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Trace Metals Contamination in Bread Ingredients and Bread from Bakeries in Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author VOEA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VOEA and APO managed the analyses of the study. Author APO managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Bread loaves and bread ingredients (wheat flours, salt, sugar, yeast and water) were randomly sampled from ten bakeries within Akure metropolis of south western Nigeria and analysed to determine their safety levels for human consumption with respect to trace metal contents. The trace metals (Cu, Zn, Mn, Cr, Cd and Pb) were analysed in the samples using flame atomic absorption spectrophotometer. Results obtained revealed that toxic trace metals such as Cr, Cd and Pb were not detected in any of the samples. However, the levels of essential trace metals such as Cu, Zn and Mn had range of values in mg/kg: (0.039 – 0.091), (0.837 – 3.310) and (0.035 – 3.148); (0.056 – 0.091), (0.034 – 2.755) and (0.054 – 1.054) in the wheat flours and bread samples analysed respectively. This study revealed that the bread ingredients and loaves of bread sampled contained essential trace metals at levels that could not threaten the health of consumers over prolonged regular consumption.

Keywords: Trace metals; food safety; bread; ingredients; bakeries.

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1. INTRODUCTION

Bread is a kind of food made from flour, water and other ingredients, usually combined with a leavening agent, kneaded, shaped into loaves and baked [1,2]. Bread is an important staple food in many countries of the world, especially in African countries and south east part of Asia [3]. Bread is consumed extensively in Nigeria in homes, restaurants and hotels with predominant consumption among the poor and young ones who constitute more than 70% of the over 150 million populations [4].

Metal elements are widespread in the environment as a consequence of both natural and anthropogenic processes [5]. Anthropogenic sources such as mining, industrial processing, pesticides, chemical fertilizers and automobile exhaust are the main sources of trace metals contamination in the environment [6,7]. The natural source of trace metals contamination in the environment mainly include natural occurrence derived from parent materials [8]. In line with industrial development, pollution in the environment and consequently, in agricultural raw materials are emerging as major concerns of safety across the world. Environmental pollution has been frequently due to heavy metals and is highly threatening to food safety [9].

Trace metal introduction in bread production may arise from a number of sources which include: bioaccumulation in bread ingredients (wheat and/or flour, water, salt, sugar and yeast), baking fuel types and metal pans used for baking. A number of studies have shown that these factors have contributed to the introduction of trace metals into bread samples [10,11,12].

The effect of environmental pollution on contamination of foods and on their safety for human consumption is a global menace and widely addressed [10,13,9,14]. Trace elements are toxic elements which can enter into the food and affect the health of people who consume the contaminated foods [15]. These metals may accumulate to a toxic concentration level which can lead to impairment in the quality of human life [16,17]. Bioaccumulation of trace metals in humans has been attributed to be the cause of severe diseases such as tubular growth, kidney damage, cancer, diarrhoea, bone effects and fractures [18,19,20,2]. The main threats to human health from trace metals are associated with exposure to Pb, Cd, As and Hg [21]. However, some trace metals such as Cr, Cu, Ni, Mn and Zn have no-carcinogenic hazardous effects to human health when exposures exceed the tolerable reference dose [22]. In small amounts they are required for maintaining good health but in larger amounts they can become toxic or dangerous.

The toxicity of trace metals manifests themselves in many ways at different organizational levels (molecular, cellular, organ and whole organism) and in different degrees of severity. Trace metals toxicity can lower energy levels and damage the functioning of brain, lungs, kidney, liver, blood composition and other important organs. Most serious incidents of trace metal toxicity to organisms have involved ingestion of contaminated food [23,24,25]. The greatest health risk comes from those heavy metals that are less toxic at levels which may be harmless to the plants but could be harmful to the animals and humans if ingested. Cadmium is the metal of major concern in this respect but mercury is also more toxic to humans and animals than to plants [15].

2. MATERIALS AND METHODS

Wheat flour, sugar, salt, yeast, water and bread samples were collected from five conventional bakeries (using electric or gas oven) and five non-conventional bakeries (using local oven) within Akure metropolis, Nigeria. A total of sixty samples were collected from bakeries cited at different locations and sites of production within the study area. These samples were collected in Ziploc polyethylene bags and transported immediately to the laboratory for preliminary treatment and subsequent analysis.

2.1 Sample Digestion

One gram of the sample (wheat flour, bread, sugar, salt or yeast) was digested with 10 ml concentrated nitric acid and 5 ml of 70% perchloric acid (HNO_3 and $HClO_4$) at 80^oC in a fume hood. The water sample (50 ml), however, was digested using only 10 ml concentrated nitric acid at 80^oC in a fume hood until the solution was clear. The solution was filtered through Whatman No.42 filter paper into 50 ml volumetric flask and made up to mark with distilled water. The digested samples were analysed in triplicates using Atomic Absorption Spectrophotometer, AAS (APHA 301A, model: 5100 PC, Perkin-Elmer, Boston, USA) for Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Zinc (Zn), and Manganese (Mn) concentration. All reagents used were of analytical grade.

2.2 Quality Control Measures Adopted

2.2.1 Blank determination

Blank determination was carried out to ascertain the background level of the analytes of interest in the materials and reagent used for analysis. This was done by running a separate determination under the same experimental condition employed in the actual analysis of the sample, but excluding the sample. The values obtained from running blank determinations were used to correct the analyte values as applicable.

2.3 Calibration of Atomic Absorption Spectrophotometer (AAS)

The AAS calibration was carried out using serial dilutions of working standards solutions of the metals prepared from concentrated stock solution (1000 mg/L) of the standard elements (Merck, Darmstadt, Germany) with 0.25 M nitric acid. The most appropriate wavelength, hollow cathode lamp current, gas mixture flow rate, slit width and other AAS instrument parameters for metals were selected as provided in the instrument guides and background correction was used during the analysis. The calibration graph obtained was used to estimate the levels of trace metals in the samples. Procedural reagent blank test was carried out over the entire procedure. Measurements were made within the linear range of working standard used for calibration. The results were expressed as mean ± standard deviation (SD).

2.4 Recovery Experiment for Trace Metals

The method described by Oyekunle et al. [26] was used for recovery analysis. Two 1.0 g portions of each sample (wheat flour and bread) from the same source were used for recovery analysis. One portion (A) was spiked with 12.5 mg/kg standard mixture of the trace metal solution while the other portion (control, E) was left unspiked. The two portions were separately but similarly taken through the procedures outlined earlier for sample digestion. The resulting solutions were subjected to AAS analysis. The percentage (%) recoveries of trace metals were determined by comparing the concentration value of each metal from the spiked and the unspiked samples using the relationship as indicated in equation 1.

$$\%R = \frac{D-E}{F} \times 100 \quad (1)$$

Where,

D = trace metal concentration in spiked sample
E = trace metal concentration in unspiked sample
F = the amount of trace metal used for spiking

2.5 Health Risk Assessment

The risk to human health as a result of eating bread from the selected bakeries was evaluated by calculating the estimated daily intake, hazard quotient and hazard index.

2.6 Estimated Daily Intake

The risk for human health as a result of eating bread is evaluated by calculating estimated daily intake (EDI) [27].

$$\text{EDI} = \frac{C_{bread} \times D_{bread}}{BW} \quad (2)$$

Where,

C_{bread} = average trace metal concentration in bread (mg/kg dry weight)
D_{bread} = the average daily bread consumption (kg/day) which is 0.170 kg/person
BW = average body weight (kg). US-EPA risk analysis, considering an adult average body weight of 70 kg [20].
EDI = Estimated Daily Intake

2.7 Hazard Quotient

The Hazard quotient is calculated by dividing the estimated daily intake (EDI) of each metal by the established reference dose (RfD) to assess the health risk from bread consumption.

$$\text{HQ} = \frac{EDI}{RfD} \quad (3)$$

Where;

EDI = Estimated Daily Intake
RfD = Reference Dose for each trace metal
HQ= Hazard Quotient

2.8 Hazard Index

The Hazard Index for residents ingesting these trace metals by consuming bread in all selected bakeries will be calculated by summation of HQ of all trace metals for each bakery.

$$HI = HQ(Cu + Zn + Mn) \quad (4)$$

Where,

HI = Hazard Index
HQ = Hazard Quotient

When HI ≤ 1 and HQ ≤ 1, there would be no obvious health risk [27].

2.9 Statistical Analysis

Data obtained were subjected to statistical analysis using the Statistical Package for the Social Sciences (SPSS 17.0). The data were expressed as mean ± standard deviation. A one-way analysis of variance was performed at $p<0.05$, and means were separated using Tukey's post Hoc test.

3. RESULTS AND DISCUSSION

3.1 Recovery Studies of Trace Metals in Wheat Flour and Bread Samples

The reliability of the analytical method adopted in this study was tested in terms of sensitivity and recovery. The percentage recovery for the trace metals in wheat flour and bread samples respectively are reported in Tables 1 and 2. Mean recoveries of trace metals in wheat flour and bread samples ranged from 81 - 84 %, 79-100%, 80-98%, 89-90%, 88-90% and 81-88% for Cu, Zn, Mn, Cr, Cd and Pb respectively. These percentage of standard elements detected were within the values adjudged acceptable.

3.2 Trace Metals Concentration in Wheat Flour

Trace metals content in the various wheat flour samples are presented in Table 3. Three metals (Cu, Zn and Mn) were detected in appreciable quantities while Cr, Cd and Pb were below the limits of detection (0.0001 – 0.0005) mg/kg. The levels of Cu, Zn and Mn in the wheat flour samples in this study were 0.039 -0.091, 0.837 - 3.310 and 0.035 - 3.148 (mg/kg) respectively. The trace metals were found to be within the limit of nutritional importance that are beneficial to human health. The values obtained were similar to the findings of Doe et al. [11] who assessed the levels of selected heavy metals in two wheat flour in the Ghanaian market (2.9-3.55 and 1.88 – 3.24 mg/kg for Mn and Zn respectively) and stated them to be below the minimum allowable intake with no health risks to the consumers. Thus, the Nigerian sample is considered non-toxic for consumption.

3.3 Concentration of Trace Metals in Other Bread Ingredients

The concentrations of trace metals in other bread ingredients analysed are presented in Figs. 1-3. Copper ranged from 0.005 to 0.150 mg/kg, with the lowest value obtained in salt and highest value in water. The concentrations of Cu in the bread ingredients were below the tolerable level of Cu in food stuffs (10 mg/kg; 0.008 – 9.00 mg/kg) [28,29]. Copper is vital at low concentration for biological systems and plays strategic roles in the maintenance of the body system, growth, anti-inflammatory actions and production of red blood cells, haemoglobin and bones [30]. The result of this study is higher than the findings of several authors [14,2] who worked on the metal content of some ingredients in bread samples in Turkey and Nigeria (0.10 mg/kg and 0.098 – 0.13 mg/kg) respectively. However, values reported in this study are lower than 2.134-2.331 mg/kg reported by Feyzi et al. [30] in bread products in Iran.

Table 1. Percentage recovery of trace metals (%) in wheat flour from selected bakeries in Akure

Bakery	Cu	Zn	Mn	Cr	Cd	Pb
A	83.37	85.06	83.38	89.24	89.65	81.34
B	82.09	91.88	80.63	89.69	89.72	81.24
C	82.98	87.56	81.94	89.01	88.92	86.91
D	81.98	96.69	94.38	90.24	89.46	81.78
E	81.51	91.63	95.94	89.52	89.77	81.69
F	81.59	79.13	92.69	89.30	89.77	81.68
G	82.02	80.06	89.63	89.64	89.69	81.97
H	83.41	97.94	96.88	89.76	89.51	81.42
I	82.37	98.00	98.31	89.82	89.60	81.78
J	81.65	85.75	90.31	89.84	89.23	81.86

Values are means of triplicates analysis

Table 2. Percentage recovery of trace metals (%) in bread from selected bakeries in Akure

Bakery	Cu	Zn	Mn	Cr	Cd	Pb
A	82.38	96.44	96.65	89.60	89.69	88.32
B	81.95	95.88	98.14	89.69	89.80	81.24
C	81.70	85.56	97.81	89.84	88.38	82.14
D	80.87	95.62	92.70	89.87	89.88	81.66
E	82.12	97.25	93.19	89.74	89.72	81.79
F	81.11	90.69	92.63	89.94	89.40	81.61
G	81.38	100.00	90.94	89.64	89.72	81.90
H	84.12	85.25	89.75	89.80	89.79	81.69
I	83.59	93.81	85.81	89.44	89.79	84.32
J	83.10	95.75	95.38	89.70	90.35	82.70

Values are means of triplicates analysis

Table 3. Trace metal concentration (mg/kg) in wheat flour from selected bakeries in Akure

Bakery	Cu	Zn	Mn	Cr	Cd	Pb
A	0.063^{cd}±0.007	0.837^{d}±0.173	0.148^{g}±0.008	<LOD	<LOD	<LOD
B	0.062^{cd}±0.019	<LOD	0.035i±0.004	<LOD	<LOD	<LOD
C	0.039^{d}±0.004	2.591^{ab}±0.120	0.078^{h}±0.011	<LOD	<LOD	<LOD
D	0.077^{bc}±0.019	1.564^{cd}±0.101	1.102^{b}±0.010	<LOD	<LOD	<LOD
E	0.073^{bc}±0.004	0.950^{d}±0.076	0.687^{f}±0.012	<LOD	<LOD	<LOD
F	0.0845^{b}±0.009	1.862^{bc}±0.128	3.148^{a}±0.027	<LOD	<LOD	<LOD
G	0.056^{cd}±0.011	0.904^{d}±0.004	0.983^{c}±0.012	<LOD	<LOD	<LOD
H	0.049^{cd}±0.007	2.487^{b}±0.104	0.857^{e}±0.012	<LOD	<LOD	<LOD
I	0.091^{a}±0.002	1.597^{cd}±0.016	0.851^{e}±0.017	<LOD	<LOD	<LOD
J	0.074^{bc}±0.017	3.310^{a}±0.823	0.915^{d}±0.004	<LOD	<LOD	<LOD

Data are mean ± standard deviation of three replicate values. Means followed by the same superscript within the column are not significantly different ($P \leq 0.05$) from one another. Limit of detection (0.0001 – 0.0005) mg/kg

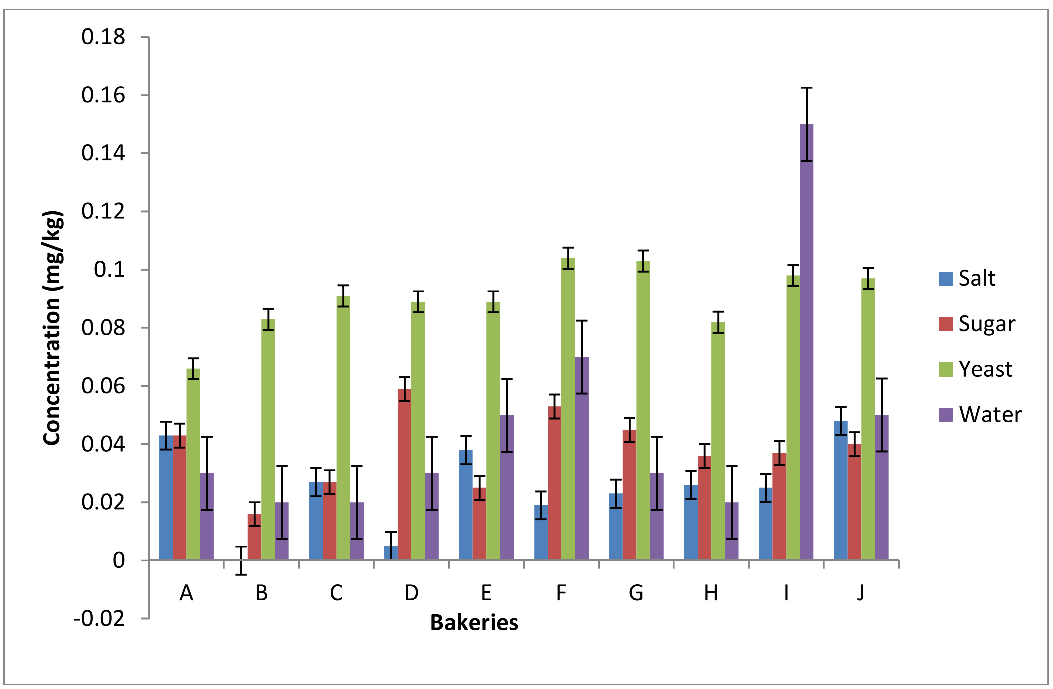

Fig. 1. Concentration of copper (mg/kg) in other bread ingredients from selected bakeries in Akure

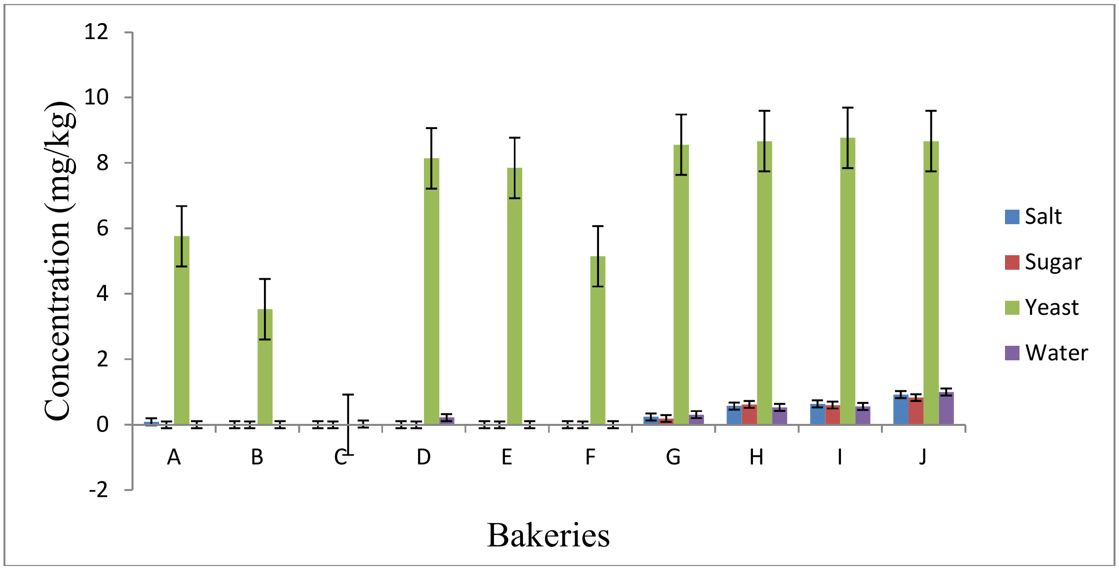

Fig. 2. Concentration of zinc (mg/kg) in other bread ingredients from selected bakeries in Akure

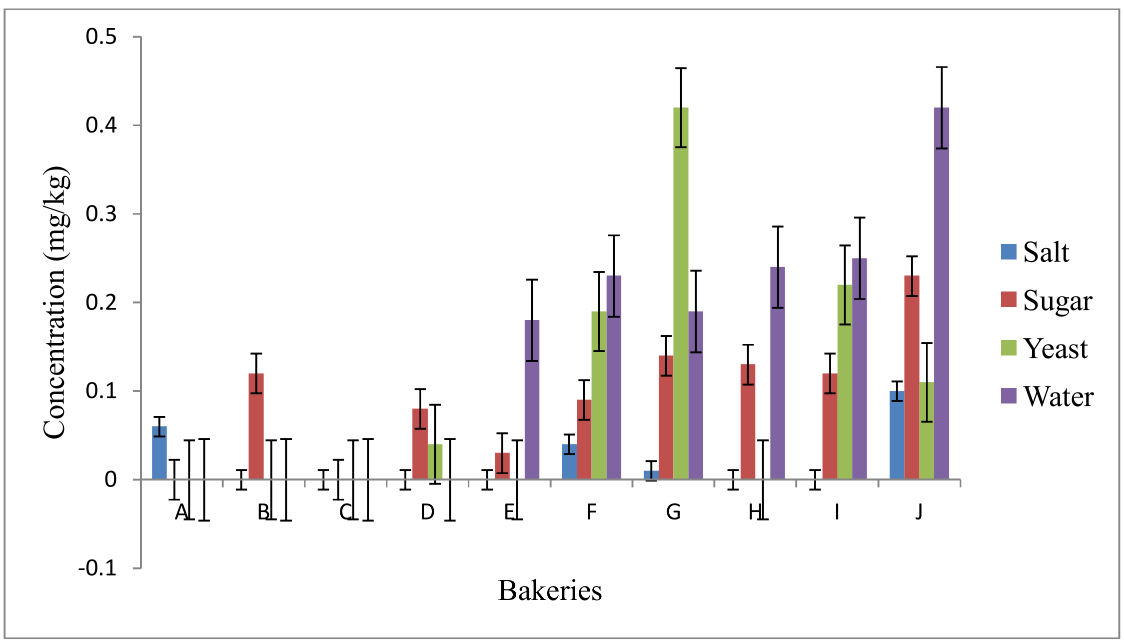

Fig. 3. Concentration of manganese (mg/kg) in other bread ingredients from selected bakeries in Akure

The mean concentration of zinc in salt, sugar, yeast and water in the bakeries ranged from 0.020 to 8.765 mg/kg. The lowest concentration was recorded in water while the highest concentration of zinc was recorded in yeast. The concentration of zinc in yeast from all the bakeries were significantly higher and different ($P>0.05$) from other bread ingredient samples. This could be adduced to strategic roles (catalytic, structural and regulatory functions) of zinc in yeasts. Zinc in yeast has been discovered to build stronger bones and muscles in human system when consumed in staple foods [31]. The values were similar to the report of Al-kamil, (2011) who reported 2.96-4.6 mg/kg for zinc concentration of Iraqi bread samples.

Table 4. Trace metal concentration (mg/kg) in bread from selected bakeries in Akure

Bakery	Cu	Zn	Mn	Cr	Cd	Pb
A	0.058[a]±0.013	1.501[c]±0.077	0.260[c]±0.338	<LOD	<LOD	<LOD
B	0.091[a]±0.004	0.836[d]±0.281	0.054[c]±0.005	<LOD	<LOD	<LOD
C	0.079[a]±0.020	2.743[a]±0.098	0.062[c]±0.013	<LOD	<LOD	<LOD
D	0.056[a]±0.003	1.092[cd]±0.022	0.718[b]±0.001	<LOD	<LOD	<LOD
E	0.079[a]±0.011	1.034[d]±0.170	0.819[ab]±0.018	<LOD	<LOD	<LOD
F	0.075[a]±0.010	0.304[e]±0.154	0.917[ab]±0.103	<LOD	<LOD	<LOD
G	0.073[a]±0.012	2.736[a]±0.015	0.871[ab]±0.052	<LOD	<LOD	<LOD
H	0.066[a]±0.026	2.585[a]±0.197	0.749[ab]±0.017	<LOD	<LOD	<LOD
I	0.090[a]±0.009	1.983[b]±0.214	0.880[ab]±0.001	<LOD	<LOD	<LOD
J	0.077[a]±0.007	2.755[a]±0.037	1.054[a]±0.009	<LOD	<LOD	<LOD

The data were presented as mean± standard deviation of three replicate values. Means followed by the same superscript within the column are not significantly different (P≤ 0.05) from one another. Limit of detection (0.0001 – 0.0005) mg/kg

Table 5. Correlation analysis of trace metals (Cu, Zn and Mn) in bread samples and its ingredients

		Correlations		
		Cu	Zn	Mn
Cu	Pearson Correlation	1	-0.029	.265**
	Sig. (2-tailed)		0.699	0
	N	180	180	180
Zn	Pearson Correlation	-0.029	1	0.091
	Sig. (2-tailed)	0.699		0.224
	N	180	180	180
Mn	Pearson Correlation	.265**	0.091	1
	Sig. (2-tailed)	0	0.224	
	N	180	180	180

*** Correlation is significant at the 0.01 level (2-tailed)*

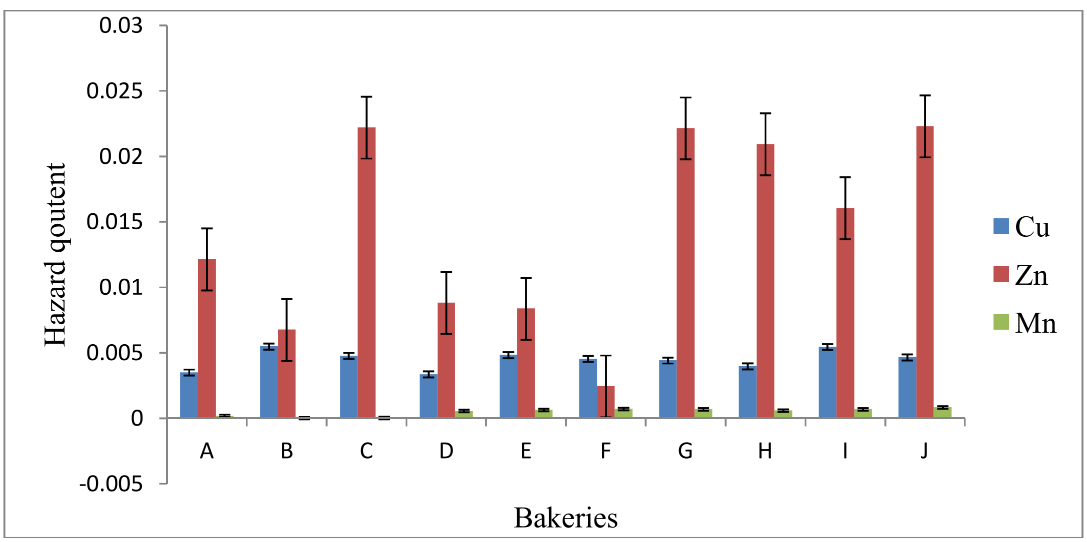

Fig. 4. Estimated Daily Intake (mg/day) of metals by humans through bread consumption from all the selected bakeries

Estimated daily intake was calculated on the basis of 0.170 kg of bread consumed daily. Assuming a reference body weight (bw) of 70kg. EDI = Cbread X Dbread/Body weight. Oral reference dose (Rfd) (Cu = 0.04, Zn = 0.30 and Mn = 3.00) mg/Kg/day

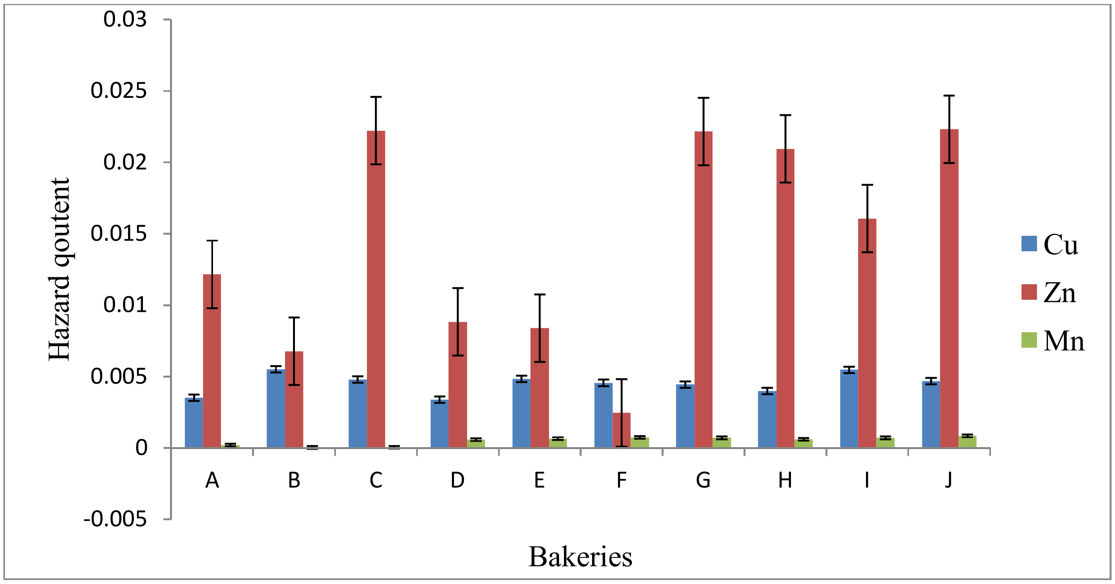

Fig. 5. Hazard Quotient (HQ) of metals through bread consumption from all the selected bakeries

The mean concentration of manganese (Mn) in salt, sugar, yeast and water from sampled bakeries ranged from 0.010 to 0.420 mg/kg. The study revealed that manganese levels were below detection limit (0.0001 – 0.0005) mg/kg in salt, sugar, yeast and water obtained from some of the bakeries. However, highest concentration of Mn (0.420 mg/kg) recorded in yeast and water are within dietary tolerable intake (32, EFSA, 2013). The level of manganese recorded in this study could be attributed to the availability and natural presence of manganese in wide variety of foods. Manganese is an essential nutrient needed by metallo-enzymes in the human body. It is also essential in bone formation, amino acid, cholesterol, and carbohydrate metabolism.

3.4 Concentration of Trace Metals in Bread Samples

The values of trace metals: Cu, Zn, Mn, Cr, Cd and Pb in bread samples analysed are presented in Table 4. Pb, Cd and Cr are among the most abundant trace metals and are particularly toxic [33]. The presence of these metals in food beyond tolerable limits is associated with a number of diseases, especially of the cardiovascular, renal, nervous and skeletal systems [19]. They are also implicated, in carcinogenesis, mutagenesis and teratogenesis [33]. The results obtained revealed that Cr, Cd and Pb were not detected in the samples analysed in this report, but Cu, Zn and Mn were found to be present at various considerable levels (below dietary tolerable intakes) in the bread samples analysed.

Copper levels obtained in the bread samples in this study were in the range of 0.056 to 0.091 mg/kg and are within tolerable dietary intakes which are adequate for man daily needs [28]. The concentration of Cu could have emanated from the various raw materials used in the production of bread samples. Copper must have leached from copper water pipes used.

Zinc (Zn) content of bread samples in this study was in the range of 0.304 to 2.755 mg/kg. These values are within the permissible level of zinc in foods (50 mg/kg) established by United States Department of Agriculture [34] and the daily requirement for human adult (22 mg/day) by WHO [35]. In humans, zinc levels within tolerable dietary intake is very essential in cognitive human function, reproductive health, especially in maintaining immune and prostate health [36,37,38].

Manganese (Mn) levels in the bread samples ranged from 0.054 to 1.054 mg/kg. These values are lower than 25.83 to 75.53 mg/kg obtained by Oyekunle et al. [26] and exceeds the daily intake of 2 – 3 mg/day recommended by WHO [39]. The levels of Mn found in the bread samples were adequate for daily needs of humans as its deficiency leads to impaired growth [40].

3.5 Correlation Analysis of Trace Metals

The result of the correlation analysis of metals (Cu, Zn and Mn) in bread samples and ingredients showed that there was a significant positive correlation ($p<0.01$, 2-tailed) between Cu and Mn in the ingredients and bread samples. This suggests that the metals may be of similar sources. However, there was no correlation between Zn and other metals in bread samples and ingredients.

3.6 Estimated Daily Intake (EDI)

The estimated daily intake of three metals (Cu, Zn and Mn) in bread from the present study was calculated and presented in Fig. 4. Daily intake of trace metals was estimated on the basis of the concentration of each trace metal measured in bread and daily bread consumption. Average body weight of an adult was assumed to be 70 kg [22] and the rate of bread consumption was taken as 0.170 kg/day. The EDI values of the metals ranged from 0.00014 to 0.00551 mg/kg and were lower than the daily requirement of 0.170 kg/day. This shortfall and low consumption rate of the nutritionally essential metals in bread could adversely affect the human health in the long run except they are adequately present in different food items consumed per day.

3.7 Hazard Quotient (HQ)

Hazard quotient (HQ) values were calculated on the basis of the oral reference dose of Cu, Zn and Mn. The Hazard Quotient in the studied bread ranged from 0.0001 to 0.0223. When HQ exceeds one (1.00), there is concern for health effect. There was no obvious risk associated with the consumption of the bread samples in all the bakeries as the HQ in this study was less than one (HQ <1). Therefore, the consumption of bread samples from the sampled bakeries is considered safe, fit and healthy to the local populace. The result corroborates the findings of several authors [41,42] and who obtained similar values of HQ for Nigerian bread and food fish respectively. This is considered safe for consumption.

3.8 Hazard Index

The Hazard Index (HI) for residents ingesting these trace metals by consuming bread in all the selected bakeries were calculated by the summation of hazard quotients (HQ) of all studied trace metals for individual bakery and is presented in Fig. 6.

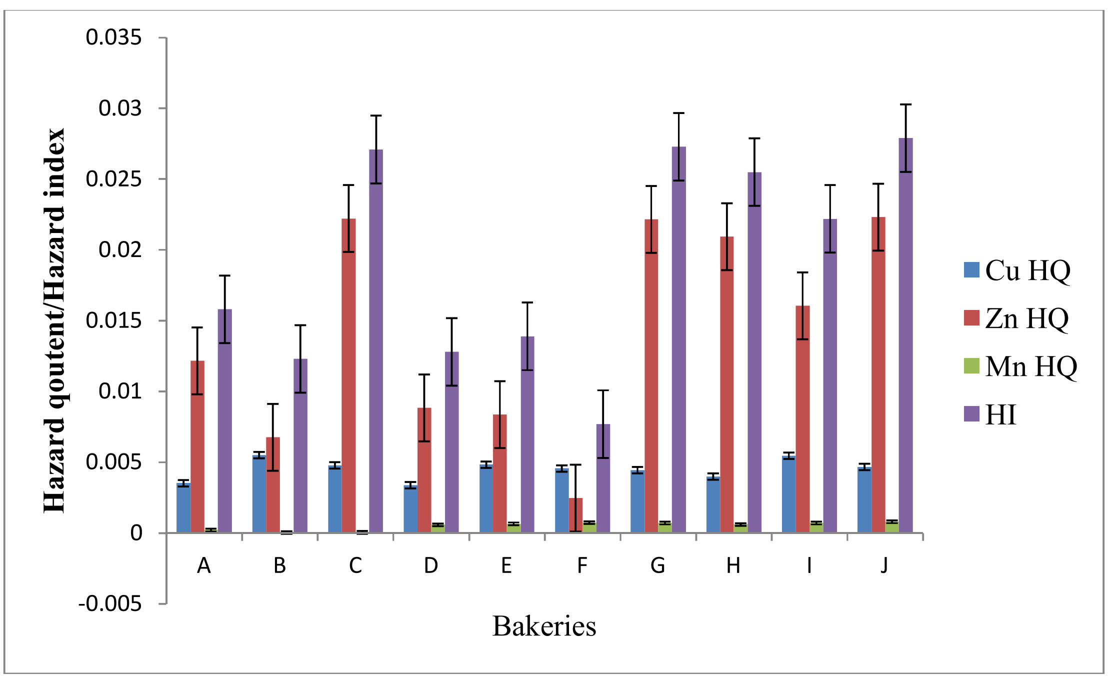

Fig. 6. Hazard Index (HI) to the population of Akure from some trace metals through bread consumption

The hazard index to average bread consumers ranged from 0.0077 to 0.0279. The observed range of hazard quotients and hazard indices were also lower than the acceptable safe risk level (HI ≤ 1) with no adverse effect on human health during consumption. Hence, the consumption of these loaves of bread is considered fit and safe to consumers.

4. CONCLUSION

The trace metals found in varying proportions in the bread ingredients may have contributory effects in the bread samples. However, the levels in the bread ingredients were lower than permissible limits and do not pose any concern to consumers. The observed levels of Cu, Zn and Mn in bread samples were also within safe limits. The daily intakes of the metals analysed were not higher than the tolerable daily intakes (PTDI). The hazard quotients (HQ) and hazard indices (HI) of the metals were lower than the acceptable safe risk level (HQ≤1 and HI≤1). Hence, the average consumption of bread at 0.170 kg/day from selected bakeries in Nigeria would not pose any health risk to the consumers.

5. RECOMMENDATION

Trace metal concentrations obtained in this study indicated that average consumption of bread was of no risk to health. Nonetheless, constant monitoring of the contaminants in bread and bakery ingredients is essential in food quality control in bread production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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