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Proximate Composition and Human Health Risk Assessment of Trace Metals in Selected Foodstuffs Sold in Abakaliki Metropolis, Nigeria

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Abstract

The study evaluated the proximate composition and human health assessment of trace metals in selected foodstuffs sold in Abakaliki metropolis, Nigeria. Moisture content, ash content, crude protein, crude fiber, dry matter, nitrogen-free extracts (NFE), and human health risks of trace metals were determined. The results of the proximate analysis of Bambara nut, maize, and wheat which were represented with sample IDs A, B, and C respectively showed that moisture contents ranged from 6.75±0.8 in sample A to 10.64±0.1 % in sample B, crude fat had a range of 1.8±0.2 in sample C to 3.35±0.2 % in sample A. Range of values for crude fiber, crude protein, crude ash, dry matter, and NFE (%) were 0.78±0.3 in sample A to 2.13±0.5 % B, 8.60±1.6 in B to 12.20±2.8 % in sample C, 5.06±0.1 in sample B to 9.22±0.4 % in sample A, 89.35±4.2 in sample B to 93.24±5.9 % in sample A and 75.85±3.7 in sample A to 81.63±2.5 in sample B respectively. The results of the trace metal analysis (mg/kg) showed that Fe ranged from 289.29±34.38 in sample A to 398.38±38.48 in sample C. Cd was not detected in any of the samples while Pb was detected in sample C only. Cr was detected in samples A and B but not obtained in sample C. The health risks assessment such as estimated daily intake (EDI), health risk index (HRI), and targeted hazard quotient (THQ) were all obtained and the results obtained showed that samples A and B had adverse health effects while samples C had no adverse health effects. The foodstuffs analyzed were found to be rich in nutrients, however, some posed health risk of cancer, prompting a recommendation from health authorities to establish mitigation measures to protect consumers.

Keywords: Foodstuffs, Hazard, Health, Index, Metals and Proximate.

Introduction

Cereal crops are the harvested seed of grasses including wheat, oats, rice, and maize. Other essential cereal crops consist of sorghum, millet, rice, barley etc. Globally, grains are known as cereals. They are the maximum essential staple food. Humans get an average of 48 % of their food energy from grains [1]. Grains and seeds are plant products containing proteins and may be categorized as nutraceuticals. Nutraceutical is stated to be a food extract with health and clinical benefits, especially to humans. Grain and seed

proteins are essential additives in food systems that assist fight protein-calorie malnutrition in growing countries. Grain and seed proteins create windows of possibility via way of means of decreasing poverty levels, development in nutrients and health status, development in food security, and sustenance of natural resources. Grain and seed proteins are a staple supply of energy, carbohydrates, minerals, vitamins B, and proteins [2].

Wheat is one of the oldest essential crops known to humanity. Thousands of kinds of wheat recognized nowadays include but are not confined to the following: common wheat (*Triticum aestivum*) used in making bread, durum wheat (*T. durum*), used in making pasta including spaghetti and macaroni and club wheat (*T. compactum*), used in the making of flours. The dietary cost of wheat varies as the environmental factor varies. On average, the kernel contains 12 % water, 70 % carbohydrates, 12 % protein, 1.8 % minerals, and 2.2 % crude fibers [3].

Maize is one of the most cultivated cereals in the world, corresponding to a maximum of 20 % of the overall energy fed by humans [4]. This species has a high-quality genetic variety for plant and grain characteristics. Besides being a supply of protein and carbohydrates, maize can be a source of different important compounds for human health, including carotenoids with pro-vitamins [4]. A good maize grain consists of starch and proteins, there are numerous other substances, including anthocyanins and carotenoids, commonly associated with pigmentation and observed in greater abundance in landrace varieties [4].

Bambara nut is a legume indigenous to Africa and is cultivated throughout the semi-arid sub-Saharan Africa region. It is a hardy crop and has been identified as an essential nutritious food supply when food is scarce. This may be attributed to its climate-smart features, consisting of its ability to repair nitrogen, and to develop under unfavorable environmental conditions including terrible soils and drought. This nutrient-dense legume is once in a while termed a “complete food” because of its balanced macronutrient composition [5]. Bambara nut contains 64.4 % carbohydrate, 23.6% protein, 6.5%, and 5.5% fiber and is rich in minerals [5]. It is exceptionally underutilized in comparison with foremost cash crops and has frequently been related to small-scale, subsistence farming, with women being the foremost producers and processors.

Trace metals are essential nutrients required by the body in small quantities for various physiological functions in the human body. However, excessive exposure to certain trace metals can lead to adverse health effects. Cd, Pb, Cr, and Fe were the trace metals investigated in the selected foodstuffs sold in the Abakaliki metropolis, and the concentrations of the trace metals obtained were used to ascertain the human health impacts of the trace metals like targeted hazard quotient (THQ), health risk index (HRI), estimated daily intake (EDI), hazard index

(HI) and targeted carcinogenic risk (TCR) were all evaluated.

Materials and Methods

Study Area and Sample Location

The study was carried out in the Abakaliki metropolis, Nigeria in October, 2023. Triplicate samples of Bambara-nut (*Vigna subterranean*), maize (*Zea mays*), and wheat (*Triticum aestivum*) labeled A, B, and C respectively were purchased in the international market in Abakaliki. The samples were ground using a metallic grinding machine and were sent for proximate and trace metal analysis after the digestion was done in the Industrial Chemistry Laboratory, Ebonyi State University, Abakaliki for the trace metal analyses. Both the digest and the ground samples were sent for analysis at Docchy Analytical and Environmental Services Ltd located at Awka, Anambra State, Nigeria.

Methods

The proximate analysis was done using the methods of the Food and Agricultural Organization/World Health Organization [6]. This involved the determination of moisture content, ash content, crude protein, crude fiber, dry matter, and Nitrogen Free Extracts (NFE).

Determination of Moisture Content

A dry crucible was weighed and the value was recorded. The sample was placed on the crucible and weighed before drying it in an oven at a temperature of 105 ^{o}C for 2 hours. It was cooled in a desiccator and weighed. The drying procedure was continued until a constant weight was achieved.

$$MoistureContents(\%) = \frac{W1 - W2}{W3} X\ 100$$

W1 = weight of crucible and sample before drying

W2 = weight of crucible and sample after drying

W3 = weight of the sample

Determination of Ash Content

The crucible was preheated in a muffle furnace at a temperature of 550 ^{o}C and was cooled in a desiccator and weighed. This was repeated until a constant weight was obtained. 2 g of the dried sample was weighed into the crucible and placed in the furnace and heated at 550 ^{o}C for 3 hours. It was allowed to cool and then transferred into a desiccator. The crucible and its contents were weighed for ash content determination.

$$Ashcontent(\%) = \frac{W3 - W2}{W1} X\ 100$$

W1 = weight of the sample

W2 = weight of crucible and sample before ashing

W3 = weight of crucible and ash.

Determination of Dry Matter

This was obtained using the formula:

$$100 - \ moisture\ contents$$

Determination of Crude Fat

The extraction flasks were heated in a laboratory kiln and then removed from the kiln, cooled in a desiccator and weighed without touching them with fingers. 3 g of the sample was weighed into an extraction thimble and placed in an extraction unit with thongs. The extractor was connected to the flask containing petroleum ether (2/3 of the flask capacity) and the flask was boiled to obtain about

10 refluxes per hour. At the end of the extraction, the ether was distilled off and the flask was cooled in a desiccator and weighed.

$$Crudefatcontent(\%) = \frac{100\ (W2 - W1)}{W3}$$

W1 = weight of the dry flask

W2 = weight of flask and fat

W3 = weight of the sample

Determination of Crude Fibre

The dry sample 3 g was weighed and boiled under reflux for 30 minutes with 200 ml of a solution containing 1.25 g of H_2SO_4 per 100 ml of solution. The solution was filtered through Whatman number 42 filter paper placed on the thistle funnel. The residue was washed with boiling water to a neutral pH of 7.0 and was transferred to a beaker and boiled for 30 minutes with 200 ml of the solution containing 1.25 g of carbonate-free NaOH per 100 ml. The residue was filtered and transferred into a pre-weighed crucible and dried in an oven at a temperature of 105-110 ^oC for 1 hour, then incinerate in a muffle furnace at a temperature of 300 ^oC for 30 minutes, allowed to cool and weighed. The loss in weight after incineration multiplied by 100 is the percentage of the crude fibre.

$$Crudefibre(\%) = \frac{weightloss}{weightofsample} x\ 100$$

Determination of Crude Protein

Dry sample 2 g was homogenized and placed into a Kjeldahl flask followed by the addition of 10 g of K_2SO_4 0.7 g HgO and 20 ml concentrated H_2SO_4. The content of the flask was digested at a temperature not below 420 ^oC until the solution became clear followed by further heating for 30 minutes. The solution was allowed to cool by gradually adding 90 cm^3 of distilled water after was cooled 25 ml of H_2SO_4 was added and the solution was stirred. A glass bead and 80 cm^3 of 40 % NaOH solution were added and the flask was quickly connected to the distillation unit and the solution distilled. A 50 cm^3 portion of the distillate was collected in a flask containing 50 cm^3 of boric acid indicator solution. The resulting solution was filtered with a standard chlorhydric acid solution.

$$Nitrogeninthesample(\%) = \frac{100(W1\ XW2\ X\ 0.014)}{W3}$$

$$Crudeprotein(\%) = nitrogeninthesampleX\ 6.25$$

W1 = chlorhydric acid used in the titration (ml)

W2 = molarity of standard acid (mol/dm^3)

W3 = weight of sample

Nitrogen-Free Extract (NFE)

This was obtained using the formula:

$$NFE(\%) = 100 - (\%\ crudeprotein + \%\ crudefat + \%\ crudefibre + \%\ ash)$$

Trace Metal Analysis

Aqueous stock solutions (1 ppm) of metals of interest (Cd, Pb, and Fe) were prepared with appropriate salts of these metals. Five standard solutions for each metal were prepared in triplicate from their respective stock solutions by serial dilution. The absorbance obtained from the AAS

instrument for each standard of a particular metal was used in drawing the calibration curve [7].

Metal Validation

The procedure and method were validated by use of a standard reference food sample from the International Atomic Energy Agency (IAEA) obtained through Docchy Analytical Laboratory and Environmental Services LTD Awka. The standard reference material was digested and analyzed with the method used for food samples. The percentage recovery was calculated using Nnaji [8] method.

$$\%\ Recovery = \frac{ARM}{CRS} X100$$

ARM = analyzed metal concentration in reference standard

CRS = metal concentration indicated in reference standard

Digestion and Analysis of the Sample

The method used by Nnaji [8] was adopted. The samples were digested in triplicate. 10 ml of the concentrated HNO3 was added to 2 g of the sample contained in 250 beakers. The beaker was covered with watch glass for the initial reaction to subside. The content of the beaker was then evaporated to near dryness after which 10 ml of HNO3 was added and the suspension was filtered through Whatman number 42 filter paper into a 50 ml volumetric flask. The filtrate was diluted to volume with distilled-deionized water and transferred into a sterile plastic bottle which was stored in a refrigerator at 4 oC before being transported for metal analysis. The sample digested was aspirated into FAAS and the absorbance was recorded.

Conversion of Results

Metal concentrations obtained were on a weight/volume basis (mg/L) and were converted to a weight/weight basis (mg/kg) using the formula [7,8]

$$Conc.(mg/kg) = \frac{conc.(mg/L)\ x\ d.f}{wt\ of\ sample}$$

d.f = dilution factor, that is, volume (ml) of the digest solution (metal analysis)

Statistical Analysis

Mean and standard deviation were calculated and the results were analyzed using single factor analysis of variance (ANOVA).

Health Risk Assessment

Estimated Daily Intake (EdI) of the Trace Metals

The EDI of the trace metal was calculated in accordance with USEPA [9] as stated:

$$EDI = \frac{C\ x\ FIR}{BW}$$

BW = average body weight (70 adult)

C = concentration of the metal in the sample (mg/kg)

FIR = daily average consumption of foodstuffs in Nigeria (g/person/day) and it is estimated to be 100 g/day [10]. The EDI was expressed as (µg/kg BW/day) for trace metals and were compared with each metal reference oral does (RfD) [11]

Health Risk Index (HRI)

HRI was obtained by dividing the EDI with the corresponding reference oral does (RfD) for each metal.

$$HRI = \frac{EDI}{RfD}$$

RfD for Fe, Cd, Pb were 0.7, 0.001, and 0.0035 mg/kg/day respectively [11]

Non-Carcinogenic Risk Assessment (NCRA)

The NCRA method was adopted by USEPA [12,13] based on targeted hazard quotient (THQ) and hazard index (HI). The THQ is a ratio of determined does of pollutants to reference does level.

$$THQ = \frac{ED\ X\ C\ X\ FIR\ X\ EF\ X\ 10^{-3}}{RfD\ X\ BW\ AT}$$

THQ = targeted hazard quotient

C = trace metal concentration in foodstuffs

FIR = food ingestion rate (g/day)

ED = exposure duration (55 years average life time for Nigeria) [11]

EF = exposure frequency (365 days/year)

AT = average exposure time (365 days/year x exposure year assume 55 years)

BW = average body weight for adult (70 kg)

RfD = oral reference does for metal

The hazard index (HI) was generated to evaluate the non-carcinogenic risk potential effects of the combined metal as follows:

$$HI = \sum HQi$$

Where i represent each metal

Table 1: Risk Categories of Trace metal Contamination[14,15]

S/N	THQ	HI	Risk Categories
1	>1.0	>1.0	Potential adverse effect
2	<1.0	<1.0	No adverse effect

Carcinogenic Risk Assessment (CRA)

The equation below can be used to estimate the carcinogenic risk factor (life time cancer)

$$TCR = \frac{EF\ XED\ X\ FIR\ X\ C\ X\ CSF_O\ X\ 10^{-3}}{BW\ X\ AT}$$

TCR = target carcinogenic risk over a life time

EF = exposure frequency (365 days/year)

AT = average time for carcinogens (365 days/year x ED)

CSFo = oral carcinogenic slope factor from the integrated risk information system data base [16] which was 0.0085, 0.5, 1.7 mg/kg/day for Pb, Cr, and Ni respectively. The cumulative target carcinogenic risk (CTCR) can be calculated as:

$CTCR = \sum TCRi.$

Results and Discussion

Results of Proximate Analysis

Table 2. Proximate Composition (% dry matter) of maize, bambra nut and wheat

S/N		Sample IDs		
	Parameters	A Bambaranut	B Maize	C Wheat
1	Moisture content	6.75±0.8	10.64±0.1	10.24±0.4
2	Crude fat	3.35±0.2	2.51±0.5	1.80±0.2
3	Crude fibre	0.78±0.3	2.20±0.3	2.13±0.5
4	Crude protein	10.80±2.8	8.60±1.6	12.20±2.8
5	Crude ash	9.22±0.4	5.06±0.1	6.09±0.6
6	Dry matter	93.24±5.9	89.35±4.2	89.75±5.6
7	NFE	75.85±3.7	81.64.63±2.5	77.78±4.1

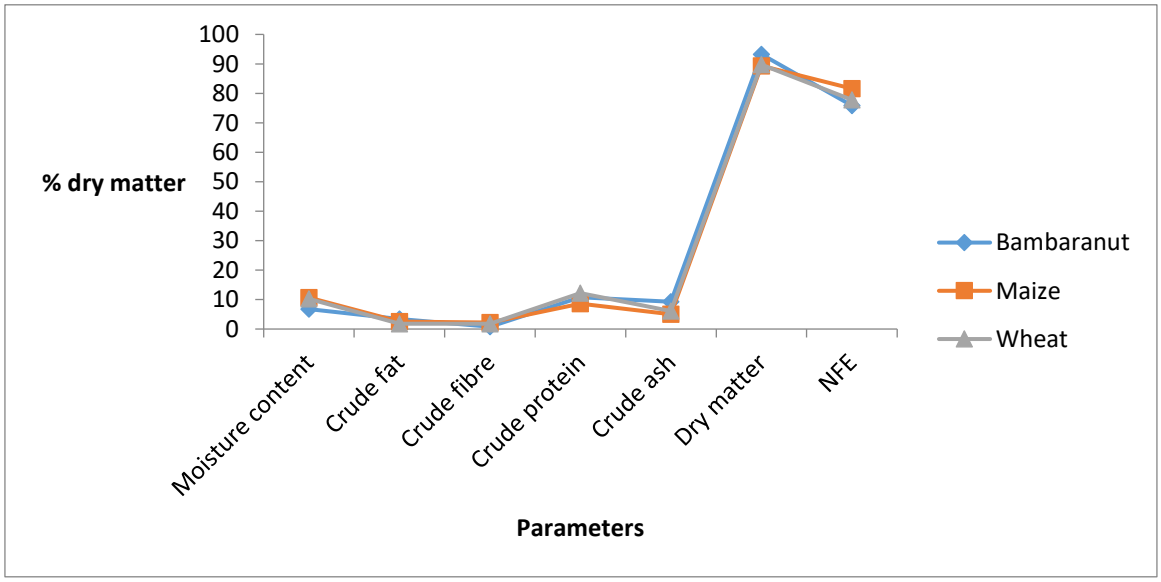

Figure 1: Proximate Composition (% dry matter) of the foodstuffs

Table 2 and Figure 1 showed the results of proximate analysis of Bambara-nut, maize, and wheat represented with sample IDs as A, B, and C respectively. Moisture content ranged from 6.75±0.8 in sample A to 10.64±0.1 % in sample B while crude fat had a range of 1.8±0.2 in sample C to 3.35±0.2 % in sample A. Range of values for crude fiber, crude protein, crude ash, dry matter, and NFE were 0.78±0.3 in sample A to 2.13±0.5%, 8.60± in B to 12.20±2.8 % in sample C, 5.06±0.1 in sample B to 9.22±0.4 % in sample A, 89.35±4.2 in sample B to 93.24±5.9 % in sample A and

75.85±3.7 in sample A to 81.63±2.5 in sample B respectively. Among the samples studied, sample B had the highest moisture content of 10.64±0.1 % but had the lowest value in terms of crude protein and crude ash which were respectively 8.60±1.6 and 5.06±0.1 %.

Sample A had the highest dry matter, crude fat, and crude ash content. Whereas sample C had the lowest crude fat content of 1.8±0.2 %. Sample B had the highest NFE content of 81.63±2.5 %. Olaoye [17] in their study of Assessment of the Functional and Pasting Properties of Flour Blends from wheat and Bambara-nut and its Potential in Bread Making found % crude ash, crude fiber, crude fat, and crude protein in wheat to be respectively 3.75, 1.51, 6.01 and 16.21 %. From the study, it was found that bambara-nut, maize, and wheat are very rich in protein, vitamins, and carbohydrates among foodstuffs made of grains.

Results of Trace Metal Analysis

Table 3: Results of trace metal concentrations in the different foodstuffs

S/N	Sample ID's	Foodstuffs	Metal Concentrations (mg/kg) Pb	Cr	Fe	Cd
1	A	Bambra-nut	ND	11.26±0.41	289.28±34.38	ND
2	B	Maize	ND	9.96±0.39	327.35±36.21	ND
3	C	Wheat	0.14±0.00	ND	398.38±38.48	ND
WHO/FAO (2018)			0.2	-	-	0.1

ND = not detected

Table 3 shows the results of the trace metal concentrations obtained in the different foodstuff samples sold in the international market in Abakaliki, Ebonyi State Nigeria. Cd was not detected in all the samples. Pb was detected in sample C while Cr was detected in samples A and B but not detected in sample C while the value for Fe ranged from 289.28±34.38 sample A to 398.38±38.48 mg/kg sample C. Wheat has the highest concentration of Fe followed by maize. The concentration of Fe in the studied foodstuffs follows the decreasing order as C < B < A. The results obtained by Nnaji [8] in human health risk assessment of heavy metal in foodstuffs processed with diesel-powered metallic disc grinders in Umuahia, Nigeria within the same range.

Results of Health Risk Assessment of Trace Metals in Foodstuffs

Human health risk assessment of trace metals in foodstuffs involves the evaluation of the potential health risk of trace metals ingested in doses through

one or more exposure pathways [13] Carcinogenic and non-carcinogenic methods were used in this study to assess the potential human health risks posed by trace metals to consumers of foodstuffs in Abakaliki, Nigeria [8].

Table 4: Result of the Estimated Daily Intake of trace metals in the studied foodstuffs

S/N	Sample ID's	Foodstuffs	EDI (µg/BW/day)			
			Pb	Cr	Fe	Cd
1	A	Bambra-nut	-	1.6×10^{-5}	4.1×10^{-4}	-
2	B	Maize	-	1.4×10^{-5}	4.7×10^{-4}	-
3	C	Wheat	2.0×10^{-7}	-	5.7×10^{-4}	-

The estimated daily intake (EDI) of the trace metal concentration in the studied foodstuffs was depicted in table 4. The EDI for Fe ranged from 5.7×10^{-4} sample C to 4.7×10^{-4} sample B. Cd was not detected in all the foodstuffs studied. Sample A had 1.6×10^{-5} for Cr, 1.4×10^{-5} was obtained in Cr for sample B while sample was not detected for Cr. Pb only detected in sample C with value of 2.0×10^{-7}.

Table 5: Result of the health risk index (HRI) of the trace metal in the studied foodstuffs

S/N	Sample ID's	Foodstuffs	HRI			
			Pb	Cr	Fe	Cd
1	A	Bambra-nut	-	5.0×10^{3}	5.9×10^{4}	-
2	B	Maize	-	3.5×10^{2}	6.7×10^{4}	-
3	C	Wheat	5.7×10^{-5}	-	8.1×10^{4}	-

The result of the health risk index is shown in Table 5. HRI for Fe ranged from 5.9×10^{4} sample A to 8.1×10^{4} sample C. Sample A for Cr had an HRI value of 5.0×10^{3} while B had 3.5×10^{2}. Pb was not detected in samples A and B while sample C had the value of 5.7×10^{-5}. These values obtained were much lower than the values obtained by Nnaji [8]. The EDI values obtained in Table 4 were below the reference oral dose (RfD) of the individual trace metals Pb, Cr, and Cd which are respectively 0.0035, 0.003, and 0.7. Not that if HRI < 1, it means there is no health risk for consumers of the foodstuffs but if the HRI > 1, this indicates potential health risk to the consumers. However, the results obtained for the HRI were below the value of 1, indicating that there is no health risk implication to the consumers of the foodstuffs in Abakaliki, Nigeria.

Tables 6 and 7 show the targeted hazard quotient (THQ) and targeted carcinogenic risk (TCR) values for the different trace metal concentrations in the studied foodstuffs respectively with their corresponding hazard index (HI) and cumulative target carcinogenic risk (CTCR) values.

Table 6: Result of the THQ and HI of trace metal in the foodstuffs

S/N	Sample ID's	Foodstuffs	THQ				
			Pb	Cr	Fe	Cd	HI
1	A	Bambra-nut	-	5.36	0.59	-	5.95
2	B	Maize	-	4.74	0.67	-	5.41
3	C	Wheat	0.057	-	0.81	-	0.88

The values of THQ for Cr in samples A and B were above 1 while that of Fe were below 1and the value of sample C for Pb was also below 1. The values of HI for samples A and B were greater than 1 and sample C was less than 1. This is an indication that sample C had no adverse health effects in terms of carcinogenic to the consumers but samples A and B which the THQ and HI were greater than 1 had potentially adverse effects on the consumers in terms of their degree of carcinogenicity and this calls for maximum attention as a mitigation measure is urgently required from the health authorities from Ebonyi state and Nigeria to safeguard the consumers from cancer in consuming the selected foodstuffs. Meanwhile, a study of metals in different foodstuffs (cereals, vegetables, fruits, fish, and meat) in Beijing concluded that total THQ (HI) for Cr, Pb, and Cd were 0.96, 0.54, and 0.50 respectively [18]. The value of HI was less than 1 and they concluded that the Beijing populace does not face significant potential health risks from the intake of single metal through the consumption of the foodstuffs.

Table 7:Result of the TCR and CTCR of trace metal in the foodstuffs

S/N	Sample ID's	Foodstuffs	TCR				
			Pb	Cr	Fe	Cd	CTCR
1	A	Bambra-nut	-	0.08	NC	-	8.0×10^{2}
2	B	Maize	-	0.007	NC	-	7.0×10^{3}
3	C	Wheat	1.7×10^{-6}	-	NC	-	1.7×10^{-7}

NC= non-carcinogenic

TCR represents the carcinogenic risk over time and the acceptable range for cancer risk is $10^{-6} – 10^{-4}$ and the risk of developing cancer over time is 1 in 1,000,000 [16]. Only Pb in sample C had this range but Cr was above this range. This indicates possible carcinogenic to the consumers of these foodstuffs over a long time apart from wheat (sample C).

Table 8: Comparison of the results of the health risk assessment of trace metals in foodstuff

Parameters	Sample A			Sample B			Sample C		
	Pb	Cr	Fe	Pb	Cr	Fe	Pb	Cr	Fe
EDI	-	1.6×10^{-5}	4.1×10^{-4}	-	1.4×10^{-5}	4.7×10^{-4}	2.0×10^{-7}	-	5.7×10^{-4}
HRI	-	5.0×10^{3}	5.9×10^{4}	-	3.5×10^{2}	6.7×10^{4}	5.7×10^{-5}	-	8.1×10^{4}
THQ	-	5.36	0.59	-	4.74	0.67	0.057	-	0.81
TCR	-	0.08	NC	-	0.007	NC	1.7×10^{-6}	-	NC

Table 8 shows the comparisons of the health risk assessment of the trace metals in the studied foodstuffs. Cr was not found in sample C and the values of the HRI and TCR which are less than 1 is an indication that sample C has no adverse health effect to the consumers in Abakaliki. Samples A and B were greater than 1 for Cr in HRI and THQ. This showed that there is a possible health risk to the consumers of these foodstuffs in Abakaliki.

Conclusion

Proximate composition and human health assessment of trace metals in selected foodstuffs sold in the Abakaliki metropolis were investigated. The result of the proximate composition showed That crude fat, crude fiber, crude protein, and moisture content recorded in the study are advantageous in ensuring nutritional value and can help prolong shelf life. The different foodstuffs studied in terms of proximate composition also indicate that the foodstuffs are very rich in protein, vitamins, and carbohydrates among foodstuffs made of grains. Results of the health risk assessment showed that some of the samples studied had no adverse health effects on the populace while some had health risks to the consumers. This calls for maximum attention as mitigation measure is urgently required from the health authorities from Ebonyi state and Nigeria to safeguard the consumers from cancer deposition in consuming the selected foodstuffs.

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