

Atomic Absorption Spectroscopic (AAS) Analysis of Heavy Metals and Health Risks Assessment of some Common Energy Drinks

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ABSTRACT

Background and Purpose: Soft drinks are commonly consumed globally. Due to high demand, regulatory protocols may be breached thereby leading to contamination with heavy metals. In this study, we determined the content of five heavy metals in some Nigerian soft drinks and estimated their potential health risks.

Methods: Atomic absorption spectroscopy (AAS) was used to determine the concentrations of magnesium, aluminum, lead, arsenic, and zinc. Twelve (12) soft drinks (labeled B1–B12) were used. After the AAS, parameters such as target health quotient (THQ), average daily intake (EDI), and chronic daily intake (CDI) were evaluated.

Results: The level of arsenic found in all soft drinks ranged from 0.001-0.0603 mg/L, as against the 0.01 mg/L maximum contaminant level (MCL) standard. Lead was 0.001-0.023mg/L (MCL is 0.01 mg/L) and within the MCL except in one sample (0.023 mg/L). Aluminum levels ranged from 0.001-2.0491 mg/L except in one sample (0.001 mg/L) and the WHO limit is 0.02 mg/L. The concentration range for magnesium was 0.6954-2.4341 mg/L versus the standard limit of 0.02 mg/L. The THQ values of aluminum were significantly different from the values of other heavy metals. The order of abundance of the metals was: Mg > Zn > Al > Pb > As. Arsenic and lead were found in relatively small concentrations compared to the other metals. The THQ value of aluminum in most of the samples was higher than normal.

Conclusion: The concentration of magnesium was above the acceptable limits in all the soft drinks. All the heavy metals, except aluminum, were within acceptable THQ limits. The high values of magnesium and aluminum underscores the need for quality control measures during production.

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INTRODUCTION

INTRODUCTION

Energy or soft drinks are refreshing non-alcoholic drinks with a sweet taste. They are consumed globally regardless of age, gender, or socioeconomic status due to affordability, flavor, and rehydrating effect (Sikalidis *et al.*, 2020). Carbonated water is combined with sugary syrup made from fruit or plant extracts (Ferraro *et al.*, 2013). Due to the strong demand for soft drinks, production is high, and good manufacturing practices may be compromised thereby leading to possible contamination with heavy metals. These metals are often difficult to clear from the body, and thus build up over time, causing diseases like cancers, cardiovascular disorders, neurological damage, lung disorders, kidney failure, memory loss, and mental disabilities (Malik *et al.*, 2006). The drinks are frequently served at festivals, funerals, and wedding ceremonies, as well as at public places like markets, motor parks, hospitals, and restaurants (Tchounwou *et al.*, 2012). Soft drinks provide vitamins, phosphates, acids, antioxidants, and other nutrients to the body (Kregiel, 2015). It is conceivable that the quality of the beverages may be compromised during the fast production process due to high demand. Previous studies have detected some heavy metals above the acceptable limits in soft drinks (Tchounwou *et al.*, 2012). Contamination of water with heavy metals occurs during the manufacturing of beverages (Izah *et al.*, 2016; Balali-Mood *et al.*, 2018).

The global soft drink market was expected to increase by 449.6 billion dollars in 2019, with an annual growth rate of about 5.8%. In 2007, Nigerians consumed more than 159.85 g of soft drinks per day (Godwill *et al.*, 2015). As the rate of consumption of soft drinks is on the rise there are continuous product innovations in terms of ingredients, formulations, exceptional taste, and other aspects to satisfy the consumer market (Dorota, 2015).

Heavy metals are among the natural elements with high atomic numbers, which have densities five times greater than that of water (Tchounwou *et al.*, 2012; Izah *et al.*, 2016). A wide variety of foods, such as tea, burgers, herbal drinks, bread, cakes, fisheries, condiments, fruits, vegetables, and beverages contain them (Mattila *et al.*, 2000; Ubuoh, 2013). Although human health and that of other living organisms depend on certain essential elements (Singh *et al.*, 2011; Woyessa *et al.*, 2015), their levels in food and soft drinks frequently consumed ought to be monitored due to their biotoxic effect and potential carcinogenicity (Izah *et al.*, 2016). These metals have been shown to disrupt nucleic acids, cause mutations, and mimic hormones to disrupt the endocrine and reproductive

systems as well as cause cancer (Jarup, 2003; Monisha *et al.*, 2014; Duffus, 2015).

Some of the most important heavy metals are iron, zinc, copper, cobalt, chromium, and manganese (Singh *et al.*, 2011; Izah *et al.*, 2016). Daily requirements for these metals are: manganese (2 to 5 mg); chromium (0.005 mg to 0.0001 mg); zinc (15 to 20 mg); iron (1 to 2 mg); and copper (2 to 5 mg) (Singh *et al.*, 2011; Izah *et al.*, 2016). Food and water are the main sources of heavy metal ingestion (Ubuoh, 2013; Magomya *et al.*, 2015). Regulatory agencies such as the National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria, and the US Environmental Protection Agency routinely monitor the spectrum of heavy metal toxicity in beverages such as soft drinks, using the maximum contaminant level goal (MCLG) to ensure health protection (Iloms *et al.*, 2018; Tchounwou *et al.*, 2012). As a result, it is permissible to allow for marginal safety while establishing an enforceable public goal and maximum contamination level (MCL), which is the maximum quantity of a pollutant that can be present in water (Adepoju-Bello *et al.*, 2012; Engwa *et al.*, 2015; Woyessa, 2015). Thus, heavy metals exceeding the limits prescribed in Nigeria's water quality standards are deemed harmful to human health (Izah 2016). The oxidative states of the ions (Zn^{2+} , Cd^{2+} , Pb^{2+} , As^{2+} , As^{3+} , Ag^+ , Hg^{2+} , etc.) that are formed in the acidic medium of the stomach may easily create strong and stable interactions with biological molecules like proteins and enzymes. The most prevalent functional groups that heavy metals bind to are thiol groups (SH of cysteine and SCH_3 of methionine). In vitro studies have shown that cysteine residues in the active sites of thiol-transferases including thioredoxin reductase, glutathione reductase, and thioredoxin, as well as glutathione itself, inhibit human enzymes (Chrestensen *et al.*, 2000).

To know the type and concentrations of heavy metals in soft drinks, several analytical methods including atomic absorption spectrometry (AAS) are used (Ere *et al.*, 2020). The purpose of this study was to use AAS to quantify the heavy metals present in some Nigerian soft drinks and to assess the health concerns they might pose.

METHODS

Sample Collection and Decontamination

The following twelve (12) brands of soft drinks were used for the study: Coke, Schweppes, Lucozade boost, 5-Alive, Zero Fanta, Lacasera, Nutri-milk, Blue-bullet, Ribena, Sprite, Amstel malt, and Fearless energy drink. They were purchased from several supermarkets in Lokoja, Kogi State, Southwest Nigeria, and were each assigned a code ranging from B1 to B12. Lokoja has three major markets which are the New Market (International Market), the Old

Market, and Kpata Market. The city lies about 7.8023° North of the equator and 6.7333° East of the Meridian. It is about 165 km Southwest of Abuja (The Federal Capital Territory of Nigeria), and 390 km Northeast of Lagos State. To avoid contamination, the samples were handled with care. All glassware used in this study were decontaminated by washing with filtered water and then soaking in a 10% v/v nitric acid solution for 24 h. They were further rinsed many times with deionised water, dried at room temperature, and stored in a clean environment.

Atomic Absorption Spectroscopy

Digestion procedure

The method reported by Otim *et al.* (2019), with some modifications was used for the digestion process. Samples (25 mL) of each well-mixed beverage and 20 mL of distilled water were measured into a 150 mL beaker. The sample was treated with 4 mL of conc. HNO₃ and 2 mL of perchloric acid. To break the complicated bond and release the sample into the solution, the mixture was heated until the volume was reduced to 15 mL. The solution was allowed to cool before being filtered into a beaker and analyzed using AAS.

Health risk assessment

Following sample preparation, the 15 mL digest was analyzed for the metals using the Solar Thermo Elemental Atomic Absorption Spectrometer, Model SN - SG 718960. To optimize the performance of the instrument, a solution containing 10 mg/L each of As, Pb, Zn, Al, and Mg was used. The accuracy of measurement was confirmed by analysis of standards from another batch.

Estimation of Average or Estimated daily intake (ADI/EDI) and chronic daily intake (CDI)

By calculating the Average or Estimated Daily Intake (ADI/EDI mg/kg/day) of the five metals investigated, an exposure assessment was undertaken to evaluate the intensity, frequency, and duration of human exposures to soft drinks containing heavy metals. According to US EPA, 1986) the formula for calculating EDI is:

$$EDI = C_m \times C_{factor} \times D_{food\ intake} / B_w$$

Where: C_m = individual metal concentrations in mg/kg in soft drinks; C_{factor} = conversion factor (0.085) (Jan *et al.*, 2010); D_{food intake} = daily intake of soft drinks (30 cL per person/day); B_w = average body weight (20 kg for children and 70 kg for adults) (NSW Centre for Public Health Nutrition, 2009). Chronic daily intake was calculated using the formula:

$$CDI = C \times EDi \times EFi \times IR / Bw \times AT$$

Where: C = metal concentration in soft drinks; EDi = exposure duration (6 years for children, and 24 years for adults); EFi = exposure frequency (365 days/yr); IR = intake rate (0.012 kg/person/day for children and 0.030 kg/person/day for adults); B_w = average body weight; AT = average time for non-carcinogens (365 days x number of exposure years [6 years for children and 24 years for adults]) (United States Environmental Protection Agency - USEPA, 2000).

Reference Oral Dose (RfD) is derived from animal research (USEPA, 1989), and is an estimate of the maximum acceptable oral dose of a toxic substance for a human population through daily exposure, taking into account, sensitive group overtime (USEPA, 2017).

A Toxicological (Target) health quotient (THQ) is calculated by comparing a pollutant level in a matrix of interest to a US EPA-defined oral reference dosage of that pollutant, accounting for length and frequency of exposure, consumer body weight, and the amount consumed. As a result, the THQ was used to calculate the risk associated with soft drink intake in Nigeria (USEPA, 1986; USEPA, 2000). The estimate was calculated using the formula:

$$THQ = CDI / RfD$$

Where: CDI = chronic daily intake; RfD = reference oral dose. A range of 0.6954 - 2.4341 mg/L of magnesium was found in all the soft drink samples.

Statistical data analysis

Data generated were analyzed using the statistical packages: Paleontological statistics software version 3.01; GraphPad 9.0; and Microsoft Excel version 2013. Statistical significance was defined as a P-value of less than 0.05. In addition, the Jitter cluster plot and diversity correlation analysis were used to assess the strength of the linear association between the heavy metal contents in the soft drinks (Hammer *et al.*, 2001).

RESULTS

Atomic absorption spectroscopy result

Table 1 shows the concentration of magnesium, aluminum, zinc, lead, and arsenic in the soft drinks. Arsenic levels range from 0.001-0.0603 mg/L, being less than the MCL (0.01 mg/L) in all samples except B9 which had 0.0603 mg/L. Lead was between 0.001-0.023 mg/L which was also within the MCL (0.01 mg/L), except B4 which had 0.023 mg/L. Zinc was from 0.5658 to 2.9071 mg/L, while the MCL is 5 mg/L, hence all samples were within the acceptable limit. The level of aluminum was from 0.001 - 2.0491 mg/L, with MCL of 0.02 mg/L and all samples were above this limit except B8 which was 0.001

mg/L. All the samples had more than the WHO MCL (0.02 mg/L) for magnesium.

The diversity profile of heavy metal concentration in soft drinks samples are shown in Figure 1. The Jitter cluster

plot of the relative abundance of the heavy metals in the soft drinks are shown in Figure 2. The order of abundance of the analyzed heavy metals was Mg > Zn > Al > Pb > As (Paleontological statistics software).

Table 1: Mean heavy metal concentration in soft drink samples.

| Samples Code | The concentration of heavy metals (mg/L) | | | | |
|--------------|--|----------------------|----------------------|----------------------|-----------------------|
| | Arsenic (As) | Lead (Pb) | Zinc (Zn) | Aluminum (Al) | Magnesium (Mg) |
| B1 | 0.0005 | 0.0132 | 2.4116 | 1.3217** | 2.0164** |
| B2 | 0.0029 | 0.0005 | 1.8767 | 0.7149* | 1.9872** |
| B3 | 0.0005 | 0.0005 | 0.5658 | 0.4864* | 2.4341** |
| B4 | 0.0005 | 0.0234* | 2.1148 | 1.6523** | 0.6954* |
| B5 | 0.0043 | 0.0005 | 0.9756 | 0.6942* | 2.3147** |
| B6 | 0.0032 | 0.01032 | 2.9071 | 1.4289** | 0.6972* |
| B7 | 0.0005 | 0.0069 | 0.7169 | 0.2361* | 1.5127** |
| B8 | 0.0005 | 0.01072 | 2.0852 | 0.0005 | 2.0652** |
| B9 | 0.0603*** | 0.0124 | 1.6537 | 1.5694** | 3.8216*** |
| B10 | 0.0005 | 0.0141 | 0.0005 | 1.7802** | 1.9671** |
| B11 | 0.0005 | 0.0132 | 1.6343 | 0.9520* | 2.6114*** |
| B12 | 0.0042 | 0.0072 | 2.3965 | 2.0491*** | 1.8659** |
| MCL (WHO) | 0.01 | 0.01 | 5.00 | 0.02 | 0.02 |
| RfD | 3.0×10^{-4} | 3.5×10^{-3} | 3.0×10^{-1} | 1.2×10^{-5} | 7.38×10^{-1} |

*Slightly higher than expected level; **Greatly higher than expected level; ***Significantly higher than expected level. MCL= Maximum Concentration Level (mg/L) of potentially toxic metals in drinking water (US EPA, 2011).

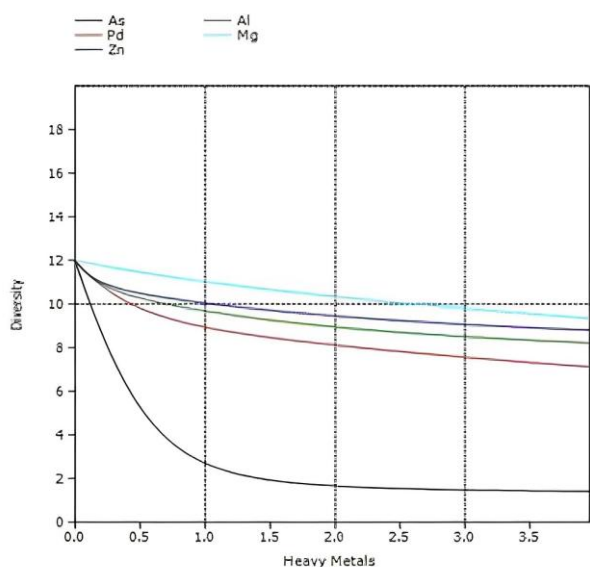


Figure 1: The diversity profile of heavy metals in the samples of soft drinks

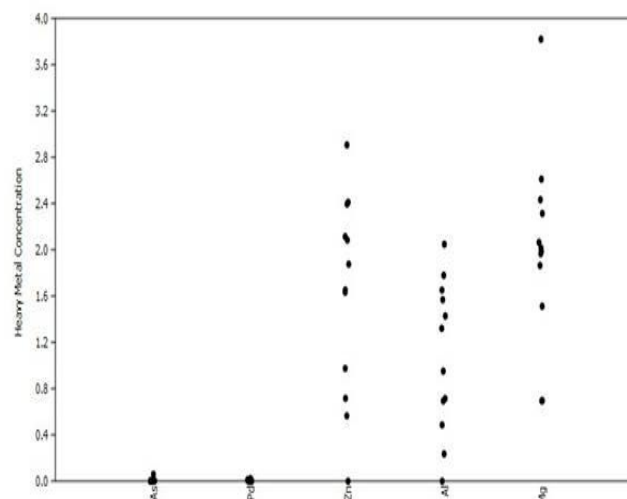


Figure 2: Jitter cluster plot of heavy metal abundance in soft drinks. Order of abundance of the heavy metals: Mg > Zn > Al > Pb > As

Health risk assessment quantification

The data of Estimated Daily Intake (EDI) of selected toxic metals in soft drinks sold in Lokoja, Kogi State, Nigeria are shown in Table 2. Arsenic and lead showed relatively low EDI compared to zinc, aluminum, and magnesium.

Table 3 shows the Chronic Daily Intake (CDI) of the analyzed samples, arsenic, and lead showed relatively low CDI compared to zinc, aluminum, and magnesium.

In Table 4, the Target Health Quotient (THQ) values of selected heavy metals in soft drinks are shown.

Table 2: The Estimated Daily Intake (EDI) values of the heavy metals in the soft drinks.

| Samples Code | EDI ($\times 10^{-5}$ mg/L) | | | | | | | | | |
|--------------|------------------------------|----------|--------|----------|----------|----------|----------|----------|-----------|----------|
| | Arsenic* | | Lead* | | Zinc | | Aluminum | | Magnesium | |
| | Adult | Children | Adult | Children | Adult | Children | Adult | Children | Adult | Children |
| B1 | 0.0182 | 0.0638 | 0.4809 | 1.6830 | 87.8511 | 307.4790 | 48.1476 | 168.5168 | 73.4546 | 257.0910 |
| B2 | 0.1056 | 0.3698 | 0.0182 | 0.0638 | 68.3655 | 239.2793 | 26.0428 | 91.1498 | 72.3909 | 253.3680 |
| B3 | 0.0182 | 0.0638 | 0.0182 | 0.0638 | 20.6113 | 72.1395 | 17.7189 | 62.0160 | 88.6708 | 310.3478 |
| B4 | 0.0182 | 0.0638 | 0.8524 | 2.9835 | 77.0391 | 269.6370 | 60.1909 | 210.6683 | 25.3324 | 88.6635 |
| B5 | 0.1566 | 0.5483 | 0.0182 | 0.0638 | 35.5397 | 124.3890 | 25.2887 | 88.5105 | 84.3212 | 295.1243 |
| B6 | 0.1166 | 0.4080 | 0.3759 | 1.3158 | 105.9015 | 370.6553 | 52.0528 | 182.1848 | 25.3980 | 88.8930 |
| B7 | 0.0182 | 0.0638 | 0.2514 | 0.8798 | 26.1156 | 91.4048 | 8.6008 | 30.1028 | 55.1055 | 192.8693 |
| B8 | 0.0182 | 0.0638 | 0.3905 | 1.3668 | 75.9609 | 265.8630 | 0.0182 | 0.0638 | 75.2323 | 263.3130 |
| B9 | 2.1966 | 7.6883 | 0.4517 | 1.5810 | 60.2419 | 210.8468 | 57.1710 | 200.0985 | 139.2154 | 487.2540 |
| B10 | 0.0182 | 0.0638 | 0.5136 | 1.7978 | 0.0182 | 0.0638 | 64.8501 | 226.9755 | 71.6586 | 250.8053 |
| B11 | 0.0182 | 0.0638 | 0.4809 | 1.6830 | 59.5352 | 208.3733 | 34.6800 | 121.3800 | 95.1296 | 332.9535 |
| B12 | 0.1530 | 0.5355 | 0.2623 | 0.9180 | 87.3011 | 305.5538 | 74.6458 | 261.2603 | 67.9721 | 237.9023 |

*Lower EDI compared to other metals.

Table 3: The Chronic Daily Intake (CDI) values of selected toxic heavy metals in the selected soft drinks.

| Samples Code | EDI ($\times 10^{-5}$ mg/L) | | | | | | | | | |
|--------------|------------------------------|---------|---------|----------|---------|---------|----------|-----------|-----------|----------|
| | Arsenic | | Lead* | | Zinc | | Aluminum | | Magnesium | |
| | Adult | Childre | Adult | Children | Adult | Childre | Adult | Children | Adult | Children |
| B1 | 2.1E-07 | 3.0E-07 | 5.7E-06 | 7.92E-06 | 0.0010 | 0.00145 | 0.00057 | 0.0008 | 0.00086 | 0.0012 |
| B2 | 1.2E-06 | 1.7E-06 | 2.1E-07 | 3.00E-07 | 0.0008 | 0.00113 | 0.00031 | 0.0004 | 0.00085 | 0.0012 |
| B3 | 2.1E-07 | 3.0E-07 | 2.1E-07 | 3.00E-07 | 0.0002 | 0.00034 | 0.00021 | 0.0003 | 0.00104 | 0.0015 |
| B4 | 2.1E-07 | 3.0E-07 | 1.0E-05 | 1.40E-05 | 0.0009 | 0.00127 | 0.00071 | 0.0010 | 0.00030 | 0.0004 |
| B5 | 1.8E-06 | 2.6E-06 | 2.1E-07 | 3.00E-07 | 0.0004 | 0.00059 | 0.00030 | 0.0004 | 0.00099 | 0.0014 |
| B6 | 1.4E-06 | 1.9E-06 | 4.4E-06 | 6.19E-06 | 0.0013 | 0.00174 | 0.00061 | 0.0009 | 0.00030 | 0.0004 |
| B7 | 2.1E-07 | 3.0E-07 | 3.0E-06 | 4.14E-06 | 0.0003 | 0.00043 | 0.00010 | 0.0001 | 0.00065 | 0.0009 |
| B8 | 2.1E-07 | 3.0E-07 | 4.6E-06 | 6.43E-06 | 0.0009 | 0.00125 | 2.15E-07 | 0.003E-04 | 0.00089 | 0.0012 |
| B9 | 2.6E-05 | 3.6E-05 | 5.3E-06 | 7.44E-06 | 0.0007 | 0.00099 | 0.00067 | 0.0009 | 0.00164 | 0.0023 |
| B10 | 2.1E-07 | 3.0E-07 | 6.0E-06 | 8.46E-06 | 2.2E-07 | 3.0E-07 | 0.00076 | 0.0011 | 0.00084 | 0.0012 |
| B11 | 2.1E-07 | 3.0E-07 | 5.7E-06 | 7.92E-06 | 0.0007 | 0.00098 | 0.00041 | 0.0006 | 0.00112 | 0.0016 |
| B12 | 1.8E-06 | 2.5E-06 | 3.1E-06 | 4.32E-06 | 0.0010 | 0.00144 | 0.00088 | 0.0012 | 0.00080 | 0.0011 |

*Relatively low CDI compared to zinc, aluminum, and magnesium

Table 4: The Target Health Quotient (THQ) values of heavy metals in the soft drinks.

| Samples code | Arsenic | | Lead | | Zinc | | Aluminum | | Magnesium | |
|-----------------|----------|----------|----------|----------|--------|----------|-----------|-----------|-----------|----------|
| | Adult | Children | Adult | Children | Adult | Children | Adult | Children | Adult | Children |
| B1 | 7.00E-05 | 1.00E-03 | 1.63E-03 | 2.26E-03 | 0.0034 | 0.004833 | 47.500*** | 66.667*** | 0.00117 | 0.00163 |
| B2 | 4.00E-04 | 5.67E-03 | 6.00E-05 | 8.57E-05 | 0.0027 | 0.003767 | 25.833** | 33.333** | 0.00115 | 0.00163 |
| B3 | 7.00E-05 | 1.00E-03 | 6.00E-05 | 8.57E-05 | 0.0008 | 0.001133 | 17.500** | 25.000** | 0.00141 | 0.00203 |
| B4 | 7.00E-05 | 1.00E-03 | 2.86E-03 | 4.00E-03 | 0.0030 | 0.004233 | 59.167*** | 83.333*** | 0.00041 | 0.00054 |
| B5 | 6.00E-04 | 8.67E-03 | 6.00E-05 | 8.57E-05 | 0.0014 | 0.001967 | 25.000** | 33.333** | 0.00134 | 0.00190 |
| B6 | 4.67E-04 | 6.33E-03 | 1.26E-03 | 1.77E-03 | 0.0042 | 0.0058 | 50.833** | 75.000*** | 0.00041 | 0.00054 |
| B7 | 7.00E-05 | 1.00E-03 | 8.57E-04 | 1.18E-03 | 0.0010 | 0.001433 | 8.333* | 8.3330* | 0.00088 | 0.00122 |
| B8 | 7.00E-05 | 1.00E-03 | 1.31E-03 | 1.84E-03 | 0.0030 | 0.004167 | 0.018 | 0.0250 | 0.00121 | 0.00163 |
| B9 | 8.67E-03 | 1.20E-01 | 1.51E-03 | 2.13E-03 | 0.0024 | 0.003300 | 55.833*** | 75.000*** | 0.00222 | 0.00312 |
| B10 | 7.00E-05 | 1.00E-03 | 1.71E-03 | 2.42E-03 | 0.0000 | 0.000001 | 63.333*** | 91.667*** | 0.00114 | 0.00163 |
| B11 | 7.00E-05 | 1.00E-03 | 1.63E-03 | 2.26E-03 | 0.0023 | 0.003267 | 34.167** | 50.000**v | 0.00152 | 0.00217 |
| B12 | 6.00E-04 | 8.33E-03 | 8.86E-04 | 1.23E-03 | 0.0034 | 0.004800 | 73.333*** | 100.00*** | 0.00108 | 0.00149 |

*Slightly highly than expected level; **Greatly higher than expected level; ***Significantly higher than expected level. There was a significant difference ($P < 0.05$) in the THQ of aluminum versus the other metals.

DISCUSSION

The atomic absorption spectrometry (AAS) was used to determine the concentrations of heavy metals in soft drinks selected from markets in Lokoja, Nigeria. The diversity profile and Jitter cluster plot showed the relative abundance of the heavy metals in the studied samples. The order of abundance was $Mg > Zn > Al > Pb > As$. This shows that arsenic and lead were found in relatively small concentrations when compared to the other heavy elements tested.

The arsenic content obtained was less than the MCL in all the soft drinks except for B9 which had 0.0603 mg/L. In Nigeria, exposure to arsenic is mainly via intake of food and drinking water (Izah and Srivastav, 2015) and beverages (Salako *et al.*, 2016). Arsenic causes diseases such as nerve injury and stomach aches, even at low concentrations (Izah and Srivastav, 2015). Cardiovascular, hematological, neurological, pulmonary, gastrointestinal, and developmental diseases, dermatitis and cancers, diabetes, and hearing loss have all been linked to high arsenic exposure (Jarup, 2003; Tchounwou *et al.*, 2012; Pflaum *et al.*, 2016).

The lead contents of the soft drinks were within the MCL except in B4 which had 0.023 mg/L. The toxicity of lead is

due to its bioaccumulation potential in tissues leading to headache, irritability, abdominal pain, impairment of hemoglobin synthesis, encephalopathy and various symptoms related to the nervous system (Ahmed *et al.*, 1987). Children may exhibit behavioral disturbances and learning and concentration difficulties (Ogundele, 2018). Lead has been found above MCL limits in beverages (Quinn and Sherlock, 1990).

Zinc levels in all the soft drink samples were within the permissible limits. Zinc is a necessary mineral with an MCL of 3 mg/L but its ingestion over the recommended daily intake may reduce the amount or level of copper absorbed into the body, resulting in anemia (Chasapis *et al.*, 2012). Zinc is relatively nontoxic, particularly when administered orally, but with extreme high intakes or concentrations, toxicity symptoms, such as nausea, vomiting, epigastric pain, lethargy, and fatigue usually occur (Plum *et al.*, 2010).

The WHO tolerable limit of aluminum is 0.02 mg/L. All the values obtained were above this limit for this metal except B8, which had a concentration 0.001 mg/L. High exposure to aluminum can cause health problems including, dementia, lethargy, and severe trembling (Eno-Obong and Ukoha, 2013). Nausea, vomiting, diarrhea,

hypotension, confusion, slow respiratory rate, coma, cardiac arrhythmia, itchy red rashes, headache, muscle and joint pain, poor memory, insomnia, depression, asthma, irritable bowel syndrome, and death from cardiac arrest are the most common symptoms of aluminum toxicity (Rahimzadeh *et al.*, 2022). Aluminum was classified as a non-carcinogen by the US Department of Health and Human Services in 1988, but a multi-element toxicology review in 2014 found no serious deleterious effects of aluminum consumed in amounts less than 40 mg/kg/day, although people with kidney disease may develop vitamin D-resistant osteomalacia, erythropoietin-resistant microcytic anemia, CNS alterations, among other symptoms (Dolara, 2014). This may be due to the fact most is excreted in feces and urine (ATSDR, 2016). Aluminum has also been found to penetrate the blood-brain barrier, particularly in Alzheimer's disease patients, where it is thought to function electrostatically, cross-linking various proteins and thereby down-regulating genes in the superior temporal gyrus (Crapper *et al.*, 1989; Xu *et al.*, 1992). It is therefore a suspected or contributory cause of Alzheimer's disease (Klotz *et al.*, 2017). It has also been reported to increase estrogen-related gene expression in breast cancer and at very high doses, associated with altered function of the blood-brain barrier (Banks and Kastin, 1989; Darbre, 2006).

Magnesium is an essential element but its concentration in the test samples was above the WHO MCL. This would eventually cause magnesium toxicity that usually develops after serum concentrations exceed 1.74-2.61 Mmol/L. Symptoms include hypotension, nausea, vomiting, facial flushing, urine retention, ileus, depression, and lethargy. Chronic or advanced magnesium toxicity includes muscle weakness, difficulty in breathing, extreme hypotension, and cardiac arrhythmias, among other clinical symptoms (Elin, 1988). The US Environmental Protection Agency (USEPA) created a method for evaluating exposure risk in 1986 (with further supplemental modifications in 2000), and the target or toxicological health quotient (THQ system) was designed for this purpose. A Nigerian industrial standard established a desirable limit for magnesium at 0.02 mg/L in soft or energy drinks, but there is no recent WHO recommendation. Hence, this range exceeds the maximum contamination limit.

Apart from sample B8 which showed aluminum level as the acceptable target health quotient (THQ), all other samples were likely to cause aluminum intoxication when consumed frequently. These toxic metal ions are present in a concentration above the maximum contamination level and others are present in concentrations lower than the maximum contamination level. THQ value <1 indicates the acceptable health risk to human populations (WHO, 2017; Kamkara *et al.*, 2021).

CONCLUSION

This study has shown that some soft drinks in Nigeria contain heavy metals (e.g., aluminum) that exceed allowable limits. Although some of these metals serve crucial functions in human health, long-term exposure or buildup in different parts of the body can cause various diseases. Aluminum with THQ >1 is evidently the most dangerous of the five heavy metals and production processes should be closely monitored to keep the heavy metals within acceptable limits.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

BUE designed the study; SBJ and CBI carried out the experiments. SBJ and HFK analyzed the data and wrote the manuscript. The entire process was supervised by BUE, AJK, and ENV. All authors read the manuscript and approved its submission.

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REFERENCES

- Adepoju-Bello AA, Oguntibeju OO, Onuegbu MT, Ayoola GAA, Coker HAB (2012). Analysis of selected metallic impurities in the soft drinks market in Lagos, Nigeria. *African Journal of Biotechnology* 11: 4676-4680.
- Ahmed NS, el-Gendy KS, el-Refaie AK, Marzouk SA, Bakry NS, el-Sebae AH, Soliman SA (1987). Assessment of lead toxicity in traffic controllers at Alexandria, Egypt, road intersections. *Archives of Environmental Health* 42(2): 92-95.
- ATSDR (2016). Public Health Statement: Aluminum; www.atsdr.cdc.gov. Archived from the original on 12 December 2016. Retrieved 18 July 2018.
- Balali-Mood M, Riahi-Zanjani B, Mahdizadeh A, Moradi V, Fazeli-Baktiyari R (2018). Arsenic and lead

contaminations in commercial fruit juices from markets in Mashhad. *Iranian Journal of Toxicology* 12(3): 15-20.

Banks WA, Kastin AJ (1989). Aluminum-induced neurotoxicity: alterations in membrane function at the blood-brain barrier. *Neuroscience & Biobehavioral Review* 13 (1): 47-53.

British Pharmacopoeia (2005). Atomic spectrophotometry emission and absorption. Appendix IID, CD.

Chasapis CT, Loutsidou AC, Spiliopoulou CA, Stefanidou ME (2012). Zinc and human health: An update. *Archives of Toxicology* 86(4): 521-534.

Chrestensen CA, Starke DW, Mieyal JJ (2000). Acute cadmium exposure inactivates thioltransferase (Glutaredoxin), inhibits intracellular reduction of protein-glutathione-mixed disulfides, and initiates apoptosis. *The Journal of Biological Chemistry* 275: 26556-26565.

Crapper DR, Lukiw WJ, Kruck TPA (1989). New evidence for an active role of aluminum in Alzheimer's disease. *Canadian Journal of Neurological Sciences* 16 (4 Suppl): 490-497.

Darbre PD (2006). Metalloestrogens: an emerging class of inorganic xenoestrogens with the potential to add to the oestrogenic burden of the human breast. *Journal of Applied Toxicology* 26 (3): 191-197.

Dolara P (2014). Occurrence, exposure, effects, recommended intake, and possible dietary use of selected trace compounds (aluminum, bismuth, cobalt, gold, lithium, nickel, silver). *International Journal of Food Sciences and Nutrition* 65(8): 911-924.

Duffus JH (2015). Heavy metal – a meaningless term? *Pure & Applied Chemistry* 74: 793-807.

Elin RJ. (1988). Magnesium metabolism in health and disease. *Disease-a-month: DM* 34(4): 161-218. [https://doi.org/10.1016/0011-5029\(88\)90013-2](https://doi.org/10.1016/0011-5029(88)90013-2)

Eno-obong SN, Ukoha PO (2013). Tin and aluminium concentration in canned foods, drinks, and beverages sold in Nigerian markets. *Chemistry and Materials Research* 3(13): 32-40.

Ere D, Bunu SJ, Alabo CE (2020). Qualitative determination of urine iodine concentration and related intelligence quotient among high school teenagers. *European Journal Advanced Chemistry Research* 1(3): 1-5.

Ferraro PM, Taylor EN, Gambaro G, Curhan GC (2013). Soda and other beverages and the risk of kidney stones. *Clinical Journal of the American Society of Nephrology* 8 (8): 1389-1395.

Godwill EA, Jane IC, Scholastica IU, Marcellus U, Eugene AL, Gloria OA (2015). Determination of some soft drink constituents and contamination by some heavy metals in Nigeria. *Toxicology Reports* 2: 384-390. <https://doi.org/10.1016/j.toxrep.2015.01.014>

Hammer O, Harper DAT, Ryan PD (2001). PAST: Paleontological statistics software package for education and data analysis. *Paleontologia Electronica* 4(1): 9.

Iloms E, Ololade OO, Ramganes S (2018). Investigating industrial effluent impacts on municipal wastewater treatment plant in Burke. Maphanga R, van Rensburg JJ, Bogaers A, Das S (eds). Proceedings of the 2018 International Women in Science Without Borders (WISWB) – Indaba Johannesburg, 1st edn, ISBN: 978-0-620-78656-0, Pp. 87-88. https://researchspace.csir.co.za/dspace/bitstream/handle/.../Burke_20974_2018.pdf

Izah SC, Srivastav AL (2015). Level of arsenic in potable water sources in Nigeria and their potential health impacts: A review. *Journal of Environmental Treatment Techniques* 3(1):15-24.

Izah SC, Inyang IR, Angaye TCN, Okowa IP (2016). A review of heavy metal concentration and potential health implications of beverages consumed in Nigeria. *Toxics* 5(1): 1. <https://doi.org/10.3390/toxics5010001>

Jan FA, Ishaq M, Khan S, Ihsanullah I, Ahmad I, Shakirullah M (2010). A comparative study of human health risks via consumption of food crops grown on wastewater irrigated soil and relatively clean water irrigated soil. *Journal of Hazardous Materials* 179(1-3): 612-621. <https://doi.org/10.1016/j.jhazmat.2010.03.047>

Jarup L (2003). Hazard of heavy metal contamination. *British Medical Bulletin* 68(1): 167-182.

Kamkara UM, Rabiun N, Nuraddeen NG, Jamila MK, Umar MK (2021). Assessment of heavy metal concentration in vegetables dried along Funta-Danja highway Katsina State, Nigeria. *FUDMA Journal of Sciences* 5(1): 436-444.

Klotz K, Weistenhöfer W, Neff F, Hartwig A, van Thriel C, Drexler H (2017). The health effects of aluminum exposure. *Deutsches Arzteblatt International* 114(39): 653-659. <https://doi.org/10.3238/arztebl.2017.0653>

Kregiel D (2015). Health safety of soft drinks: contents, containers, and microorganisms. *Biomed Research International* 2015 (2015): 128697, <http://dx.doi.org/10.1155/2015/128697>

Magomya AM, Yebpella GG, Okpaegbe UC (2015). An assessment of metal contaminant levels in selected soft

drinks sold in Nigeria. *International Journal of Innovative Science, Engineering & Technology* 1(10): 517-522.

Malik VS, Schulze MB, Hu FB (2006). Intake of sugar-sweetened beverages and weight gain: a systematic review. *The American Journal of Clinical Nutrition* 84 (2): 274-288.

Mattila P, Suonpää K, Piironen V (2000). Functional properties of edible mushrooms. *Nutrition* 16(7-8): 694-96.

Monisha J, Tenzin T, Naresh A, Blessy BM, Krishnamurthy NB (2014). Toxicity, mechanism, and health effects of some heavy metals. *Interdisciplinary Toxicology* 7(2): 60-70.

NSW Centre for Public Health Nutrition (2009). Soft drinks weight status and health: A review. <http://www.cphn.mmb.usyd.edu.au>. Accessed 12th March 2022.

Ogundele MO (2018). Behavioural and emotional disorders in childhood: A brief overview for paediatricians. *World Journal of Clinical Pediatrics* 7(1): 9-26. <https://doi.org/10.5409/wjcp.v7.i1.9>

Otim O, Juma T, Otunnu O (2019). Assessing the health risks of consuming 'sachet' alcohol in Acoli, Uganda. *PLoS ONE* 14(2): e0212938. <https://doi.org/10.1371/journal.pone.0212938>

Pflaum T, Hausler T, Baumung C, Ackermann S, Kuballa T, Rehm J, Lachenmeier DW (2016). Carcinogenic compounds in alcoholic beverages: An update. *Archives of Toxicology* 90(10): 2349-2367. <https://doi.org/10.1007/s00204-016-1770-3>

Plum LM, Rink L, Haase H (2010). The essential toxin: Impact of zinc on human health. *International Journal of Environmental Research and Public Health* 7(4): 1342-1365. <https://doi.org/10.3390/ijerph7041342>

Quinn MJ, Sherlock JC (1990). The correspondence between UK 'action levels' for lead in blood and water. *Food Additives and Contaminants* 7: 387-424.

Rahimzadeh MR, Rahimzadeh MR, Kazemi S, Amiri RJ, Pirzadeh M, Moghadamnia AA (2022). Aluminum poisoning with emphasis on its mechanism and treatment of intoxication. *Emergency Medicine International* 2022: 1480553. <https://doi.org/10.1155/2022/1480553>

Salako SG, Adekoyeni OO, Adegbite AA, Hammed TB (2016). Determination of metals content of alcohol and non-alcoholic canned drinks consumed at Idiroko border town Ogun State Nigeria. *British Journal of Applied Science & Technology* 12(6): 1-8.

Sikalidis AK, Kelleher AH, Maykish A, Kristo AS (2020). Non-alcoholic beverages, old and novel, and their potential effects on human health, with a focus on hydration and cardiometabolic health. *Medicina* (Kaunas, Lithuania) 56(10): 490. <https://doi.org/10.3390/medicina56100490>

Singh R, Gautam N, Mishra A, Gupta R (2011). Heavy metals and living systems: An overview. *Indian Journal of Pharmacology* 43(3): 246-253. <https://doi.org/10.4103/0253-7613.81505>

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012). Heavy metal toxicity and the environment. *Experientia Supplementum* 101: 133-164. https://doi.org/10.1007/978-3-7643-8340-4_6

The United States Environmental Protection Agency (1986). Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185): 34014-34025. Available at https://www.epa.gov/sites/production/files/2014-11/documents/chem_mix_1986.Pbf. Accessed 12th March 2022.

The United States Environmental Protection Agency (1989). Risk assessment guidance for superfund Volume 1: Human Health Evaluation Manual (Part A); Office of Emergency and Remedial Response: Washington, DC. Available at https://www.epa.gov/sites/production/files/2015-09/documents/rags_a.Pbf. Accessed 12th March 2022.

The United States Environmental Protection Agency (2000). Supplementary guidance for conducting a health risk assessment of chemical mixtures. Risk Assessment Forum, Washington, DC, EPA/630/R-00/002, 2000. Available at <https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=20533>. Accessed 18th January 2022.

The United States Environmental Protection Agency (2017). Regional screening levels (RSLs)—User's Guide. Available from: <https://www.epa.gov/risk/regional-screening-levels-rsls-users-guide-june-2017>.

Ubuoh EA (2013). Analysis of metal concentrations in selected canned beers consumed in Owerri Urban, Imo State, Nigeria. *International Journal of Chemistry and Material Science*, 1(5): 90-95

World Health Organization - WHO (2017). Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum, World Health Organization, Geneva, Switzerland. Available at: <https://www.who.int/publications/i/item/9789241549950>. Accessed 2nd December, 2022.

Woyessa GW, Kassa SB, Demissie EG, Srivastava BBL (2015). Determination of the level of some trace and heavy metals in some soft drinks of Ethiopia. *International Journal of Current Research in Chemistry & Pharmaceutical Sciences*, 2: 84-88.

Xu N, Majidi V, Markesbery WR, Ehmann WD (1992). Brain aluminum in Alzheimer's disease using an improved GFAAS method. *Neurotoxicology* 13 (4): 735-743.