR for consumption of Pb, Cd, and As in honey, *Manihot esculenta*, and *Vernonia amygdalina*. The cancer risk of heavy metals ranged from 5.10*E*−06 to 1.20*E*−02 and from 8.60*E*−07 to 1.07*E*−01 for adults and children, respectively, while the Σ ILCR values for adults and children ranged from 8.79*E*−03 to 3.84*E*−02 and from 5.76*E*−02 to 1.06*E*−01, respectively.

For *Vernonia amygdalina*, the ILCR values of Pb among adults in Uzo-Uwani, 9th Mile, and Obollo are all above 1.0*E*−04 but less than 1.0*E*−04 among children. The ILCR for Cd in Uzo-Uwani is less than 1.0*E*−04 in both adults and children but above 1.0*E*−04 in 9th Mile and Obollo. The ILCR for As in all the sites is less than 1.0*E*−04 in both adults and children. The Σ ILCR values for Pb, Cd, and As in Uzo-Uwani, 9th Mile, and Obollo are above 1.0*E*−04 in both adults and children.

Table 1 Heavy metals (mg kg^{-1}) in honey from rural and urban communities in Enugu State, Nigeria

Heavy metals (mg kg^{-1})	Sample sites				
	Rural		Urban		
	Enugu-Ezike	Uzo-Uwani	Opi-Nsukka	Obollo	9th Mile
Cd	0.011 ± 0.00	0.05 ± 0.06	0.098 ± 0.04	0.08 ± 0.02	0.08 ± 0.03
Pb	0.007 ± 0.00	0.005 ± 0.00	0.081 ± 0.01	0.06 ± 0.01	0.04 ± 0.03
As	0.001 ± 0.00	0.001 ± 0.00	0.0025 ± 0.02	0.06 ± 0.05	0.02 ± 0.01
Cu	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.02	0.02 ± 0.02	0.05 ± 0.02
Ni	0.11 ± 0.09	0.11 ± 0.09	0.11 ± 0.03	0.10 ± 0.01	0.27 ± 0.06
Mn	0.44 ± 0.09	0.44 ± 0.09	0.46 ± 0.10	0.52 ± 0.16	3.17 ± 0.57
Fe	2.35 ± 0.98	2.35 ± 0.98	4.28 ± 0.87	2.44 ± 0.58	3.65 ± 1.16
Cr	0.002 ± 0.00	0.002 ± 0.00	0.01 ± 0.00	0.002 ± 0.00	0.02 ± 0.02
Zn	0.20 ± 0.18	0.20 ± 0.17	0.58 ± 0.14	0.17 ± 0.07	0.64 ± 0.12

Data are presented as mean ± SD

The ILCR values of Pb in *Manihot esculenta* among adults in Uzo-Uwani, 9th Mile, and Obollo were all above 1.0E−04 while the ILCR for Pb in children was less than 1.0E−04 in all the three sites. The ILCR for Cd in Uzo-Uwani is less than 1.0E−04 in both adults and children but above 1.0E−04 in 9th Mile and Obollo. The ILCR for As in all the sites is less than 1.0E−04 in both adults and children. The Σ ILCR values for Pb, Cd, and As in all the sites are above 1.0E−04 in both adults and children in exception of the children in Uzo-Uwani with the Σ ILCR of 6.72E−05.

Statistical analysis and PCA

A total of 9 normality tests were run for soil, bitter leaf, and cassava tuber against three locations of Uzo-Uwani, 9th Mile, and Obollo, respectively. In all cases, the probability versus probability (*P*-*P*) plots were not normally distributed. Figure 1 served as a typical example as other plots follow a similar trend of abnormality. Thus, analysis of variance (ANOVA) and PCA are non-parametric methods. The choice of ANOVA is that of Friedman's 2-way approach to identify any significant difference in heavy metal concentration (i) in soil, bitter leaf, cassava tuber, and honey for each of the three locations of Uzo-Uwani, 9th Mile, and Obollo and (ii) in locations and each of the four variables, i.e. soil, bitter leaf, cassava tuber, and honey. The summary output on ANOVA is presented in Tables 6 and 7.

Further analyses were carried out on the collected data set to identify the heavy metals that set soil, cassava tuber, honey, and bitter leaves apart. Thus, PCA was employed. Figure 2 presents the biplot of the sampled variables (honey, soil, bitter leaves, and cassava tuber) from the selected community and the principal heavy metal(s) that define the variables. Table 8 presents the output from the PCA after the application of varimax rotation which tends to optimize the relationship between the variables and the extracted factors in the PCA. The values styled in italics found in Table 8 represent the principal factors needed for multiple regression modeling.

Based on the principal component, a regression analysis (that is principal component regression, PCR) was applied to develop a model to predict the concentration of heavy metals, assuming the honey and soil as dependent variables while the cassava tuber and bitter leaves as independent variables. In the model development, the average values of the concentration of the heavy metals in the soil, honey, bitter leaves, and cassava tuber from all the communities sampled were employed.

Based on the output of the principal component analysis, regression modeling was applied to develop a model to predict the concentration of heavy metals, assuming multiple linear regression in the first instance in which the dependent variable was interchanged between soil, bitter leaf, and cassava tuber for selection of the best model, with the highest goodness of fit and least value of MSE; thus, the multiple regression modeling was repeated for quadratic equivalent. The R^2 values for both linear and

Table 2 Heavy metals (mg kg^{-1}) in soil, *V. amygdalina*, and *M. esculenta* from rural and urban communities in Enugu State, Nigeria

Sample sites	Cd	Pb	As	Cu	Ni	Mn	Fe	Cr	Zn
Soil									
Rural									
Uzo-Uwani	0.0026 ± 0.0	6.92 ± 0.83	0.0060 ± 0.00	7.87 ± 2.26	3.08 ± 1.21	45.36 ± 4.33	48.62 ± 4.58	1.90 ± 0.32	21.64 ± 3.20
Urban									
9th Mile	0.0063 ± 0.0	15.84 ± 2.93	0.014 ± 0.00	13.05 ± 2.36	6.06 ± 0.74	57.73 ± 3.64	170.52 ± 8.70	4.76 ± 0.47	51.00 ± 6.30
Obollo	0.0044 ± 0.0	16.78 ± 1.56	0.013 ± 0.00	9.84 ± 1.55	5.40 ± 1.06	46.71 ± 4.27	106.95 ± 1.30	2.94 ± 0.69	38.86 ± 1.92
Vernonia amygdalina									
Rural									
Uzo-Uwani	0.0016 ± 0.0	7.60 ± 1.070	0.0017 ± 0.00	3.56 ± 0.50	2.25 ± 0.60	12.79 ± 2.00	65.07 ± 7.29	1.04 ± 0.50	21.11 ± 3.00
Urban									
9th Mile	0.0076 ± 0.0	11.78 ± 2.88	0.0027 ± 0.00	8.56 ± 1.09	5.64 ± 0.64	22.35 ± 2.28	126.48 ± 6.38	2.60 ± 0.78	31.55 ± 4.60
Obollo	0.0053 ± 0.0	10.78 ± 1.27	0.0019 ± 0.00	6.94 ± 1.56	2.47 ± 0.55	16.77 ± 2.52	99.95 ± 7.52	2.22 ± 0.61	30.57 ± 3.77
Manihot esculenta									
Rural									
Uzo-Uwani	0.0015 ± 0.0	3.97 ± 0.35	0.0012 ± 0.00	2.98 ± 1.15	1.79 ± 0.33	7.30 ± 0.92	41.79 ± 5.62	0.067 ± 0.04	21.83 ± 2.46
Urban									
9th Mile	0.0059 ± 0.0	7.90 ± 1.42	0.0022 ± 0.00	7.16 ± 2.48	4.93 ± 1.30	10.94 ± 0.30	80.63 ± 9.50	1.72 ± 0.35	32.93 ± 4.00
Obollo	0.0038 ± 0.0	8.14 ± 2.49	0.0016 ± 0.00	6.94 ± 1.56	2.27 ± 0.63	7.80 ± 1.31	60.68 ± 5.03	1.48 ± 0.79	28.77 ± 4.51

Table 3 Estimated daily intake (EDI) of metals in honey, *V. amygdalina*, and *M. esculenta* among adults and children

Heavy metals	Sample sites					
	Rural		Urban			
	Uzo-Uwani		9th Mile		Obollo	
	AD	CH	AD	CH	AD	CH
Cd in honey	6.0*E*−06	8.75*E*−06	9.6*E*−06	1.4*E*−05	9.6*E*−06	1.4*E*−05
Cd in *Vernonia*	7.9*E*−06	1.5*E*−05	3.74*E*−05	7.3*E*−05	2.61*E*−05	5.1*E*−05
Cd in *Manihot*	7.5*E*−06	8.77*E*−06	2.95*E*−05	3.45*E*−05	1.90*E*−05	2.22*E*−05
Pb in honey	5.9*E*−07	8.58*E*−07	4.80*E*−06	7.00*E*−06	7.20*E*−06	1.05*E*−05
Pb in *Vernonia*	0.03745	0.07346	0.058050	0.11387	0.05313	0.10420
Pb in *Manihot*	0.0199	0.0232	0.0396	0.0462	0.0408	0.0476
As in honey	1.2*E*−07	1.72*E*−05	2.4*E*−04	3.5*E*−06	7.2*E*−06	7.2*E*−06
As in *Vernonia*	8.3*E*−06	1.64*E*−05	1.3*E*−05	2.6*E*−05	9.3*E*−06	1.83*E*−05
As in *Manihot*	6.0*E*−06	7.0*E*−06	1.1*E*−05	1.3*E*−05	8.0*E*−06	9.4*E*−06
Cu in honey	2.4*E*−06	3.5*E*−06	6.0*E*−06	8.75*E*−06	2.4*E*−06	3.5*E*−06
Cu in *Vernonia*	1.8*E*−02	0.03441	0.042189	0.08274	0.034204	0.06708
Cu in *Manihot*	1.5*E*−02	0.017	0.036	0.042	0.035	0.041
Ni in honey	1.3*E*−05	1.93*E*−05	3.24*E*−05	4.73*E*−05	1.20*E*−05	1.75*E*−05
Ni in *Vernonia*	1.1*E*−02	0.02175	0.027797	0.05452	0.012174	0.02388
Ni in *Manihot*	0.009	0.010	0.025	0.029	0.011	0.013
Mn in honey	5.28*E*−05	7.7*E*−05	3.80*E*−04	5.6*E*−04	6.24*E*−05	9.1*E*−05
Mn in *Vernonia*	0.062593	0.122767	0.110154	0.21605	0.082652	0.16211
Mn in *Manihot*	0.036	0.043	0.055	0.064	0.039	0.046
Fe in honey	2.82*E*−04	4.1*E*−04	4.38*E*−04	6.39*E*−04	2.93*E*−04	4.27*E*−04
Fe in *Vernonia*	0.320702	0.62901	0.623366	1.22264	0.492611	0.96618
Fe in *Manihot*	0.209	0.244	0.404	0.471	0.304	0.355
Cr in honey	2.4*E*−07	3.5*E*−07	2.4*E*−06	3.5*E*−06	2.4*E*−07	3.5*E*−07
Cr in *Vernonia*	0.00512	0.010053	0.01281	0.010053	0.01094	0.010053
Cr in *Manihot*	0.0003	0.0004	0.0086	0.0100	0.0074	0.0086
Zn in honey	2.4*E*−05	3.5*E*−05	7.68*E*−05	1.12*E*−04	2.04*E*−05	2.98*E*−05
Zn in *Vernonia*	0.10404	0.017592	0.10404	0.026292	0.10404	0.025475
Zn in *Manihot*	0.109	0.128	0.165	0.192	0.144	0.168

AD adult (70 kg), *CH* children (24 kg)

quadratic options for soil, bitter leaf, and cassava tuber as dependent variables are presented in Table 9.

Honey has been cherished by many due to its several health benefits and, of course, a favorite of many especially children because of its natural sweet taste. The levels of Pb and Cd in most of the samples showed a 100% violation when compared with the standard permissible levels of 0.005 mg kg^{-1} for Pb and 0.005 mg l^{-1} for Cd set by the WHO and USEPA. However, the Pb levels in honey in this study were lower than the levels reported by Iwegbue et al. (2015) who found Pb levels of 0.50 mg kg^{-1} to 10.02 mg kg^{-1} in already bottled honey obtained in south-eastern Nigeria. In another study, higher Pb levels of 10.2 mg kg^{-1} obtained in honey from south and east regions of Turkey have been reported (Altun et al. 2017). The proximity of farm produce and farmlands to vehicular emissions is known to have an impact on the metal burden of foods (Orisakwe et al. 2012). Children are particularly vulnerable to the toxic effects of Pb and can suffer profound and permanent adverse health effects, particularly

Table 4 Target hazard quotient (THQ) and hazard index (HI) of metals in honey, *V. amygdalina*, and *M. esculenta* among adults and children

Heavy metals	Sample sites					
	Rural		Urban			
	Uzo-Uwani		9th Mile		Obollo	
	AD	CH	AD	CH	AD	CH
Cd in honey	6.0*E*−03	9.0*E*−03	9.6*E*−03	1.4*E*−02	9.6*E*−03	1.4*E*−02
Cd in *Vernonia*	0.008	0.015	0.037	0.073	0.026	0.051
Cd in *Manihot*	0.008	0.009	0.030	0.034	0.019	0.022
Pb in honey	1.50*E*−04	2.1*E*−04	1.20*E*−03	1.75*E*−03	1.80*E*−03	2.63*E*−03
Pb in *Vernonia*	9.364	18.367	14.515	28.468	13.283	26.051
Pb in *Manihot*	4.970	5.800	9.889	11.54	10.19	11.89
As in honey	4.0*E*−04	5.8*E*−04	8.0*E*−03	1.2*E*−02	2.4*E*−02	3.5*E*−02
As in *Vernonia*	0.028	0.055	0.044	0.087	0.031	0.061
As in *Manihot*	0.020	0.023	0.037	0.043	0.027	0.031
Cu in honey	6.0*E*−05	8.75*E*−05	1.5*E*−04	2.2*E*−04	6.0*E*−05	8.75*E*−05
Cu in *Vernonia*	0.439	0.860	1.055	2.069	0.855	1.677
Cu in *Manihot*	0.373	0.435	0.896	1.046	0.869	1.014
Ni in honey	6.6*E*−04	9.6*E*−04	1.6*E*−03	2.4*E*−03	6.0*E*−04	8.8*E*−04
Ni in *Vernonia*	0.554	1.088	1.390	2.726	0.609	1.194
Ni in *Manihot*	0.448	0.523	1.234	1.44	0.568	0.663
Mn in honey	1.6*E*−03	2.3*E*−03	1.2*E*−02	1.7*E*−02	1.9*E*−03	2.8*E*−03
Mn in *Vernonia*	1.897	3.720	3.338	6.547	2.505	4.912
Mn in *Manihot*	1.108	1.293	1.66	1.937	1.184	1.382
Fe in honey	4.0*E*−04	5.9*E*−04	6.3*E*−04	9.1*E*−04	4.2*E*−04	6.1*E*−04
Fe in *Vernonia*	0.458	0.899	0.891	1.747	0.704	1.380
Fe in *Manihot*	0.299	0.349	0.577	0.673	0.434	0.507
Cr in honey	1.6*E*−07	2.33*E*−07	1.6*E*−06	2.33*E*−06	1.6*E*−07	2.33*E*−07
Cr in *Vernonia*	0.003	0.007	0.009	0.007	0.007	0.007
Cr in *Manihot*	0.0002	0.0003	0.0057	0.0067	0.0049	0.0057
Zn in honey	8.0*E*−05	1.7*E*−04	2.6*E*−04	3.7*E*−04	6.8*E*−05	9.9*E*−05
Zn in *Vernonia*	0.347	0.0587	0.347	0.0876	0.347	0.0849
Zn in *Manihot*	0.364	0.425	0.55	0.641	0.480	0.560
HI for honey	*9.80E−3*	*1.39E−02*	*7.25E−02*	*1.06E−01*	*9.65E−02*	*1.41E−01*
HI for *Vernonia*	*13.098*	*25.068*	*21.624*	*41.811*	*18.366*	*35.419*
HI for *Manihot*	*7.590*	*8.857*	*14.877*	*17.360*	*13.776*	*16.075*

Italicized values represent summation of ILCR of honey, Vernonia or Manihot

affecting the development of the brain and nervous system (Bellinger et al. 1992). Pb also causes long-term harm in adults, including an increased risk of high blood pressure and kidney damage. Exposure of pregnant women to high levels of Pb can cause miscarriage, stillbirth, premature birth, and low birth weight ((Bellinger et al. 1992; Kalia and Flora 2005); Amadi et al., 2017).

The Ni level in all the honey samples violated the 0.02 mg kg^{-1} permissible limit set by EFSA (EFSA 2008). Meanwhile, the Ni level in our study is lower than the level 0.88 mg kg^{-1} observed in the assessment of trace element levels in *Rhododendron* honeys of the Black Sea Region in Turkey (Silici et al. 2008). Similarly, Ni levels in our samples are lower than those reported by Iwegbue

Table 5 ILCR in honey, *Manihot esculenta*, and *Vernonia amygdalina* among adults and children

Heavy metals	Sample sites					
	Rural		Urban			
	Uzo-Uwani		9th Mile		Obollo	
	AD	CH	AD	CH	AD	CH
Cd in honey	3.78*E*−02	5.67*E*−02	6.05*E*−02	8.82*E*−02	6.05*E*−02	8.82*E*−02
Cd in *Vernonia*	4.96*E*−05	9.45*E*−05	2.36*E*−04	4.60*E*−04	1.64*E*−04	3.21*E*−04
Cd in *Manihot*	4.73*E*−05	5.53*E*−05	1.86*E*−04	2.17*E*−04	1.20*E*−04	1.40*E*−04
Pb in honey	5.10*E*−06	8.60*E*−07	1.02*E*−05	1.49*E*−05	1.53*E*−05	2.24*E*−05
Pb in *Vernonia*	3.18*E*−04	2.71*E*−06	4.93*E*−04	4.19*E*−06	4.52*E*−04	3.84*E*−06
Pb in *Manihot*	1.69*E*−04	1.44*E*−06	3.37*E*−04	2.86*E*−06	3.47*E*−04	2.95*E*−06
As in honey	6.00*E*−04	8.70*E*−04	1.20*E*−02	1.80*E*−02	3.60*E*−02	5.25*E*−02
As in *Vernonia*	1.25*E*−05	2.46*E*−05	1.95*E*−05	3.90*E*−05	1.40*E*−05	2.75*E*−05
As in *Manihot*	9.00*E*−06	1.05*E*−05	1.65*E*−05	1.95*E*−05	1.20*E*−05	1.41*E*−05
Σ ILCR for honey	*3.84E−02*	*5.76E−02*	*7.25E−02*	*1.06E−01*	*9.65E−02*	*1.41E−01*
Σ ILCR for *Vernonia*	*3.80E−04*	*1.22E−04*	*7.49E−04*	*5.03E−04*	*9.65E−02*	*1.41E−01*
Σ ILCR for *Manihot*	*2.25E−04*	*6.72E−05*	*5.39E−04*	*2.40E−04*	*4.79E−04*	*1.57E−04*

Σ ILCR is the total incremental lifetime cancer risk

AD adults, *CH* children

Italicized values represent summation of ILCR of honey, Vernonia or Manihot

et al. (2015) and Leblebici and Aksoy (Leblebici and Aksoy 2008). Although the trace amount of Ni may be beneficial as an activator of some enzyme systems, at higher levels, it accumulates in the lungs and may cause bronchial hemorrhage (Underwood 1977). Ni may also act as a promoter in carcinogenesis (WHO 1991).

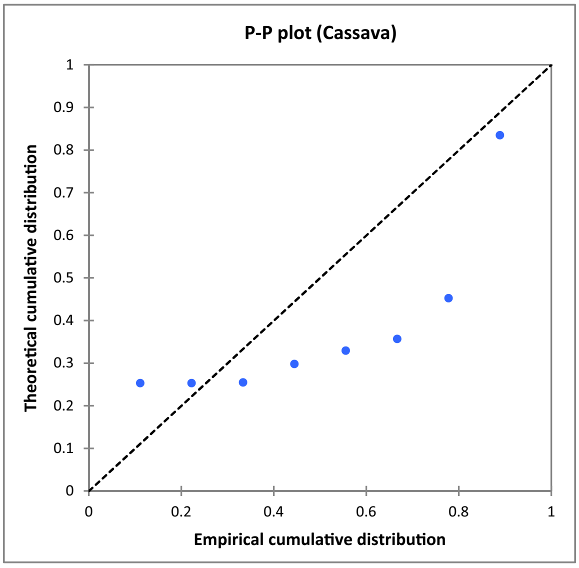

Fig. 1 *P-P* plot for cassava sampled from Uzo-Uwani

Heavy metal contaminants in the biospheres are mostly found to settle on ground. In this study, all the heavy metals assayed were detected in the soil samples. This study shows that Cd level in soil is within the permissible limits set by NEPAC (NEPAC 2005) and USEPA (USEPA 2005). The Cd level in bitter leaf (*Vernonia amygdalina*) was significantly higher ($p < 0.05$) than that in cassava (*Manihot esculenta*) harvested from 9th Mile or Obollo. Cd is associated with itai-itai disease with other symptoms like gastrointestinal pain, nausea, respiratory distress, diarrhea, being impaired reproductively, kidney damage, and hypertension (Underwood 1977).

Heavy metal (except Pb) levels in bitter leaf (*Vernonia amygdalina*) were lower than the tolerable limits set by the FAO/WHO (FAO/WHO 2011). However, the Pb level from our study is higher than the levels reported by Adedokun et al. (Adedokun et al. 2016), who reported a Pb level in vegetables at the limit of 0.40–5.44 mg kg^{-1}. The high Pb levels in these vegetables may be from runoff water that irrigates the farm lands (Oluwole et al. 2013; Orisakwe et al. 2012). Pb levels in honey and cassava (*Manihot esculenta*) were higher than the permissible standards set by the WHO and EFSA.

Table 6 Summary of the significant differences of heavy metal concentration in 2-way ANOVA

Location	Variable			
	Cassava versus soil	Bitter leaf versus honey	Soil versus bitter leaf	Honey versus soil
Uzo-Uwani	NS	S	S	NS
9th Mile	S	NS	NS	S
Obollo	S	NS	NS	S

NS not significant, *S* significant

Essentially, soil, plant root or tuber, and vegetables constitute sources and pathways of exposure of contaminants in man through the food chain (Díez et al. 2009; Gallmann et al. 2009). Food is a major source of intake of toxic metals by man (Llobet et al. 2003). Among food varieties, vegetables are the most exposed food to environmental pollution due to aerial burden (Ioannidou et al. 2005). Vegetables absorb heavy metals and accumulate them in their edible and non-edible parts at quantities of public health concern to both animals and man (Jolly et al. 2013). Excessive content of these metals beyond the maximum permissible level (MPL) leads to an array of health disorders (WHO, 1992; Steenland and Boffetta, 2000; Jarup, 2003). Cassava (*Manihot esculenta*) as a root crop is in constant contact with soil and water where it obtains its nutrients (Osu et al. 2015). There is, therefore, ready transfer of heavy metals from water and soil to the cassava (*Manihot esculenta*) (Mahmood and Malik 2014). In the present study, Pb, Cu, Ni, and Zn levels were above the permissible limit set by various regulatory organizations.

The EDIs of Cd, Cu, Mn, Ni, Fe, Cr, and Zn from cassava (*Manihot esculenta*) and bitter leaf (*Vernonia amygdalina*) were below the TDI in both adults and children as set by the FAO, WHO, and USEPA. The THQ estimates the non-carcinogenic health risk associated with the consumption of individual heavy metals in honey in both adults and children, but none of the metals in honey presented potential health risk since their THQs were less than 1 (THQ < 1). The HI estimates the cumulative non-carcinogenic health risk associated with the consumption of all heavy metals in honey in both adults and children (Cao et al. 2010). It gives an additive effect of all the individual metals The THQ value in honey and values greater than 1 mean that there is a probability of an adverse health effect associated with exposure to such metals in honey. In this study, the HI of metals in honey for both adults and children was less than 1, suggestive of no adverse effect.

The THQ values for all the individual metals detected in the *Vernonia amygdalina* in Uzo-Uwani were all below 1. However, the THQs for most metals detected in *Vernonia amygdalina* in 9th Mile were greater than 1 (THQ > 1) with the THQ of Pb having the highest level in Uzo-Uwani, 9th Mile, and Obollo. The HI estimates for all the metals in Uzo-Uwani, 9th Mile, and Obollo were greater than 1 (THQ > 1). This is an indication of possible adverse health effects of these heavy metals. The HI of the metals in *Manihot esculenta* was more than the threshold value of 1 in both adults and children. Therefore, there is an increased health risk to the exposed population. However, the THQs for Cd, As, Cu, Ni, Mn, Fe, Cr, and Zn were less than 1 (THQ < 1), indicating no adverse effect level. Ingestion of As especially of large amounts may result in acute intoxication causing disturbances in the gastrointestinal, cardiovascular, and nervous systems, and death.

Carcinogenic risk is an estimation of the probability of an individual developing cancer over a lifetime estimated as 55 years. Risk values exceeding 1×10^{-4} are

Table 7 Summary of the significant differences of heavy metal concentration in 2-way ANOVA

Variable	Location		
	Uzo-Uwani versus 9th Mile	9th Mile versus Obollo	Obollo versus Uzo-Uwani
Soil	S	NS	S
Bitter leaf	S	NS	S
Cassava tuber	S	NS	S
Honey	NS	NS	NS

S significant, *NS* not significant

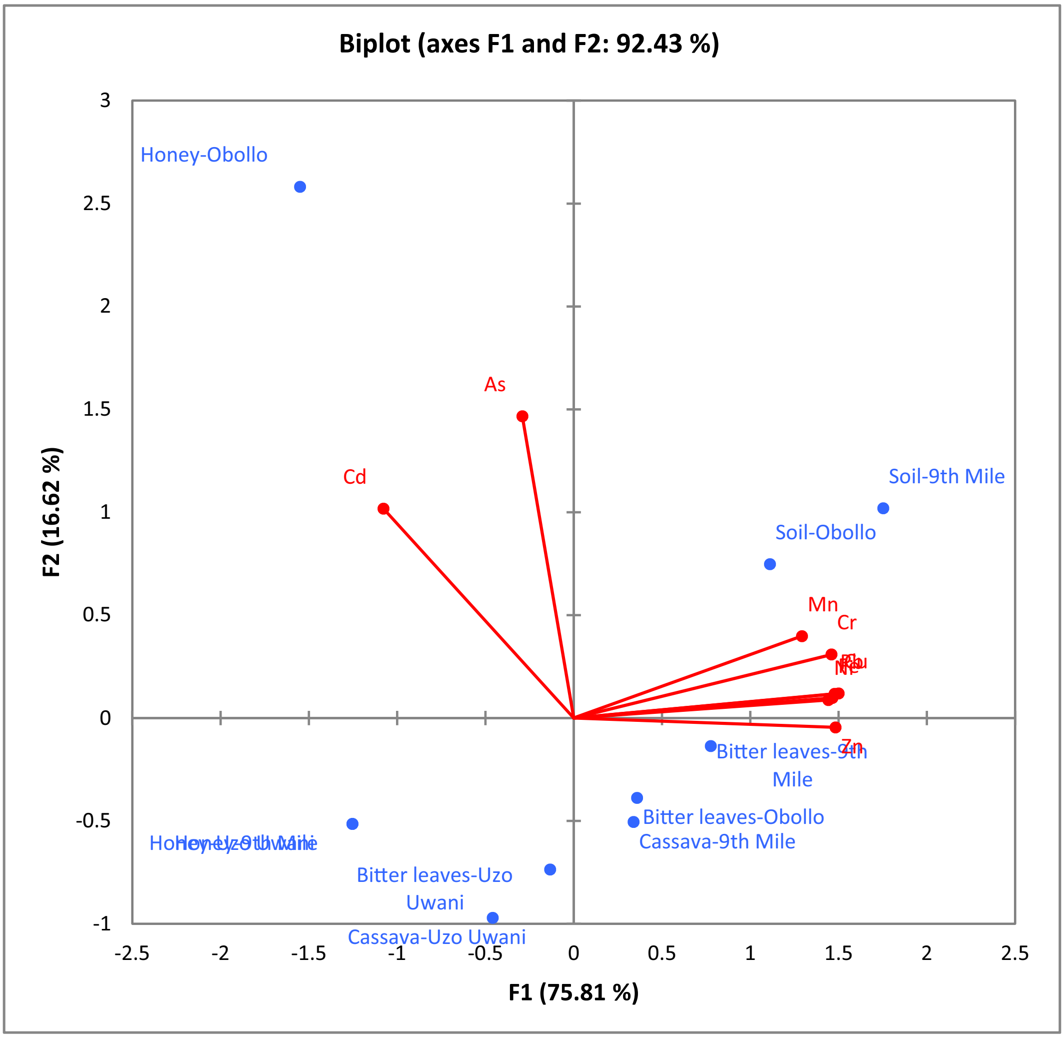

Fig. 2 Biplot of principal heavy metals on sample variables from selected communities

regarded as intolerable, risks less than 1×10^{-6} do not produce adverse health effects, and risks lying between 1×10^{-4} and 1×10^{-6} are generally said to be a safe range (Hu et al. 2012). Our results suggest that there is an increased cancer risk due to Cd and As from consumption of all the honey and bitter leaf (*Vernonia amygdalina*) samples since the risk exceeded the 1*E*−04 (Xia et al. 2010). Increased As exposure causes kidney, lung, and bladder cancers (Rehman et al. 2018). Similarly, our results showed an increased cancer risk due to Pb, Cd, and As from consumption of cassava (*Manihot esculenta*) having also exceeded the risk level of 1*E*−04 (Xia et al. 2010).

From Fig. 1, the blue dots did not follow the black dashed line (the major bisectors), suggesting that the collected data are not normally distributed with respect to the sampled population. Thus, further statistical analysis tended to follow a non-parametric nature.

Following the output of the normality test, Friedman's test, a non-parametric 2-way analysis of variance was employed at a 5% significance level to assess the data collected for any significance difference. From Tables 6 and 7 with respect to Uzo-Uwani community, there was a significant difference between the concentrations of the heavy metals in the sampled bitter leaves and honey and those of the sampled soil and the honey. This implied that there might exist a relationship between the concentrations of the heavy metals in the cassava tuber, the soil, and the bitter leaves sampled in the community. Thus, there was no significant connection between the heavy metal concentrations in the honey, soil, and leaves. But, this was not so from the

Table 8 Squared cosines of the observations after varimax rotation

	D1	D2
Uzo-Uwani		
Cassava (*M. esculenta*)	0.2978	0.4138
Bitter leaf (*V. amygdalina*)	0.1271	0.5675
Soil	0.0285	0.0050
Honey	*0.8323*	0.0135
9th Mile		
Cassava (*M. esculenta*)	0.0305	0.2611
Bitter leaf (*V. amygdalina*)	0.3453	0.0749
Soil	*0.8443*	0.0618
Honey	*0.8323*	0.0135
Obollo		
Cassava (*M. esculenta*)	0.0125	0.3442
Bitter leaf (*V. amygdalina*)	0.0734	0.2640
Soil	0.6779	0.0792
Honey	0.0742	*0.9087*

samples collected from 9th Mile and Obollo communities where there are significant differences between the sampled soil and cassava tuber from these communities and also between the sampled honey and the soil. But, there was no significant differences noted between the sampled honey from these communities and the bitter leaves. It can be inferred that there exists a relationship between the concentrations of heavy metals in the atmosphere (honey) and the sampled bitter leaves.

Tables 6 and 7 show that 9th Mile and Obollo communities have the same heavy metal concentration significantly in the sampled cassava tuber and soil. This reaffirms the output from the analysis of variance from Uzo-Uwani. However, it is of interest to note that there exists no significant difference between the concentrations of heavy metals in the sampled honey from the selected communities. Thus, the concentration of heavy metals in the soil sampled from Uzo-Uwani has a higher influence compared to that noted in the honey as it did not reflect its presence significantly in the bitter leaves. From the PCA, it was noted that the honey sampled from Obollo community tended to differ from the other sampled communities in terms of its cadmium and arsenic heavy metal concentrations (see Fig. 2). This was with respect to extracted factor 2 (F2) which accounted for a 16.62% variability of the data collected with respect to this study. Thus, variables associated with factor 1 (F1) account for the highest variability among the data collected. This relationship is reflected in the model development for prediction of heavy metals.

Applying regression analysis for model development (Nwaogazie 2011) with respect to prediction of heavy metal concentration, a majority of the models developed by applying the quadratic approach tended to be superior to the linear equivalent judging from the MSE, goodness of fit, and R^2 values (see Table 9). A total of eight models were developed and ranked in the order of superiority, and the applicable variables (dependent and independent) were the extracts from the PCA after varimax rotation (see Table 8). The models serve a useful purpose in forecasting the concentration level of any metal, say, honey given those of bitter leaves and cassava tuber (Table 9).

Exposures to mixtures may synergize or may be an additive, thus leading to adverse health effects

Table 9 Developed multiple regression models and overall ranking

Sample no.	Model type	R^2	MSE	Overall ranking
Linear multiple regression models				
1	Soil = 5.512 + 1.44 BL − 0.522 CT	0.9272	124.4690	8th
2	HY = − 0.034 + 0.053 BL − 0.04555 CT	0.9900	0.0079	2nd
3	BL = 0.86 + 0.355 L + 23.81 HY	0.9645	45.5371	6th
4	CT = − 0.58 + 0.52 SL	0.8781	56.0457	7th
Quadratic regression models				
5	$BL = 0.89 - 0.59\ SL + 1.98\ CT + 0.013\ SL^2 - 0.03\ CT^2$	0.9985	2.8252	3rd
6	$CT = 0.43 + 1.04\ SL - 65.77\ HY - 0.006\ SL^2 + 31.04\ HY^2$	0.9527	38.0715	5th
7	$SL = -0.686 + 5.867\ BL - 5.390\ CT - 0.043\ BL^2 + 0.007399\ CT^2$	0.9910	23.52	4th
8	$HY = 0.0197 + 0.06\ BL - 0.0738\ CT - 0.000267\ BL^2 + 0.00094\ CT^2$	0.994	0.008	1st

BL bitter leaf (*V. amygdalina*), *CT* cassava (*M. esculenta*), *HY* honey, *SL* soil exceeding those noted upon exposures to a single pollutant (Calderon et al. 2003). Although low doses of a single metal may not cause health effects, metal mixtures may present increased health risk (Calderon et al. 2003) and the USEPA recognizes this as a key gap in metal risk assessment (Abboud and Wilkinson 2013).

Conclusion

Results of non-carcinogenic and carcinogenic risk assessments of metals in *V. amygdalina* and *M. esculenta* samples from both rural and urban communities of Enugu State, Nigeria, were not within a safe range and may pose a public health concern. We recommend population-based biomonitoring of these heavy metals and advice physicians to consider a possible role of these heavy metals in the diagnosis and management of various diseases including cancer.

References

Abboud, P., & Wilkinson, K. J. (2013). Role of metal mixtures (Ca, Cu and Pb) on Cd bioaccumulation and phytochelatin production by Chlamydomonas reinhardtii. *Environmental Pollution, 179*, 33–38.

Adedokun, O. M., Kyalo, M., Gnonlonfin, B., Wainaina, J., Githae, D., Skilton, R., & Harvey, J. (2016). Mushroom: molecular characterization of indigenous species in the Niger Delta Region of Nigeria. *European Journal of Horticultural Science, 81*(5), 273–280.

Adelekan, B. A. (2010). Investigation of ethanol productivity of cassava crop as a sustainable source of biofuel in tropical countries. *African Journal of Biotechnology, 9*, 5643–5650.

Altun, S. K., Dinç, H., Paksoy, N., TemamoLullar, F. K., & Savrunlu, M. (2017). Analyses of mineral content and heavy metal of honey samples from south and east region of Turkey by using ICP-MS. *International Journal of Analytical Chemistry, 2017*, 1–6. https://doi.org/10.1155/2017/6391454.

Amadi, C. N., Igweze, Z. N., & Orisakwe, O. E. (2017). Heavy metals in miscarriages and stillbirths in developing nations. *Middle East Fertility Society Journal*, 22(2), 91–100.

Bamuwamye, M., Ogwok, P., & Tumuhairwe, V. (2015). Cancer and non-cancer risks associated with heavy metal exposures from street foods: evaluation of roasted meats in an urban setting. *Journal of Environment Pollution and Human Health, 3*(2), 24–30.

Bellinger, D. C., Stiles, K. M., & Needleman, H. L. (1992). Low-level lead exposure, intelligence and academic achievement: a long-term follow-up study. *Pediatrics, 90*(6), 855–861.

Calderon, J., Ortiz-Perez, D., Yanez, L., & Díaz-Barriga, F. (2003). Human exposure to metals. Pathways of exposure, biomarkers of effect, and host factors. *Ecotoxicology and Environmental Safety, 56*, 93–103.

Cao, H., Qiao, L., Zhang, H., & Chen, J. (2010). Exposure and risk assessment for aluminum and heavy metals in Puerh tea. *Science of the Total Environment, 408*, 2777–2784.

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., & Raskin, R. G. (1997). The value of the world's ecosystem services and natural capital. *Nature, 387*(6630), 253–260.

EFSA. (2008). Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in food. *EFSA Journal, 724*, 1–114.

FAO/WHO. (2011). *Evaluation of certain contaminants in food: seventy-second report of the joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 959*. Geneva: World Health Organization.

Gallmann, P., Stefan, B., Tomislav, J., & Robert, S. (2009). Honey for nutrition and health: a review. *Journal of the American College of Nutrition, 27*, 677–689. https://doi.org/10.1080/07315724.2008.10719745.

Hague, T., Petroczi, A., Andrews, P. L., Barker, J., & Naughton, D. P. (2008). Determination of metal ion content of beverages and estimation of target hazard quotients: a comparative study. Chemistry Central Journal, 2(1), 13.

Hernández, O. M., Fraga, J. M. G., Jiménez, A. I., Jimenez, F., & Arias, J. J. (2005). Characterization of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry. *Food Chemistry, 93*(3), 449–458.

Hu, X., Zhang, Y., Ding, Z. H., Wang, T. J., Lian, H. Z., & Sun, Y. Y. (2012). *Bio accessibility and health risk of arsenic and heavy metals (Cd, Co, Cr, Cu, Ni, Pb, Zn and Mn) in TSP and PM2. Atmospheric Environment* (Vol. 57, pp. 146–152).

Igbiri, S., Udowelle, N. A., Ekhator, O. C., Asomugha, R. N., Igweze, Z. N., & Orisakwe, O. E. (2017). Polycyclic aromatic hydrocarbons in edible mushrooms from Niger Delta, Nigeria: carcinogenic and non-carcinogenic health risk assessment. *Asian Pacific Journal of Cancer Prevention, 18*(2), 437.

Ioannidou, M. D., Zachariadis, G. A., Anthemidis, A. N., & Stratis, J. A. (2005). Direct determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry. *Talanta, 65*, 92–97.

Iwegbue, C. M., Obi-Iyeke, G. E., Tesi, G. O., Obi, G., & Bassey, F. I. (2015). Concentrations of selected metals in honey consumed in Nigeria. *International Journal of Environmental Studies*, 72(4), 713–722.

Jarup, L., (2003). Hazards of heavy metal contamination. *Br. Med. Bull*. 68, 167–182.\

Jolly, Y. N., Islam, A., & Akbar, S. (2013). Transfer of metals from soil to vegetables and possible health risk assessment. *SpringerPlus, 2*(1), 385.

Kalia, K., & Flora, S. J. (2005). Strategies for safe and effective therapeutic measures for chronic arsenic and lead poisoning. *Journal of Occupational Health, 47*, 1–21.

Komosinska-Vassev, K., Olczyk, P., Kaźmierczak, J., Mencner, L., & Olczyk, K. (2015). Bee pollen: chemical composition and therapeutic application. *Evidence-Based Complementary and Alternative Medicine*, 1–7.

Leblebici, Z. E. L. I. H. A., & Aksoy, A. H. M. E. T. (2008). Determination of heavy metals in honey samples from Central Anatolia using plasma optical emission spectrofotometry (ICP-OES). *Polish Journal of Environmental Studies, 17*(4), 549–555.

Li, Z., Zhuanga, P., McBride, M. B., Xiaa, H., & Lia, N. (2009). Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. *Science of the Total Environment, 407*(5), 1551–1561.

Llobet, J. M., Falco, G., Casas, C., Teixido, A., & Domingo, J. L. (2003). Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *Journal of Agricultural and Food Chemistry, 51*(3), 838–842.

NEPAC. (2005). *Maximum levels of contaminants in food*. Beijing, China: National Environmental Protection Agency of China.

Nwaogazie, I. L. (*2011*). *Probability and statistics for science and engineering practice* (pp. 125–126). Enugu: De-Adroit Innovation.

Oluwole, S. O., Makinde, O. S. C., Yusuf, K. A., Fajana, O. O., & Odumosu, A. O. (2013). Determination of heavy metal contaminants in leafy vegetables cultivated by the road side. *International Journal of Engineering Research and Development, 7*(3), 1–5.

Orisakwe, O. E., Nduka, J. K., Amadi, C. N., Dike, D. O., & Bede, O. (2012). Heavy metals health risk assessment for population via consumption of food crops and fruits in Owerri, South Eastern, Nigeria. *Chemistry Central Journal, 6*, 77.

Orisakwe, O. E., Blum, J. L., Sujak, S., & Zelikoff, J. T. (2014). Metal pollution in Nigeria: a biomonitoring update. *Journal of Health Pollution*, 4(6), 40–52.

Orisakwe, O. E., Igweze, Z. N., & Udowelle, N. A. (2019). Candy consumption may add to the body burden of lead and cadmium of children in Nigeria. *Environmental Science and Pollution Research, 26*(2), 1921–1931.

Osu, S. R., Solomon, M. M., Abai, E. J., & Etim, I. G. (2015). Human health risk assessment of heavy metals Intake via cassava consumption from crude oil impacted soils with and without palm bunch ash additive. *International Journal of Technical Research and Applications, 3*(4), 140–148.

Ozoani, H. A.. (2018). *Human health risk assessment of heavy metal and polycyclic aromatic hydrocarbons (PAHs) in honey, cassava and bitter leaf from Enugu State, Nigeria*. MSc thesis. Faculty of Pharmacy, University of Port Harcourt. Nigeria

Rehman, K., Fatima, F., Waheed, I., & Akash, M. S. H. (2018). Prevalence of exposure of heavy metals and their impact on health consequences. *Journal of Cellular Biochemistry, 119*(1), 157–184.

Silici, S., Uluozlu, O. D., Tuzen, M., & Soylak, M. (2008). Assessment of trace element levels in Rhododendron honeys of Black Sea Region, Turkey. *Journal of Hazardous Materials, 156*(1–3), 612–618.

Singh, A., Sharma, R. K., Agrawal, M., & Marshall, F. M. (2010). Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. *Tropical Ecology, 51*(2), 375–387.

Sitarz-Palczak, E., Kalembkiewicz, J., & Galas, D. (2015). Evaluation of the content of selected heavy metals in samples of Polish honeys. *Journal of Ecological Engineering, 16*(3), 130–138.

Steenland, K., Boffetta, P. (2000). Lead and cancer in humans: where are we now? *Am. J. Ind. Med*. 38, 295–299

Underwood, E. J. (1977). *Trace elements in human and animal nutrition* (4th ed.). New York: Academic.

US EPA (1989) (United States Environmental Protection Agency)Risk assessment guidance for superfund. Human Health Evaluation Manual (Part A). *Interim Final, vol. I, United States Environmental Protection Agency*, Washington (DC) EPA/540/1-89/002

USEPA (2000) *Supplementary guidance for conducting health risk assessment of chemical mixtures*. Risk Assessment Forum Technical Panel [EPA/630/R-00/002].

USEPA. (2005). *The limits of pollutants in food*. China: State Environmental Protection Administration.

WHO (1991) *International Programme on Chemical Safety*. Environmental Health Criteria, Vol. 108, Geneva, p. 286.

WHO (1992). Cadmium. Environmental Health Criteria, Geneva. Vol. 134.

Xia, Z. H., Duan, X. L., Qiu, W. X., Liu, D., Wang, B., Tao, S., Jiang, Q. J., Lu, B., Song, Y. X., & Hu, X. X. (2010). Health risk assessment on dietary exposure to polycyclic aromatic hydrocarbons (PAHs) in Taiyuan, China. *Science of the Total Environment, 408*, 5331–5337.

Zhan, X., Liang, X., Jiang, T., & Xu, G. (2013). Interaction of phenanthrene and potassium uptake by wheat roots: a mechanistic model. *BMC Plant Biology, 13*(1), 1–9.