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Assessment of Anthropogenic Influence in Kurmi Cocoa Farmlands and Cocoa Beans using Heavy Metals as Indicators, and the In-vitro Bioaccessibility in Locally Produced Cocoa-Based Beverages to Public Health

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Abstract - **This study was conducted to assess the level of anthropogenic-influence across cocoa farmland soils, to cocoa beans and the locally produced cocoa-based beverages (CBB) from cocoa producing communities of Kurmi, Taraba State using heavy metals (Cr, Cu, Zn Pb, Cd, Mn, and Fe) as indicators. The results show Cr, Zn, and Pb in soil samples from Abong, Gidan Makeri, and Asha on the average detected to have values above the permissible limit (PL) set by the FAO/WHO. The further assessment shows only 23%, 39% and 4% of their total concentrations are from anthropogenic-related activities (Apn%) and of low ecological risk to the environment with potential ecological risk (Er) <40 and Ri≤10. The level of heavy metals in the cocoa beans samples analyzed were observed to be above the PL set by FAO/WHO. Compared to the values detected in the cocoa beans samples, the mean concentrations for Cr (6.25±1.88), Cu (7.78±2.87), Zn (19.73±7.19), Pb (18.10±6.59). Cd (4.78±2.07), Mn (8.26±4.92), and Fe (33.79±4.07) were observed to be significantly higher in the locally produced CBB samples. The in vitro digestion of the CBB samples shows mean bioaccessibility concentrations range from 0.67-3.38 mg/kg, with Fe, Cu, Cd, and Pb showing the highest mean concentration values of 3.38±2.64, 2.68±3.51, 1.84±1.82, and 1.65±1.27 mg/kg.The results revealed Cu and Cd with mean in vitro concentrations of 2.68±3.51, and 1.84±1.82 to be highly extracted in the gastrointestinal fluids showing a percent bioaccessibility of 34%and 39%. The estimated daily intake (EDI) of the metals were observed to be above the oral reference dose (RfD) values with average contributions rates (ACR) to RfD >100%.**

Keywords: Cocoa-based beverages, Heavy metals, Bioaccessibility, In vitro gastrointestinal digestion, Risks.

I. INTRODUCTION

To combat the scourge of black pod disease, must farmers in Nigeria resorted to the use of copper-based fungicides and Heavy-metal based agrochemicals to boost production demands. These practices as reviewed by Aikpokpodion *et al*(2012) reported traces of heavy metals in some cocoa plantations soil in Nigeria. With various literature reporting the presence of Pb, Cd, V, Co, Cr, Cu, Fe, As, Ni, Mn, Sn, Zn, and Hg in agricultural soils **(**Reviewed in Nkwunonwo et al., 2020**)**. Which according to the studies transcends beyond the soils into the food chains (Opaluwa *et al.*, 2012; Nabulo *et al.*, 2010; Doherty *et al;* 2012; Babatunde *et al.*, (2014). The perspective put forward by Abt and Robi (2020) shows a relationship between heavy metals build-up in cocoa foods and anthropogenic-related chemistry. Showing several studies reporting a moderate to strong correlation between heavy metals levels and percent cocoa. A similar review by Izah*et al.*, (2017) reported the presence of Cr, Mn, Cd, Zn, and Pb in commonly consumed commercially packaged beverages in Nigeria. The concentration in some of the products according to the review exceeded the SON and WHO acceptable limits. Cocoa-based Chocolate were also observed to accumulated more Cu, Cr, Pb, Ni, and Cd than Milo, Bournvita beverages, and Candies in a separate study conducted by Dahiya *et al.*, (2005).

Though these various studies established the presence of heavy metals in the cocoa products, the studies however are limited to their total concentrations in branded and imported cocoa-based beverages and confectionaries without going further to assess their bioavailability and mobilization physiologically. This brings out the obvious exigency to further investigate the locally produced cocoa-based beverages common among the cocoa farming communities in Nigeria. A recent survey revealed that most of the smallholder farming communities produced cocoa-based products (CBB) locally to complement their dietary needs for beverages; a common

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practice observed in the cocoa farming communities of Kurmi in Taraba State. For these reasons, this study was carried out to established possible pollution trends/dynamics using heavy metals as indicators in the soil of cocoa farmlands, cultivated cocoa beans, and beverages (promton) locally produced by the cocoa farming communities of Kurmi in Taraba State and the in vitro bioaccessibility to public health. Employing In vitro-based techniques in dietary health risk assessment is considered effective in evaluating the actual extractible amount of heavy metals available for absorption.

II. MATERIAL AND METHOD

2.1 Description and Location of the Study Area

Kurmi local government area (LGA)of Taraba state as shown in Fig. 1 is located at an elevation of 265 meters above sea level and lies roughly between Latitude $6^{0}30^{1}$ and 9^{0} 36^{1}N Longitudes $9^{0}10^{1}$ and $11^{0}50^{1}$E (Zaku et al., 2019). A rich agricultural area geographically forested and characterized by mountains and waterfalls. Bounded in the south by the Republic of Cameroon, to the south-east and north-east by Sardauna and Donga LGA of Taraba State.

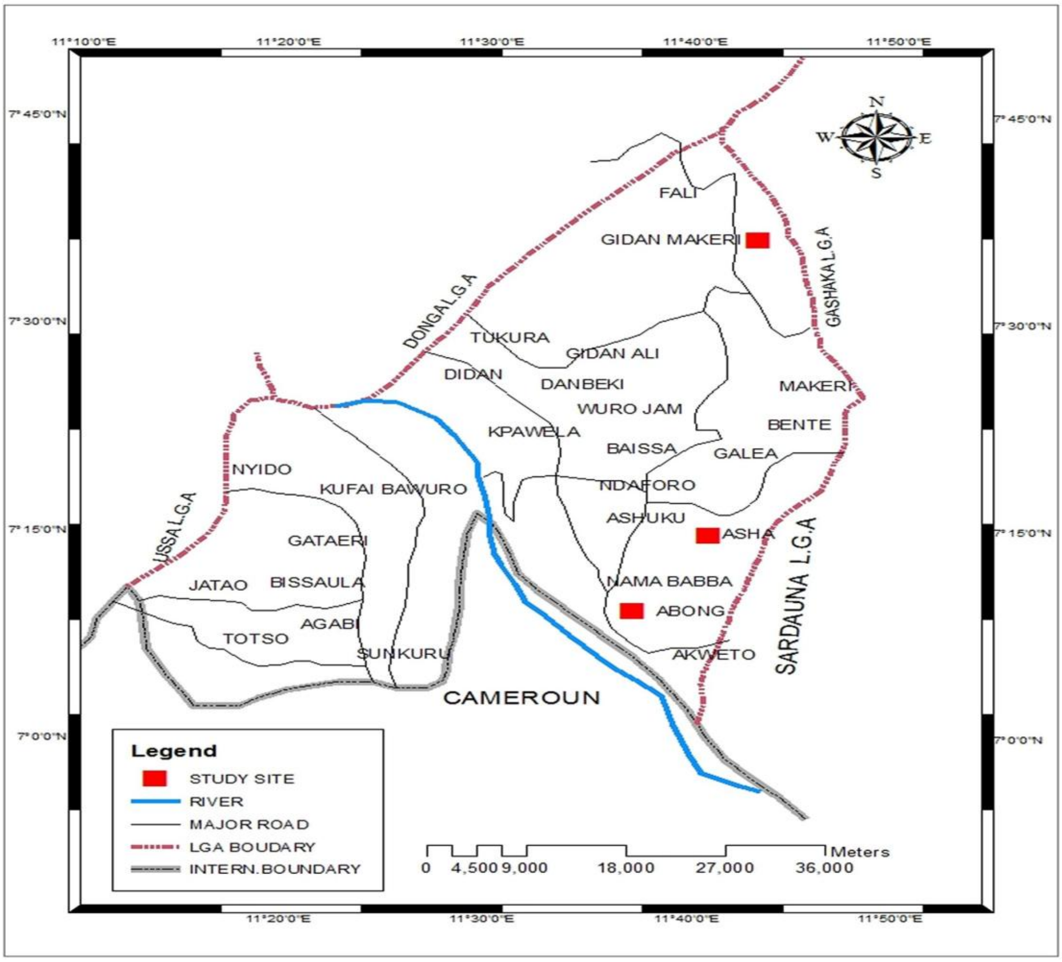

Figure 1: Map of the Kurmi LGA showing the sample locations

2.2 Sample Collection and Preparations

The soil, cocoa beans, and the locally processed cocoa-based beverages (CBB) samples were obtained from three (3) cocoa farming towns in Kurmi Local Government area Taraba State. The soils and cocoa beans were obtained directly from cocoa farmlands in the selected areas. Ten (10) samples each were collected in farmlands across Abong, Gidan Makeri, and Asha towns, making a total of thirty (30) representative sample sites. One gram of the milled sieved cocoa beans and soil samples were digested in 30ml of aqua regia (3:1 volume of HCl and HNO_3)at 200 ^{0}C for 20 minutes and analyzed for the presence of Cu, Cd, Mn, Cr, Fe, Pb, and Zn using Atomic absorption spectrophotometers (AAS) (Buck scientific spectro photometer, VGP 2010).

The bioaccessibility concentrations of the aforementioned heavy metals were analyzed from the locally produced cocoa-based beverages (CBB) commonly called Promton. The CBB samples obtained from the three samples locations were

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digested in a Simulated Gastrointestinal solution prepared following four sequential steps (Wragg and Cave, 2003**).** Briefly, Simulated Gastric Solution (A)with a pH of 1.8 ±0.1 were prepared by dissolving 10.0g of pepsin, in 800ml deionized water, then 16.4ml of dilute hydrochloride acid prepared by diluting 234ml of concentrated hydrochloric acid to 1000ml with deionized water were added to bring the total volume up to 1000ml. Furthermore, the Simulated Intestinal Solution (B) were prepared just before use by mixing an equal volume of (C and D) solution. Solution (C) is prepared by dissolving 6.8g of potassium dihydrogen phosphate in 500ml of water (pH to 6.8).The solution (D) is prepared by dissolving 10g of pancreatin in 500ml water (Sun, *et al*., 2019). Five (5) ml of the prepared gastric juice (A) were added into 0.5g of precisely weighted CBB samples and heated for 1 hour and agitated in a water bath for 30min in a 250mm^{-1}beaker. The samples were then allowed to incubate for an additional 3h at 38^{0}C and centrifuged for 10min to obtain the gastric digestion extraction solution (supernatant). To the residue obtained from the gastric juice (A) digestions, 5ml of intestinal juice (B) were added, heated for 1 hour, followed by agitation in a water bath for 30min, and incubate further for an additional 3h at 38^{0}C. The samples were centrifuged and the supernatant passed through a 0.45µm PVDF syringe-type. The obtained supernatant were then mineralized in a microwave oven in a mixture containing gastric digestion extraction solution(Pizarro *et al*., 2016) and run for heavy metal analysis using AAS.

2.3 Assessment of Anthropogenic Influence and Potential Ecological Risks of Heavy Metals in the Soil of the Cocoa Farmlands

The percentage of anthropogenic influence on the availability of the heavy metals in the farmland soils were determined using Anthropogenicity (Apn %) calculated from the expression

$$APn\% = \frac{Mc}{Bn} x100$$

Where, M_c = measured concentration, while B_n= average world background concentration. The Bn for the metals are Cr=90, Cu=45, Zn=95, Pb=20, Cd=0.30, Mn=850 and Fe=47200.

The nature or potential effects of anthropogenic-related actions on public health and the environment were further assess using the Ecological Risk factor E_r and Potential Ecological Risk Index (R_i) proposed by Hakanson (1980). The Ecological Risk factor $E_r$is expressed in the equation

$$Er = Tix\left(\frac{Cm}{Bn}\right)$$

Where E_r is an ecological risk factor, T_i is a toxic response factor for the metals. The values for each metal is in the order of Zn=Mn=1<Cr=2<Cu=Pb=5<Cd=30 (USEPA, 2017). The toxic response factor is not available for Fe and Mn, thus not included in the assessment. The Cm is the metal content in the soil and Bn is the background value of metals in soil. The following terminology are used to describe the potential ecological risk factor, E_r<40 indicate low potential ecological risk, 40<E_r<80 moderate potential ecological risks, 80<E_r<160 considerable potential ecological risks, 160<E_r<320 high potential ecological risks and E_r>320 very high potential ecological risks. The Potential Ecological Risk Index (R_i) is calculated using the equation below

$$Ri = \sum E_r^i$$

Where R_i is potential ecological risk calculated as the sum of an ecological risk factor for heavy metals in soil. E_i is an ecological risk factor. The following terminology are used to describe the potential ecological risk index; R_i<150 low ecological risks, 150≤R_i>300 moderate ecological risks, 300≤ R_i>600 considerable ecological risks, and R_i> 600 very high ecological risks.

2.4 Determination of Heavy Metals Bioaccessibility in the BCC samples and Estimation of Dietary Daily Intake rate to Public Health

The Bioaccessibility concentration which represents the fraction of the total amount of the heavy metals in the in vitro digested CBB samples were assessed in this study using the equations (Liu et al., 2017)

$$\text{Bioaccessibility \%} = \frac{[metal\ ]Bac}{[metal\ ]G} \times 100$$

Where [metal]Bac is the bioaccessible metal concentration in vitro gastrointestinal solution of CBB samples. The [Metal]G is the total metal concentration in the CBB samples before digestions.

In this study, rather than using the total metal concentrations in the CBB samples, the Dietary Daily intake of the Heavy Metals was assessed using the in vitro bioaccessibility concentrations values determined using the USEPA recommended procedure described in the equation.

$$\text{EDI}= (C\mu \times F_{IR})/B\text{w}$$

Where EDI is the average daily intake (mg/kg body weight/day); C_μ is the concentration of the elements in the CBB sample; F_{IR} is the average daily consumption of Cocoa (kg). F_{IR} of 20 g (Children) and 40g (adults) of cocoa-based products per day were used in this study (Afoakwa, 2016).

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The BW is the body weight (kg); set at 60 for an average adult and 15 for children (Bwatanglang *et al.*, 2019a). Table 1 is used as the oral reference doses (*RfD*) to compare the EDI (USEPA, 2017).

Table 1: Reference doses (*RfD*) in (mg/kg-day) for the individual heavy meta

Elements	RfD
Cr	0.003
Cu	0.037
Zn	0.300
Pb	0.004
Cd	0.001
Mn	0.140
Fe	0.70

Source: USEPA, (2017).

2.5 Statistical Analysis

All analysis was performed in triplicate and results were presented in Means ± SD. The level of significance was established using a one-way analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

3.1 Concentrations of Heavy Metals in the Farmlands Soil Samples

As shown in Fig. 2, only Cr, Zn, and Pb were detected to have values above the permissible limit (PL) set by the FAO/WHO. Mean concentrations of 21.51±0.12 mg/kg of Cr above the PL of 0.40mg/kg set by FAO/WHO were detected in the soil samples. On average, these values were significantly lower to the 40.26±52.12mg/kg recorded in a soil sample collected in Ibadan, and the 42.57±77.10mg/kg recorded in a soil sample from Northern Nigeria by Tsafe *et al.*, (2012). Chromium is a metal linked to cancer pathogenesis that found its way into the soil sample in the study location most probably from the application of Agrochemicals (Liu *et al.*, 2017). And further introduced into the soil in addition to the background values from activities such as the burning of palm trees shaft to ash, used by the locals in the production of black soap. Even though Zinc is considered the least toxic element and very essential component of a healthy diet, beyond the permissible limit of2.0mg/kg set by the FAO/WHO in agricultural soil as detected in this study with a mean value of 37.85±4.02mg/kg, could lead to health-related complications such as nausea, vomiting, diarrhea, fever, and drowsiness (Tchounwou*et al.*, 2012). In addition to engine oil spills, combustion processes, and attrition of motor vehicle tires, other activities such as the continual mechanical abrasion of brake linings could release Quantum of Zinc particles into the soil (Bwatanglang et al., 2019b). Similar activities as discussed for Zn in addition to the applications of agrochemicals are possible routes for Pb induction into the soil.

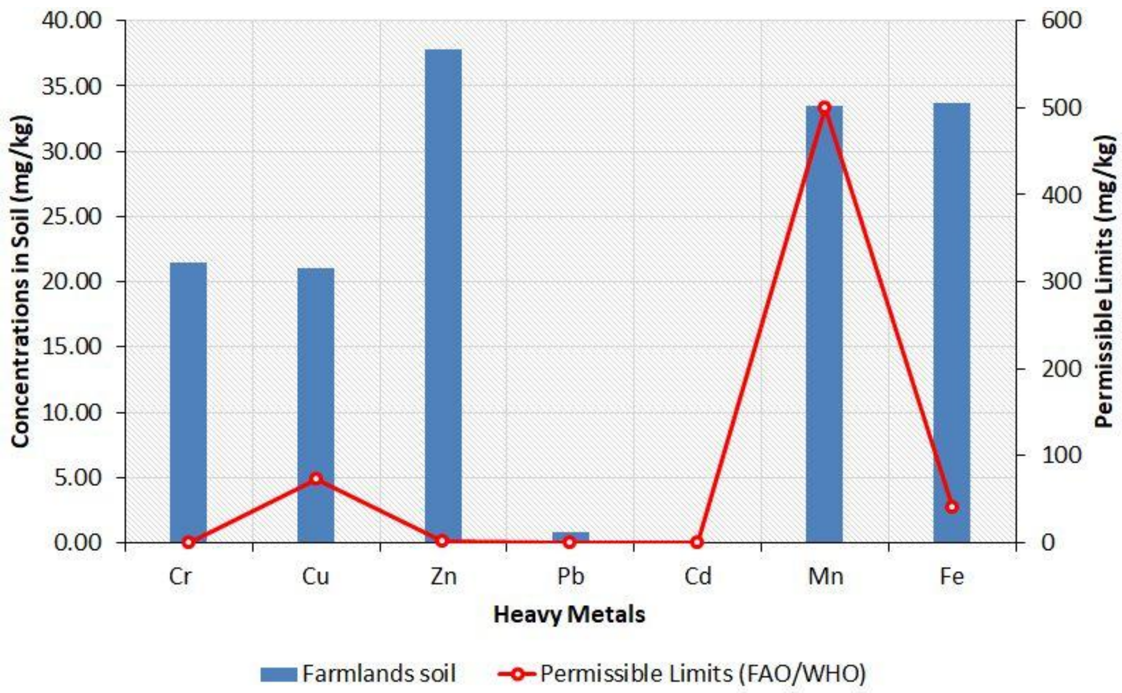

Figure 2: The Mean Concentrations of Heavy Metals in Cocoa-Farmlands Soil and the FAO/WHO Permissible Limits. Results are presented in Mean±SD

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3.2 Anthropogenic Influence and Potential Ecological Risks of Heavy Metals in the Farmlands Soil

Though the findings of this study as shown in Fig. 2 revealed the concentrations of Cr, Pb, and Zn in the soil of the cocoa-farmlands to be above the PL, the further assessment shows only 23%, 39% and 4% of their total concentrations are from anthropogenic-related activities as presented in Fig. 3. Cadmium and Cu with total mean concentrations of 0.07±0.03 mg/kg and 21.02±1.88 mg/kg were observed to be largely contributed by anthropogenic influences with an Apn of 24% and 46%. These findings further support the conclusion that the cocoa farmers used Cu-based fungicide to combat the black pod scourge (Semu and Singh, 1996). The contribution of Cd as mentioned earlier could emanate from the applications of Phosphate-based fertilizers which were reported to contain Cd-based ingredients (Grant and Sheppard, 2008; Atafar *et al*., 2010). Study shows a high correlation for these metals associated with phosphate in fertilizers (Ukpabi *et al*. 2012), and application of fungicide for Cu enrichment (Semu and Singh, 1996). From the results, as shown in the figure, the total mean concentrations of Mn (33.51±0.42 mg/kg) and Fe (33.73±3.06) are derived largely from natural geologic processes as the A pn assessment shows only 3% and 0.1% are from anthropogenic-related activities. The result further suggests that the metals may have originated from the rock type or runoff from erosion. Similar conclusions were also reported by Onisogen and Friday (2017) in soil samples from Abattoirs in Port Harcourt, Rivers State, Nigeria. This observation corroborates the findings of Osakwe (2016) in a similar environment and activity in the Niger delta area, Nigeria.

The possible risk associated with these pollutants though observed to have no or little impact on the total metal concentration in the soil, however, draws the obvious conclusion to further subjects the data to potential ecological risk assessment. The Er of the heavy metals in the soils from the study areas factored using the world background concentration and the toxic response factor was found to be less than 40, thus are of low ecological risk. Cadmium shows a mean Er of 7.47 and the second highest Er is Cu with an average mean value of 2.34. Similarly, the Ri values estimated for the metals in the surface soil from all the study locations are observed to be of low ecological risk, having values of R_i<150 with a mean value of 10.72. The values calculated showed that the metals do not pose any immediate ecological risk to the environment (Hakanson, 1980).

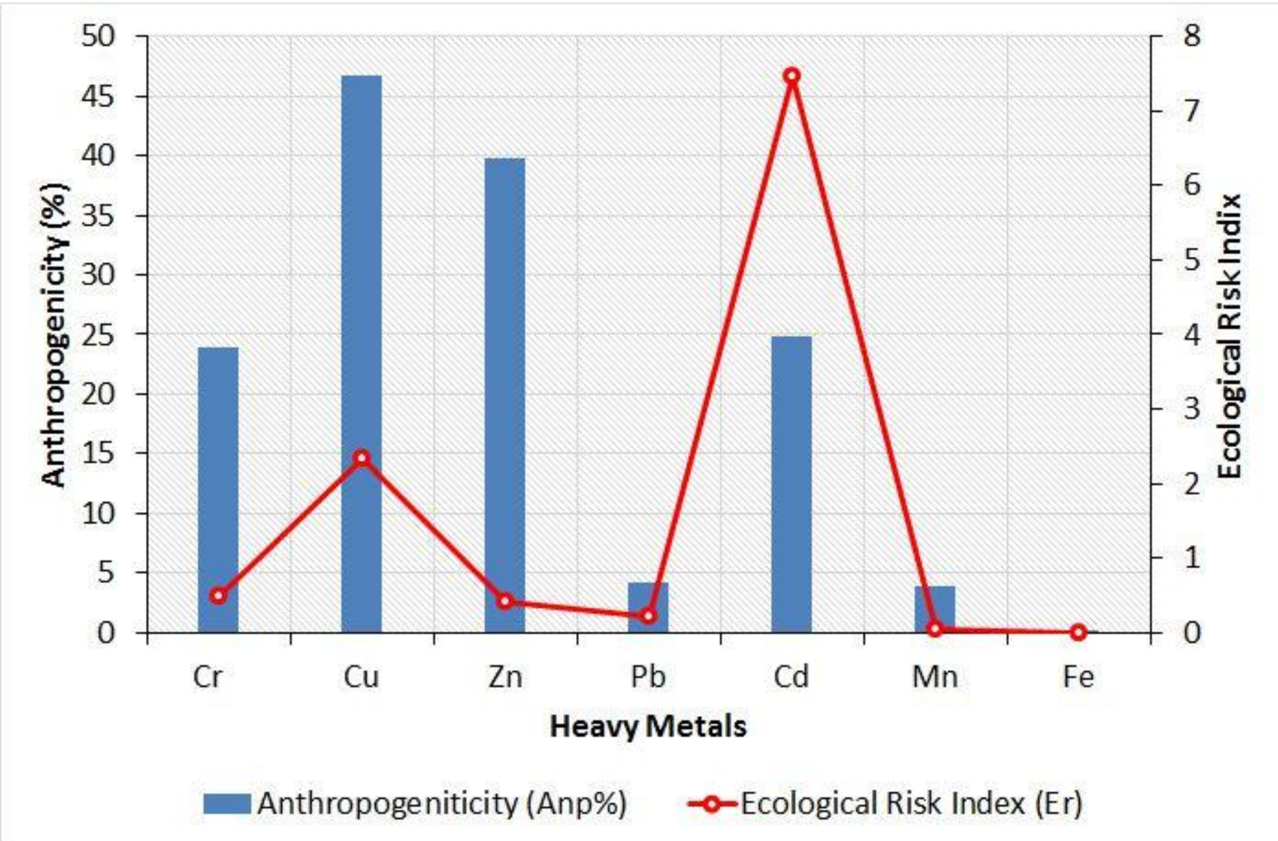

Figure 3: The Anthropogenicity and the Ecological Risk Index of Heavy Metals in the Cocoa-Farmlands Soil

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3.3 Concentrations of Heavy Metals in the Raw Cocoa Beans Samples

The results in Fig. 4 show the level of the heavy metals in the raw cocoa beans samples. The mean values of the heavy metals analyzed were observed to be above the PL, except Mn showing mean values of 9.71±0.28 mg/kg, below the PL of 500 mg/kg set by FAO/WHO for edible plants. A mean value of 4.11±0.25 mg/kg for Cr were detected in the samples. Though, Cr enhances the action of insulin, a hormone that is critical for the metabolism and storage of fat, carbohydrates, and protein in the body (Magili and Bwatanglang, 2018), at a concentration above the 0.02mg/kg set by the FAO/WHO for edible plants should be discouraged. Anthropogenic activities which include amongst others, the burning of cocoa pods to ash by the farmers in the study area and the presence of local cocoa beans processing machines and other vehicular activities could also release Cr into the environment for plant uptake. The values of Cu (2.04±0.55 mg/kg) as shown in the results fall above the PL of 0.70mg/kg set by the FAO/WHO. Minerals such as Cu, readily translocate across plants tissue via the phloem. Phloem sap were reported to easily absorb metal such as Fe, Cu, Zn, and Mn (Stephan *et al*.,1995).A study conducted in Colombo, Sri Lanka, reported about 7.05±18.44mg/kg of Cu in vegetable samples (Thilini *et al.*, 2014). A concentration as high as 27mg/kg were reported by Aikpokpodion, *et al.*, (2013a), in cocoa beans samples from Ogun and Ondo state respectively. The same author reported concentration ranges from 10-24mg/kg with an average value of 18mg/kg in cocoa samples from Cross River state. Lee and Low (1985) reported Cu levels ranging's from 15.22mg/kg-24.50mg/kg, in raw cocoa, semi-finished, and finished chocolate products. The result may be suggestive that the use of Copper-based fungicides to prevent black pod disease over time may have accumulated levels of copper in the sample. Zinc with a mean concentration of 10.53±0.38mg/kg above the PL of 0.13mg/kg set by the FAO/WHO for edible plants were detected in the samples. These values are higher than the 0.21±0.25mg/kg reported by Lawal and Audu (2011) in vegetable samples from Kano. Generated from similar anthropogenic activities stated earlier.

Higher concentrations of lead above the PL of 0.12mg/kg set FAO/WHO were detected only in the raw cocoa samples from Abong (2.33mg/kg). Cocoa farmers in the study area often apply Ridomil, Kocide, Funguran, and a host of Fertilizers to control pests and enhance cocoa bean's yield. Such practices as reported in several studies could increase the contamination level of heavy metals in the soil and translocation in plants (Thomas *et al.,* 1999). Even in low concentrations, Cd could be harmful to organisms (Tsafe et al., 2012). The concentration of Cd (mean value of 0.05±0.02 mg/kg) in the samples from all the study locations were observed to be above the PL of 0.02mg/kg set by the FAO/WHO for edible plants. The result is incongruity with the study conducted in Sri Lanka in vegetables where Cd exceeded the FAO/WHO Limit (Thilini *etal.,* 2014).The concentration of Fe were observed to be above the PL of 0.40mg/kg set by the FAO/WHO for edible plants. The result shows a mean concentration of 19.60±0.49mg/kg in cocoa beans samples. The concentrations as observed in this study could come from either geogenic origin or excessive application of agrochemical in the study areas.

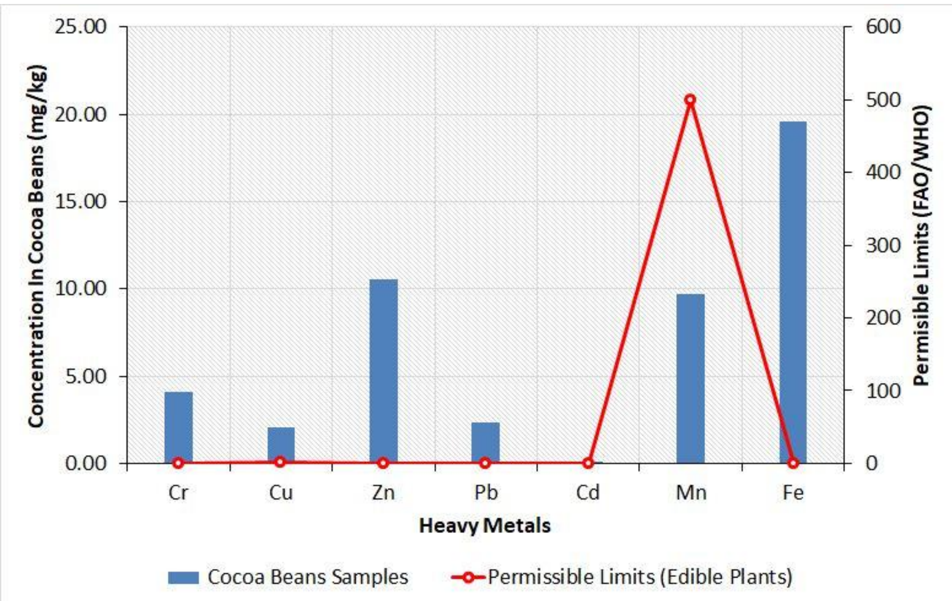

Figure 4: The Mean Concentrations of Heavy Metals in Cocoa Beans Samples and the FAO/WHO Permissible Limits. Results are presented in Mean±SD

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3.4 Concentrations of Heavy Metals in Locally Produced Cocoa-based Beverages (CBB)

Though the mean concentrations of Cr, Zn, Cd, and Fe in the raw cocoa beans samples (Fig. 4)were found to be above their PL, these values were however found to be relatively lower than the values detected in the locally processed cocoa beverages (CBB) in Fig.5. Lead were only detected in samples from Abong but the results in Fig 4 shows Pb in samples from all the study locations with a mean concentration of 18.10±6.59 mg/kg. Compared to the values detected in the cocoa beans samples, the mean concentrations (mg/kg) for Cr, Cu, Zn, Cd, Mn, and Fe were observed to be significantly higher in the CBB samples, showing mean concentrations (mg/kg) of 6.25±1.88, 7.78±2.87, 19.73±7.19, 4.78±2.07, 8.26±4.92, and 33.79±4.07.

The concentrations of Cr detected in this study, though higher than the PL and the5.37 mg/kg detected in chocolate samples from Bosnia and Herzegovina (Alagić and Huremović, 2015), and lower than the 32.0 mg/kg values reported by Alkherraz et al (2019) in samples from France. The average Cu concentration detected in this study were observed to be comparable to the 6.3 mg/kg detected in the cocoa sample from United State but much higher to the 0.8 mg/kg detected in Italian cocoa (Alkherraz et al, 2019). In the same study, the concentration of Zn determined is 0.4 mg/kg in samples from Italy and 20.9 mg/kg in French cocoa samples, relatively higher than the mean value of 19.73 mg/kg detected in this study.

The average concentrations of Pb and Cd observed in samples from this study were observed to be significantly higher than the ranged values of 1.8 mg/kg to 2.1 mg/kg detected in the Italian sample and the 0.3 mg/kg in samples from the United States (Alkherraz et al., 2019). The same authors reported 22.4 mg/kg of Mn in cocoa samples from the USA. Ruggerti et al. (1983) reported that two commercial cocoa and six chocolate products contained 1.65 to 1.85 ppm of Pb and 0.04 to 0.06 ppm of Cd. The content in the CBB samples in this study was thus higher than that reported by the author and Knezevic (1979, 1982). The higher contents of the heavy metals observed in the CBB compared to the raw concentrations detected in the raw cocoa samples suggest anthropogenic inputs other than natural sources. Heavy metal contamination could ordinarily result from the rolling machine (Alkherraz et al., 2019), another study also reported that cooking processes in chocolate formations could lead to higher metal contents. The higher the fraction of cocoa mass in the finished product, the higher its metal contents (Lee and Low, 1985).

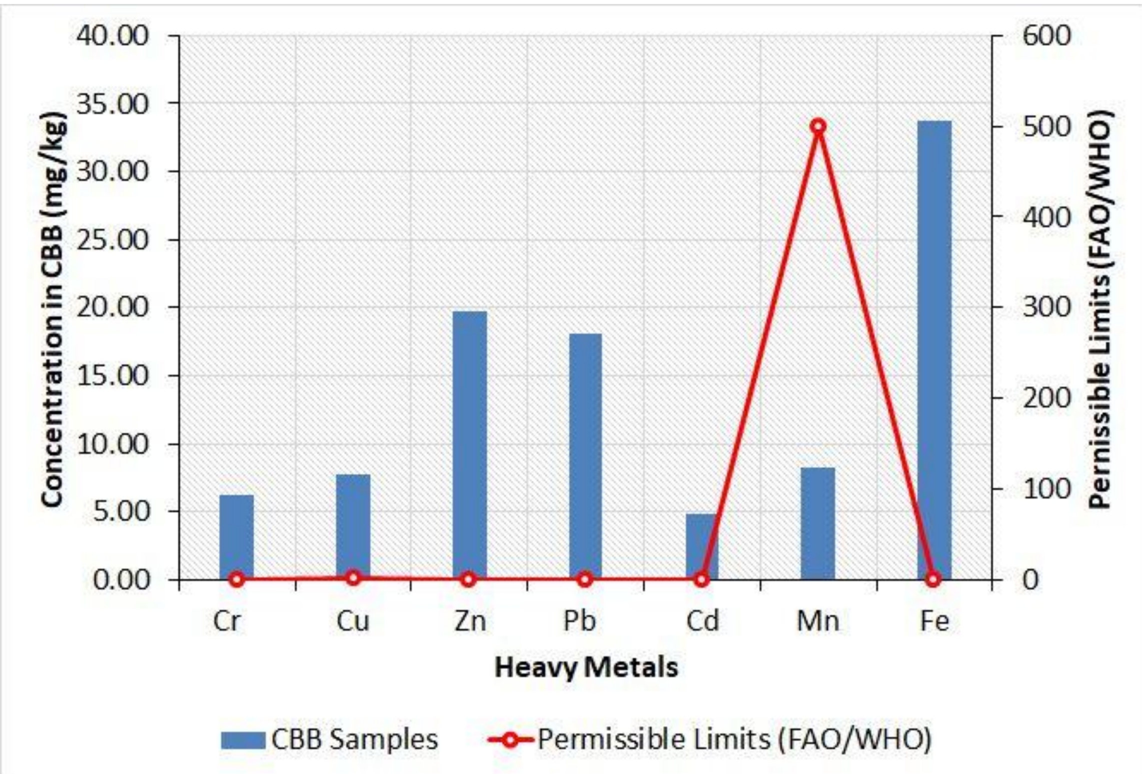

Figure 5: The Mean Concentrations of Heavy Metals in Cocoa-Base Beverages (CBB) and the FAO/WHO Permissible Limits. Results are presented in Mean±SD

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3.5 In vitro Concentration of Heavy Metals (mg/kg) in CBB Samples and their Percentage Bioaccessibility (%)

The results in Fig. 5 generate a level of concern as per the degree of contamination detected in the CBB samples. Cognate to these data, it is pertinent to track down the significant causal factors for the metal contaminations in the CBB products to mitigate them as studies show the emergence of heavy metals exposure and risks from food matrices (Reviewed in Anyimah-Ackah et al., 2018)

As presented in Fig. 6, Cr with a mean value of 6.25 mg/kg in the CBB samples were observed to show a reduction, given a mean in vitro bio concentration of 0.67±0.57 mg/kg. Cupper and Zn were detected at a concentration of 7.78 mg/kg and 19.73 mg/kg in the CBB samples shows only 2.68±3.51mg/kg and 0.69±0.43 mg/kg as the extractible concentration following the in vitro analysis. Cadmium and Pb are out rightly the most dangerous species detected in the samples, by nature are classified carcinogenic and were detected above the level of concern even after the in vitro analysis. Though only 1.65±1.27 mg/kg and 1.84±1.82 mg/kg were extracted in the gastrointestinal medium, these however draw the obvious conclusion to review and establish regulation on the CBB processing methods adopted by the cocoa farming communities. Iron is the most available element detected in samples from all the locations and Mn is one of the essential elements. The results show only 0.92±0.75 mg/kg of Mn and 3.38±2.64 mg/kg of Fe were extracted by the gastrointestinal medium from the total mean concentration of 8.26 and 33.79 mg/kg detected in the CBB samples. A trend established by Rankin *et al.* (2005) shows on average a significant level of Pb in chocolate (0.070–0.230 mg/kg) compared to the value detected in the raw cocoa beans (≤0.0005 mg/kg), largely emanated from processing methods showing the pollution levels correlating contaminant levels in cocoa to production origin. These findings were supported by Villa *et al.* (2014), reporting a strong positive correlation between lead ($R2$ = 0.955) and cadmium ($R2$ = 0.907) levels and cocoa content in chocolate. Duran *et al.*(2009) determined 1.347 mg/kg and 0.681 mg/kg of cadmium and lead in cocoa-derived candies marketed in Turkey. Similarly reported by Abt *et al.* (2018) in the US market. Lo Dico *et al.* (2018) reported 0.116 mg/kg Cd above the European Union maximum contaminant level (MCL) of 0.10 mg/kg in chocolate after microwave-assisted digestion. Other studies reported the level of Pb in chocolate to be greater than the 0.5 mg/kg set by the National Food Safety Standard, China, and the 0.05 mg/kg set by the Food Standards Australia (Kruszewski *et al.* 2018)

The bioaccessibility concentrations which represent the actual amount of the heavy metals extractible on consumption in the gastrointestinal environment. The assumption to bioaccessibility is that not all (100%) the concentrations of contaminate in the food matrix are absorbed by the gastrointestinal tract. Thus, bioaccessibility concentrations may be more accurate in assessing the risk of the heavy metal detected in the CBB samples (Jin, 2012; Huang *et al.*, 2016). Based on the result of the in *vitro* experiment conducted in this study as shown in Fig.6, the mean bioaccessibility of the heavy metals as a proportion of the total showed only 10 percent of Cr were absorbed by the gastrointestinal medium. About 34% of Cu were absorbed following the in vitro digestion of the CBB. Zinc showed absorption values of 3% while Pb and Cd uptake are9% and 38%. Iron percentage absorption was 10% while 11%were observed for Mn. As shown in the results Cd were highly bioaccessible (38%) followed by Cu (34%). The bioaccessibility of Cd with a total mean concentration of 4.78 mg/kg in the CBB samples was observed to be highly extracted in the gastrointestinal fluids showing bioaccessibility of 38% from an in vitro concentration of 1.84 mg/kg in vitro analysis. As for the exceptional bioaccessibility increase of Cu, it could be partially attributed to the good solubility of Cu-phytate complexes in the gastrointestinal fluids (Schümann, & Elsenhans, 2002). Yin et al, (2017) reported the bioaccessibility of Cu, Fe, Mn, and Zn in gastro-intestinal phases to vary within 5.7–75.5%, 17.3–50.4%, 13.3–49.1%, and 19.9–63.7%, respectively. Other studies reported the average bioaccessibility was 16% and 18% for Cu in the gastric and small intestinal phases in some leafy vegetables (Hu et al., 2013). Reported to varied within 32% and 15% in lettuce, and 38% and 26% in leaf lettuce (Pan et al, 2016)

Studies show that Food source is a key factor influencing bioaccessibility, varied among foods types. The bioaccessibility of Cd in the current study (38%) was observed to be higher than the values reported by Yang et al. (2012) for uncooked rice (16.61%), and remarkably lower than the 54% reported in shellfish (Amiard et al., 2008) and the 49–74% range reported in vegetables (Versantvoort et al., 2004). The Cd bioaccessibility percentage in this study was observed to be lower than the 87.3% reported for cabbage from major producing cities in Southwest China (Li et al, 2021) and the 71% reported by Hu et al. (2013) in cabbages samples from Hong Kong. Similarly, Fu and Cui (2013) showed Cd bioaccessibility at 65% in cabbage. Sun et al, (2019) show the bioaccessibility of Cd to be ~50% lower in the gastrointestinal phase which was also observed to agree with the studies reported by Williams et al, (2009). Zhuang et al. (2018) reported similar percentages of Cd bioaccessibility in cooked contaminated rice of 86% in the gastric phase and 51% in the intestinal phase. A study by Mounicou *et al.* (2003) reported Pb and Cd bioavailability of 14% and 50%, showing Cd to be more bioavailable than Pb. In gastric juice, Barraza *et al.* (2017) reported Cd in total, was 90% to 100% bioaccessible.

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Cadmium can accumulate in the vacuoles of plant cells and easily be released from plant tissues (Fu and Cui, 2013). Further observed to be influenced by the low pH value of the gastrointestinal phase which according to the studies facilitates the breakdown of chemical bonds between metals with the body organic components, hence can facilitate its release in the intestinal medium (Shara et al., 2019)

Due to its low solubility, the bioaccessibility of Pb was found to be low (9%) largely due to the formation of Pb-precipitates (Elless, 2000). The bioaccessibility of Zn (3%) was drastically reduced to the competing nature of the much available divalent ions present in the medium. It was reported that the uptake route for divalent ions such as Cd, Cu, Mn, and Pb detected in this study in higher concentrations followed the same route to most divalent micronutrients such as Zn ion. Thus, if the concentration of Cd is higher in the medium as observed in this study, the possibility of effective uptake of Zn ion could be hampered by the competing Cd ion (Degryse et al., 2012).

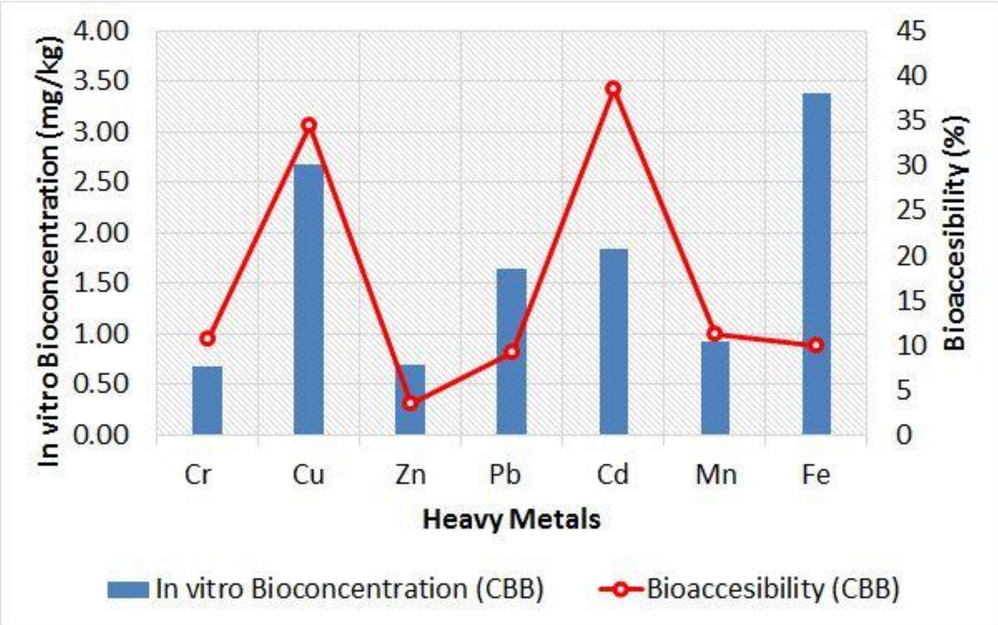

Figure 6: The Mean In vitro Concentration of Heavy Metals in CBB Samples and the Percentage Bioaccessibility

3.6 Estimated daily intakes of heavy metals using in vitro concentrations

The potential health impact on the heavy metal contaminated CBB to the locals in the study areas as shown in Fig. 7were evaluated using the in vitro concentration. In the table, EDI above the RfD (USEPA, 2017) values were observed for all the heavy metals detected. Heavy metals with the highest EDI for adults are Fe (2.25 mg/kg) followed by Cu (1.78 mg/kg) and Cd (1.23 mg/kg). The average contribution rate of EDI for all the metals to their RfD values were observed to be >100%. A similar trend was observed in the EDI values for children orally exposed to the CBB samples. The results show EDI values of 4.50, 3.57, 2.46, and 2.20 for Fe, Cu, Cd, and Pb (mg/kg). All showing an average contributions rate to RfD is >100%. Overall, the result shows children are more likely to be susceptible to a higher level of exposure dose to these metals compared to the adult on the consumption of the CBB.

A similar study reported 0.7, 8.8, 2.7, and 4.5 µg kg-1 d-1 as an average EDIs of Cu, Fe, Mn, and Zn in vegetable samples. The contributions rates of EDI to *RfD* in the study were observed to be lower than the values recorded in this present work. The study reported an average contributions rates of EDI to *RfD* of 1.8%, 1.8%, and 1.5% for Cu, Mn, and Zn, respectively (Yin et al., 2017). Hu *et al*. (2013) also reported an average EDIs for Cu and Zn to be 1.6 and 6.3 µg kg-1 d-1 in vegetable samples from Hong Kong. Pan *et al*. (2016) found higher EDI of Cu (2.8 µg kg-1 d-1) from the vegetables grown in contaminated soils. Li et al, (2021) reported EDI values >1 for Cr, having values 2.29–2.15, with As, Cd, and Pb ranging from 0.57–0.02 mg/kg in cabbage samples from major producing cities in Southwest China. A study in Saudi Arabia, on dietary exposures of contaminants from foods containing chocolate, shows similar results where the exposures decreased significantly as the age of the consumers increased (Afoakwa et al., 2016). For young children, it is their lower body weights that put them at risk (Raters, and Matissek, 2018). Studies by Duran *et al.*, (2009) and Villa *et al.*, (2014) reported a higher potential risk 8.6 % for children than adults (1.8%) while the study by Barraza *et al.* (2018) show higher potential noncarcinogenic risk (HQ > 1) amongst adults exposed to cocoa-based products.

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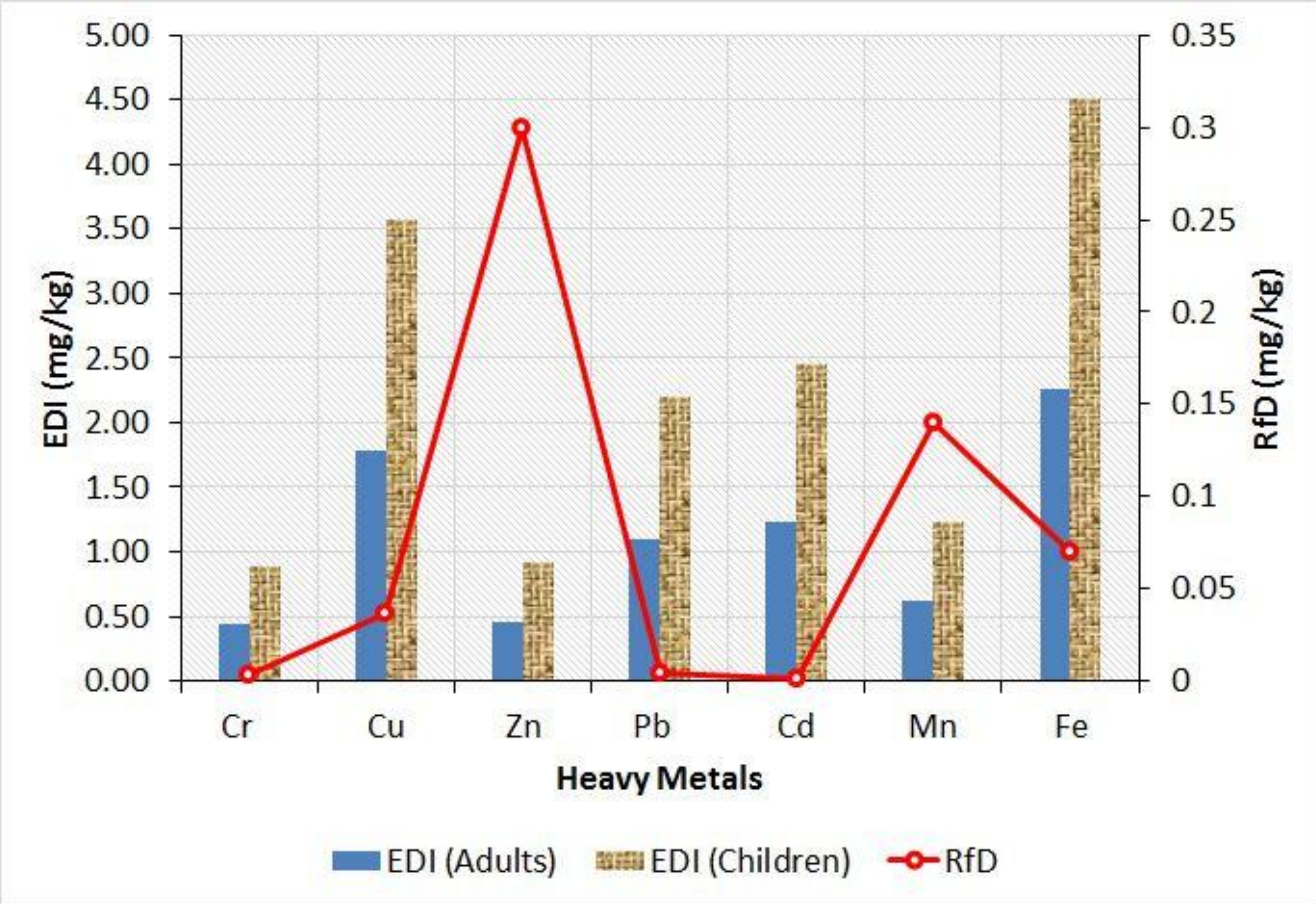

Figure 7: **The Estimated Daily Intakes of Heavy Metals in the CBB samples from the In vitro Concentrations**

IV. CONCLUSIONS

The study established minimum or low potential ecological risk from heavy metals pollution with approximately 0.1-47% of the total concentrations in the soil connected to anthropogenic-related activities. The percent bioaccessibility of the heavy metals in the CBB samples showed Cr, Cu, and Cd to be highly extracted in the gastrointestinal fluids. The EDI of the heavy metals established were observed to be above the RfD values with an ACR to RfD >100%. Cognate to the study conducted, it is pertinent to track down the significant causal factors for the metal contaminations in the CBB products to mitigate them as the results show the emergence of heavy metals exposure and risks CBB matrices. The study, therefore, recommends an analysis of critical pollution sources in the processing and production of cocoa-based products across local and industrial lines.

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