EVALUATION OF HEAVY METAL CONTAMINANTS OF BREAD IN BADE LOCAL GOVERNMENT AREA OF YOBE STATE, NIGERIA.

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ABSTRACT
Consumption of foodstuffs is among the most likely route for human exposure to heavy metals. This study was designed to investigate the heavy metals (Cd, Co, Cr, Fe, Cu, Mn, Pb, and Zn) concentrations in bread sample and then estimate the potential health risks of toxic metals via consumption to the local residents in Bade, Nigeria. Bread samples were collected and analyzed using Energy Dispersive X-ray Fluorescence Spectrometry (ED-XRFS). Most of the selected heavy metal levels in the sample were lower than the maximum allowable concentrations of bread as recommended by the World Health Organization (WHO). The health risks associated with the heavy metals were assessed based on the Target Hazard Quotients (THQs) proposed by the United States Environmental Protection Agency (USEPA). It can be deduced that the bread samples analyzed may be suitable for human consumption since the concentrations of almost all the elements found in the bread samples were below the international threshold values in the study area. However, concentrations of Pb in the two bread samples analyzed are above the international standard value, with 1.812% in Sample D and 1.731% in Sample F. Can cause a health risk, damage of organs on humans. This may be due to the raw material mixture like flour, water, yeast, salt, sugar, fats, butter oil and other preservatives.

Key words: Energy Dispersive X-Ray Fluorescence Spectrometry (ED-XRFS), Heavy metals, Health Risk Index (HI). Target Hazard Quotients (THQs).

1.0 INTRODUCTION
Heavy metal contamination in food products has become a significant public health concern globally. Bread, being a staple food, is vulnerable to contamination with heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), cobalt (Co), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) [1]. These metals can accumulate in the human body and cause various health problems, including cancer, neurological damage, and organ dysfunction. Heavy metals can contaminate food through agricultural pollutants, industrial wastewater, and food processing/packaging materials. Environmental pollution is released into the environment through industrial activities, mining, and waste disposal. Health risks: prolonged consumption of contaminated food can lead to adverse health effects [2].

In Nigerian Context, a rapid urbanization and industrialization, which increased the risk of environmental pollution and food contamination. Limited regulations and enforcement that are inadequate for monitoring and controlling heavy metal contamination in food products. High consumption of bread as a staple food in Nigeria, making contamination a significant public health concern [3].

2. THEORICAL FRAMEWORK

ED-XRF technique is normally used in quantifying elemental analysis of a wide range of organic and inorganic samples. The basis of the technique is that all elements emit secondary (fluorescent) X-rays of characteristic energy when exposed to X-rays of appropriate higher energy. Energy and intensity of emitted X-rays are used to determine elemental composition. In general, the heavier the element being analyzed, the higher the energy of x-rays required to elicit fluorescence, and then it is easier to detect fluorescence. Non-destructive testing: EDXRF analyzes samples without damaging or altering them [4]. These are some of the merit of ED-XRF Techniques:

i. Multi-element detection: EDXRF can detect multiple elements simultaneously.
ii. High sensitivity: EDXRF detects trace levels of elements (ppm to %).
iii. Rapid analysis: EDXRF provides fast results, typically within minutes.
iv. Low sample preparation: Minimal sample preparation required.
v. Cost-effective: EDXRF is relatively inexpensive compared to other analytical techniques.
vi. Portable: EDXRF instruments can be portable, allowing on-site analysis., but the illustration of the working principles shown in Figure 1.
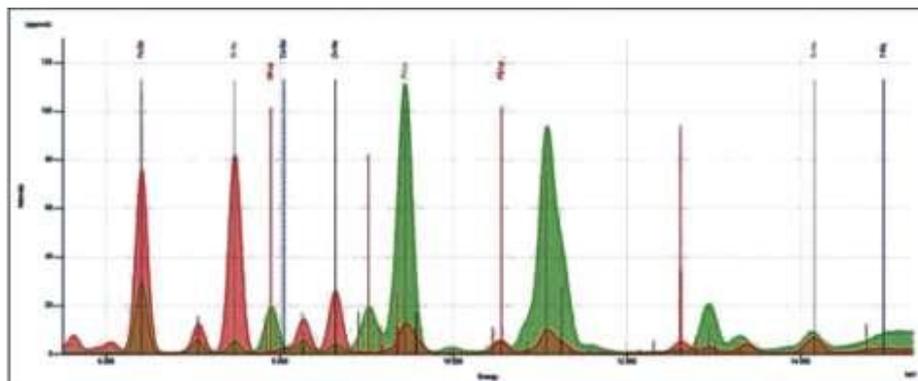

Figure 1. Spectrometer Chart [4]

ED-XRF spectrometers use X-rays in the 1 – 65 KeV energy range and employ the photoelectric effect to determine elemental composition. This effect is when incident Xrays irradiate atoms in a sample, and the sample material excites and fluoresces energy in the form of X-rays. When this phenomenon happens, electrons eject from inner atomic orbitals, causing an electron from a higher-energy orbital to transfer and fill the vacancy in the lower-energy orbital. The excess energy is emitted as an X-ray, and the spectrometer counts and measures the energies of these X-rays emitted from the sample. These energy differences between each shell are always the same for a particular element, so the spectrometer identifies and quantifies which elements are in the sample [4].

When electrons fall from the outer to the inner atomic shells, these X-ray transition lines produce peaks in a spectrum. Each transition line creates a peak at a specific energy in the spectrum of the element as shown in Figure 2.
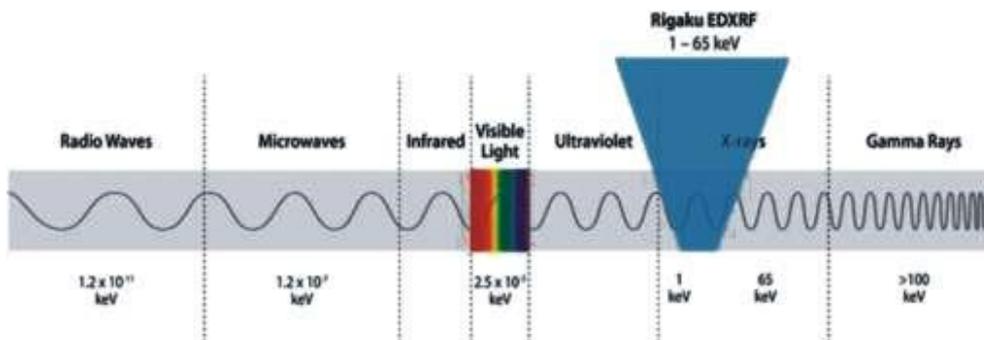
Figure 2. Electromagnetic wave spectrum [4].

2.1 Target Hazard Quotient
An estimate of the potential hazard to human health (Target Hazard Quotient- THQ) through consumption of bread along Bade, local government area of Yobe State, Nigeria.

The estimated daily metals intake from bread in this study was determined using equation (1) [5].

$$THQ = \frac{(DIB) \; X \; Cmetal}{RfD \; X \; BW} \qquad (1)$$

where *(Dib)* is the daily intake of bread (kg per day), (Cmetal) is the concentration of metal in the bread (mgkg^{-1}), RfD is the oral reference dose for the metal (mg kg^{-1} of body weight per day), and BW is the human body mass (kg).

2.2 Hazard Index
The Hazard Index (HI) will be computed as the sum of the target hazard quotients of the heavy metals under study as described in Equation (2). The health risk index (HRI) for the locals through the consumption of contaminated bread will be access based on the food chain and the reference oral dose (RfD) for each metal. The Health Risk Index from contaminated bread will be assessed based on the food chain and metal toxicity levels [8]. If the HI and THQ are both less than or equal to 1, there is no significant health risk.

$$HI = \Sigma THQ = THQ_{cu} + THQ_{Cr} + THQ_{Fe} + THQ_{Mn} + THQ_{Cd} + THQ_{Co} + THQ_{Zn} + THQ_{pd} \qquad (2)$$

where: ΣTHQ is Total Target Hazard Quotients, THQ_{cu} is the Target Hazard Quotient for coper, THQ_{Cr} is the Target Hazard Quotients for Chromium, THQ_{Fe} is the Target Hazard Quotients for Iron, THQ_{Mn} is the Target Hazard Quotients for Manganese, THQ_{Cd} Target Hazard Quotients for Cadmium, THQ_{Co} is the Target Hazard Quotients for Cobalt, THQ_{Zn} is the Target Hazard Quotients for Zinc and THQ_{pd} is the Target Hazard Quotients for Lead.

3.0 MATERIAL AND METHOD
Bread samples were collected from 10 popular varieties in Bade metropolis, selected for their widespread use and popularity within the community. These samples were then transported to the laboratory for analysis [6].

Table 1. Showing Bread Samples Name and sample code

S/N	BREAD COMPANY	SAMPLE CODE
.,1	Shukura Bread	Sample A
2	Annur Bread	Sample B
3	Al-Mustapha Bread	Sample C
4	Silver Bread	Sample D
5	Golden Boal Bread	Sample E
6	Sawaba Bread	Sample F
7	DSD Bread	Sample G
8	Royal Bread	Sample H
9	Savana Bread	Sample I
10	Resala Bread	Sample J

3.1 Materials
- Bread samples, sample cup, mask, and hand gloves, ED XRF machine, weighing machine
- Plastic container (2 liters), plastic container (200 ml), plastic bottles (50 ml), stirrer
- Wash bottle, volumetric flasks (100 ml), digestion tubes, hot plate (adjustable).

3.2 Sample Preparation
To prepare bread samples for ED-XRF analysis, first grind the sample into a fine powder using a mortar and pestle or food processor. Then, dry the powder to remove moisture (50°C, 2 hours) and mix thoroughly to ensure uniform composition. Next, create pellets or discs by mixing the powder with a binder and pressing into the desired shape (10-15 mm diameter, 1-2 mm thickness).

Ensure the sample meets ED-XRF requirements: 1-5 grams, 1-2 mm thickness, pellet or disc shape, and clean surface. Wear protective gear, handle samples in a well-ventilated area, and avoid cross-contamination.

3.3 Methods
The sample analysis was conducted at Umaru Musa Yar'adua University, Katsina State (UMYUK), which utilized energy-dispersive X-ray fluorescence (ED-XRF) analysis to assess heavy metals contamination in different varieties of bread samples such as Shukura, Al-Mustapha, Annur, Savana, Golden Goal, Silver, DSD, Sawaba, Gashua Royal, and Resala bread, detecting the presence of cadmium, cobalt, chromium, copper, iron, manganese, lead, and zinc.

ED-XRF Technique (Energy-Dispersive X-Ray Fluorescence) machine analyzer is a non-destructive technique that uses X-rays to excite atoms, emitting characteristic X-rays that are detected and measured to determine the elemental composition of a sample. The atoms in the sample material are excited by x-rays emitted from an x-ray tube or radioisotope. For increasing sensitivity, the primary excitation radiation can be polarized by using specific targets between the x-ray tube and the sample (ED-P (Polarization)-XRF). All element-specific x-ray fluorescence signals emitted by the atoms after the photoelectric ionization are measured simultaneously in a fixed-mounted semi-conductor detector or sealed gas-proportional counter [4].

3.4 Data Analysis
This study employed statistical analysis (range, mean, standard deviation) to evaluate experimental data. The analysis assessed significant variations in bread sample parameters,

comparing them to permissible limits, nutritional requirements, and standard limits, as referenced [8].

4. RESULTS AND DISCUSSION

The result obtained from experiment are presented here in tabular form and discussed accordingly.

4.1 Results of the elemental concentrations of the samples

The result of the elemental concentrations of the samples and their comparison with other studies and internationally acceptable values is shown in tables 1 and 2.

Table 2: Elemental concentrations (in ppm)of the sample analyzed.

S/N	BREAD VARIETY	HEAVY METALS							
		Cd	Co	Cr	Cu	Fe	Mn	Pb	Zn
1	Sample A	0.00	0.00	0.00001	0.00142	0.05058	0.00150	0.04000	0.00485
2	Sample B	BDL	BDL	0.000018	0.00500	0.01789	0.001474	0.05000	0.00284
3	Sample C	BDL	BDL	BDL	0.00145	0.01856	0.00136	0.04000	0.00373
4	Sample D	BDL	BDL	BDL	0.12000	0.04800	0.00200	1.81200	0.54500
5	Sample E	BDL	BDL	0.00009	0.14890	0.05500	0.00160	0.36500	0.66710
	Sample F	BDL	BDL	0.00005	0.00131	0.00813	0.00119	1.73100	0.00330
7	Sample G	BDL	BDL	BDL	0.00240	0.00790	0.00160	0.51800	0.00240
8	Sample H	BDL	BDL	BDL	0.00090	0.00940	0.00140	0.08400	0.00480
9	Sample I	BDL	BDL	BDL	0.00070	0.00670	0.00130	0.14300	0.00130
10	Sample J	BDL	BDL	0.00002	0.00073	0.00450	0.00105	0.13700	0.00206

BDL=Below Detection Limit

Table 3: Comparative Analysis with Who, Usepa, Nigeria Standard and other Study Conducted.

S/N	HEAVE METALS	LIBYA MISURATE. {a}	{b}	{c}	NIGERIA STANDARD {d}
1	Cd	-	0.2	0.005	0.1
2	Co	0.700	0.5	0.100	0.5
3	Cr	0.340	0.1	0.100	0.1
4	Cu	-	10	1.300	10
5	Fe	-	50	0.300	50
6	Mn	-	2.0	0.050	2.0
7	Pb	0.470	0.5	0.015	0.5
8	Zn	0.042	50	5.000	50

{a} [9].
{b} [10].
{c} [11].
{d} [12].

4.3 Discussions

This study investigated the levels of eight heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Pb, and Zn) in bread samples, comparing the findings to international standards and synthesizing the results with existing research to contribute to the broader understanding of heavy metal contamination in baked goods as shown in Table 3 (Nicholas *et al.*, (2024)[13].

Table 2. shows an analysis of heavy metals in various bread varieties revealed that Cadmium (Cd) and Cobalt (Co) were undetectable, while Lead (Pb) exceeded safe limits (0.1 mg/kg) in most varieties, except Shukura and Resala. Chromium (Cr) and Manganese (Mn) levels were relatively low, while Copper (Cu) and Zinc (Zn) levels varied. Comparing

bread varieties, Shukura had the lowest Pb concentration, Annur had the lowest Cr concentration, and AL Mustapha had relatively low Pb, Cu, and Zn levels. In contrast, Silver and Sawaba had high Pb concentrations, with Silver also showing elevated Cu and Zn levels.

Table 2. show the analysis revealed varying levels of heavy metals in different bread varieties as presented in graph chart below. Where, sample A, Sample B, and Sample C had relatively low concentrations of Pb, Cu, and Zn, with sample A and Sample C having the lowest Pb concentration (0.040 mg/kg) and Sample B having the lowest Cr concentration (0.000018 mg/kg). In contrast, Sample D had the highest Pb concentration (1.812 mg/kg) and elevated Cu and Zn levels, while Sample F had the second-highest Pb concentration (1.731 mg/kg), and Sample E had relatively high Pb concentration (0.365 mg/kg) and elevated Cu and Zn levels.

i.   Cadmium (Cd) and Cobalt (Co) were below detection limits (BDL) in all bread varieties.
ii. Lead (Pb) exceeded the safe limit (0.1 mg/kg) in most bread varieties, except Sample A and Sample J.
iii. Chromium (Cr) and Manganese (Mn) levels were relatively low across all bread varieties.
iv. Copper (Cu) and Zinc (Zn) levels varied among bread varieties.

4.2. Computed Results of Hazard Quotient and Health Risk Index
The computed results of the Hazard Quotients and Health Risk Index using equations 1 and 2 respectively of the populations leaving in the study area were presented in the tables below.

Table 4. Computed Value of Hazard Quotients (HQ) and Health Risk Index (HI) of Sample A.

POPULATION	HQ_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	0.00023	0.8078	1.6484	0.0692	0.9198	0.3692	3.8146
CHILDREN	0.0013	0.175	8.929	0.375	4.9826	2.00	16.4629

Table 5: Computed Value of Hazard Quotients (HQ) and Health Risk Index (HI) of Samples A.

POPULATION	HQ_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	BDL	0.8078	0.5947	0.0646	0.9198	0.746	3.1329
CHILDREN	BDL	0.175	3.2143	0.35	4.9825	4.041	12.7628

Table 6: Computed Value of Hazard Quotients (HQ) and Health Risk Index (HI) of Samples C.

POPULATION	HQ_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	0.4153	2.885	0.5947	0.0646	1.149	0.2153	5.3239
CHILDREN	2.25	15.625	3.2143	0.35	6.228	1.167	28.8343

Table 7: Computed Value of Hazard Quotients (HQ) and Health Risk Index (HI) of Sample D.

POPULATION	Q_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	BDL	0.3288	O.2176	0.0646	0.9198	0.161	1.6923
CHILDREN	BDL	1.7812	1.1786	0.35	4.9825	0.875	9.1673

Table 8: Computed Value of Hazard Quotients (HQ) and Health Risk Index (HI) of Sample E.

POPULATION	HQ_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	0.2153	8.0769	1.780	0.055	8.2783	50.769	69.175
CHILDREN	1.1667	43.75	9.6428	0.3	44.8430	275	374.70

Table 9: Computed Value of Hazard Quotients (HQ) and Health Risk Index (HI) of Sample F.

POPULATION	HQ_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	BDL	69.23	0.4945	0.069	41.41	41.53	152.734
CHILDREN	BDL	152.734	2.6785	0.375	224.21	225	827.264

Table 10: Computed Value of Hazard Quotients (HQ) and Health Risk Index (HI) of Sample G.

POPULATION	HQ_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	BDL	1.5230	0.2604	0.0785	11.9576	0.1846	14.004
CHILDREN	BDL	8.25	1.4107	0.425	64.773	1.00	75.859

Table 11: Computed Value of Hazard Quotients (HQ) And Health Risk Index (HI) of Sample H.

POPULATION	HQ_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	0.1046	0.75	0.2670	0.6857	39.09	0.2538	41.1511
CHILDREN	0.5667	4.063	1.446	0.4	211.75	1.375	219.600

Table 12: Computed Value of Hazard Quotients (HQ) and Health Risk Index (HI) of Sample I.

POPULATION	HQ_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	BDL	04615	0.3095	0.3095	1.9316	0.3692	3.1271
CHILDREN	BDL	2.500	1.6785	0.300	10.463	2.000	16.9415

Table 13: Computed Value of Hazard Quotients (HQ) and Health Risk Index (HI) of Sample J.

POPULATION	HQ_{Cr}	HQ_{Cu}	HQ_{Fe}	HQ_{Mn}	HQ_{Pd}	HQ_{Ze}	HI
ADULT	0.4615	0.4038	0.1615	0.1615	3.2193	0.1538	4.4645
CHILDREN	0.250	2.1875	0.875	0.3500	17.4389	0.8333	21.9347

Table 14. International Guideline for Hazard Quotients (HQ) and Health Risk Index (HI)

S/N	Exposure/ Group	Does (mg/kg/day)	Does (mg/kg/day)	CTE-Non cancer HQ	RME-Non cancer HQ
1	Birth to < 1 year	0.23	0.50	45	55
2	1 to < 2 years	0.095	0.27	19	39
3	2 to < 6 years	0.076	0.20	15	31
4	6 to < 11 years	0.056	0.15	11	25
5	11 to < 16 years	0.039	0.12	7.9	24
6	16 to < 21 years	0.038	0.12	7.5	24
7	Adult	0.054	0.14	11	27
8	Pregnant Women	0.042	0.12	8.4	25
9	Lactating Women	0.080	0.17	16	34

[13]

4.4 Discussions

For children, the Hazard Index (HI) value of 16.4629 signals a profoundly significant overall health risk. Notably, iron (Fe) and lead (Pb) exposure pose substantial health risks, while manganese (Mn) and zinc (Zn) exposure also present potential health concerns, emphasizing the need for urgent attention and effective mitigation measures to safeguard children's health [7]. To mitigate the identified health risks, the following recommendations are proposed: Reduce exposure to iron and lead, with a particular emphasis on protecting children, and monitor copper and manganese levels to prevent potential harm. Additionally, ensuring adequate nutrition is crucial to minimize health risks. Furthermore, regularly assessing and mitigating potential sources of contamination is essential to maintaining a safe environment and protecting public health [14]. It can be observed, from Table 13, as it

follows an International guideline for Hazard Quotient (HQ) and Health Index (HI) include: WHO (≤1: no risk, 1-10: moderate, >10: high), USEPA (≤1: no adverse effects, 1-10: potential, >10: significant), and Nigeria Standard (≤1: acceptable, 1-5: tolerable, >5: unacceptable). The HI interpretation is consistent: ≤1 (no risk), 1-10 (moderate), and >10 (high), providing a framework for assessing health risks and informing public health decisions. CTE Non-Cancer HQ (Chonic Toxicity Exposure) And RME Non-Cancer HQ (Risk Management Evaluation) [13].

Also an analysis of heavy metals in various bread varieties revealed that Cadmium (Cd) and Cobalt (Co) were undetectable, while Lead (Pb) exceeded safe limits (0.1 mg/kg) in most varieties, except Sample A and Sample J Chromium (Cr) and Manganese (Mn) levels were relatively low, while Copper (Cu) and Zinc (Zn) levels varied. Comparing bread varieties, Sample A had the lowest Pb concentration, Sample B had the lowest Cr concentration, and Sample C had relatively low Pb, Cu, and Zn levels. In contrast, Sample D and S Sample F had high Pb concentrations, with Sample D also showing elevated Cu and Zn levels.

5.1 CONCLUSION

A study on heavy metal contamination in bread varieties revealed alarming findings, including undetectable levels of cadmium and cobalt, excessive lead levels (above 0.1 mg/kg) in most varieties except Sample A and Sample J, relatively low chromium and manganese levels, and varying copper and zinc levels. Sample A and Sample C had the lowest lead concentrations (0.040 mg/kg), while Sample D (1.812 mg/kg) and Sample F (1.731 mg/kg) had the highest. A Lead contamination poses significant health risks, whereas cadmium, cobalt, chromium, and manganese pose minimal risks. Overall, the study highlights the urgent need for action to mitigate health risks associated with lead contamination in bread [15].

The bread's pH level of 5.5-5.6, slightly acidic to neutral, is generally considered safe for consumption but may favor mold growth and affect nutrient availability. The moisture content of 30-50% is moderate to high, increasing the risk of mold growth, bacterial contamination, and shorter shelf life. These factors pose health risks, including gastrointestinal issues, allergic reactions, respiratory problems, and nutrient deficiencies [16]. To ensure safety and quality, it's crucial to monitor and control moisture levels, implement proper packaging and preservation techniques, and regularly test for microbial contamination and mycotoxins to mitigate potential health implications [16].

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