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## **Research Article**

# HEAVY METALS QUANTIFICATION AND CORRELATIVE CARCINOGENIC-RISKS EVALUATION IN SELECTED ENERGY DRINKS SOLD IN BAYELSA STATE USING ATOMIC ABSORPTION SPECTROSCOPIC TECHNIQUE

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### ABSTRACT

**Objective:** The study aimed to quantify the concentrations and carcinogenic-related health risks assessment of some heavy metals in selected energy drinks frequently utilized in Bayelsa State, Nigeria.

**Methods:** Eleven energy drinks samples were purchased from the general markets in Amassoma and Yenagoa, Bayelsa State, Nigeria, and were labeled D1–D11. The samples were digested using 10 ml of nitric acid at 120-150 °C, and 2 ml of Perchloric acid was added after attaining room temperature, it was digested further until a clear solution was obtained, then made up to 25 ml with distilled water. The concentration of lead, cadmium, iron, and zinc were determined and quantified using Atomic Absorption Spectrophotometry (AAS), and the health-associated risks of these metals were evaluated using the standard Target Health Quotient (THQ).

**Results:** The EDI (Estimated Daily Intake) of lead (Pd), Cadmium (Cd), iron (Fe), and zinc (Zn) was 0.130, 0.001, 0.726, and 0.193 mg/l, respectively, all were within the World Health Organization (WHO)-acceptable range. The Chronic Daily Intake (CDI) of Pd, Cd, Fe, and Zn was obtained as 0.001 to 0.010,  $5.7 \times 10^{-5}$ , 0.001 to 0.050, and 0.0001 to 0.010 mg/l respectively.

**Conclusion:** THQ for all metals analyzed was<1, the WHO acceptable limit. All the heavy metals were within acceptable THQ limits, thus posing no carcinogenic health potential risks on long-term consumption.

Keywords: Heavy metals, Carcinogenic-Risk, Energy drinks, AAS, THQ, CDI, EDI

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## INTRODUCTION

Energy drinks are in the category of functional beverages, which also include nutraceutical and sports drinks [1]. Nutraceutical beverages help to promote and enhance health, with concentrated extracts from teas, fruits, vegetables, or herbs as part of their constituent [2]. Caffeine, one of the main ingredients in most energy drinks has a similar chemical conformation with adenosine, thus it binds and acts as an adenosine receptor blocker in the brain [3]. The blockage of adenosine to the brain neurons inhibits the sleep-inducing biochemical effects of adenosine, resulting in the neurons firing [4]. Taurine (2-aminoethyl sulfonic acid) found naturally in the retina, skeletal, and cardiac muscle tissues, is another component of some energy drinks [5]. Taurine is associated with neuro-modulation, cellular membrane stability, and modulation of intracellular calcium levels [6].

Heavy metals are considered slow, deadly toxins that are bioaccumulated in plants and animal tissues through the natural biological cycle and sometimes from contaminated farm fertilizers or animal feeds [7, 8]. These metals disrupt normal physiological processes, leading to toxicity in several body organs. Their accumulation in different tissues is due to the gradual storage in the liver, kidney, or other organs after continuous exposure because of their low rate of excretion compared with their uptake [9]. Almost all heavy metals are unfriendly to the environment. Human absorbs these metals either via inhalation or ingestion (fig. 1) [7]. Heavy metals are not easily degraded [10].

Recent research has shown that long-term exposure to Pd in children may lead to reduced intellectual capacity [11]. Excess concentrations of Zn in the body could lead to nausea, fatigue, stomach pain, allergic reactions, and obesity [12]. High levels of Cd could cause liver, kidney, and bone diseases [13]. Cadmium has been linked to skeletal and kidney damage, GIT irritation, and pulmonary abnormalities [14].

Disorders in iron (Fe) metabolism cause anemia, and there is also a possibility of neurodegenerative diseases [15, 16]. The current study aimed to quantify the concentration of some heavy metals in selected energy drinks using AAS, and evaluate their correlative health risk with Target Health Quotient (THQ).

# MATERIALS AND METHODS

# Samples procurement

Eleven (11) energy drinks (labeled D1–D11) were purchased from Supermarkets in Bayelsa State. They include King\_King®, Fearless®, Supa\_Komando®, Monster®, Predator®, Lucozade\_boost®, Bullet®, Power\_horse®, Eviron®, Tiger®, and Climax®, in no particular order. Appropriate quality assurance procedures and precautions were carried out to ensure the reliability of results. The samples were completely handled to avoid any contamination. All glassware used in this work was decontaminated by washing with purified water followed by immersing for 24 h in a dilute 10% v/v nitric acid solution. Then rinsed with purified water, dried at room temperature in a dust-free environment, and stored in a clean area.

## Samples digestion and atomic absorption spectrophotometric (AAS) analysis

About 20 ml of sample and 10 ml of nitric acid were transferred into a beaker and heated for 120-150 °C on a heating mantle inside the fume cupboard. Nitric acid was added from time to time to prevent dryness. After 40 min, the mixture was allowed to cool, and 2 ml of Perchloric acid was

added to digest further until a clear solution was obtained. After cooling, it was transferred into a 25 ml volumetric flask and made up to volume using distilled water. The solution was filtered and transferred into 5 ml sample tubes for AAS for analysis.

The 2002 edition of an American standard test method reported by Bray  $et\,al.$ , (2004), was followed in the digestion procedure [17]. 50 ml of the well-mixed selected sample was measured into a 150 ml beaker. 4 ml of concentrated HN03 and 2 ml of perchloric acid were added. The solution was evaporated to near dryness (low heat) and was placed at room temperature. A few drops of concentrated HN03 were continuously added and refluxed on a hot plate until a light clear residue was obtained, indicating digestion completion. About 2 ml of concentrated HN03 was added to the residue. The end solution was filtered, insoluble materials removed, made the volume up to 25 ml with distilled water, labeled appropriately, and stored in a 125 ml polypropylene bottle before aspirating the solution with the flame before AAS analysis. Atomic Absorption Spectrometer, Model D1971/4691 was used to quantify the heavy metals concentrations in the selected energy drinks, and data were analyzed using an Excel spreadsheet, 2016.

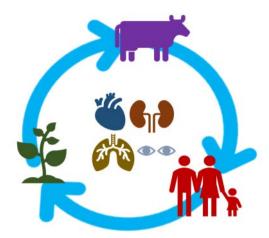


Fig. 1: Heavy metals intoxication cycle: These metals cycle in the environment is linked with the food chain as soil-plant-animal-man. Bioaccumulation affects human body organs, including the eyes, lungs, kidneys, heart, etc., [14]

## RESULTS

Table 1: Heavy metal concentrations in soft drinks sample using solar thermo elemental atomic absorption spectrometer (AAS)

Samples	The concentration of heavy metals (mg/l)				
-	Lead (Pd)	Cadmium (Cd)	Iron (Fe)	Zinc (Zn)	
D1	0.276	< 0.002	0.727	0.162	
D2	0.147	< 0.002	0.907	< 0.002	
D3	0.074	< 0.002	0.154	< 0.002	
D4	0.073	< 0.002	0.919	< 0.002	
D5	0.018	< 0.002	0.026	< 0.002	
D6	0.166	< 0.002	0.226	0.134	
D7	0.129	< 0.002	1.65	< 0.002	
D8	0.037	< 0.002	0.113	< 0.002	
D9	0.074	< 0.002	0.282	0.047	
D10	0.147	< 0.002	1.27	0.493	
D11	0.332	< 0.002	1.85	0.332	
Control (H <sub>2</sub> O)	< 0.009	< 0.002	0.026	< 0.002	
*WHO	0.01	0.003	1.0-3.0	5.00	
RfD	$3.5 \times 10^{-3}$	5 x 10-4	7 x 10 <sup>-3</sup>	3 x 10-1	

\*WHO maximum permissible limit; RfD = Reference oral Dose

Table 2: Estimated daily intake (EDI) of heavy metals in selected energy drinks (mg/l)  $\,$ 

Sample	Lead (Pd)	Cadmium (Cd)	Iron (Fe)	Zinc (Zn)
D1	0.108	0.001	0.285	0.064
D2	0.058	0.001	0.356	0.001
D3	0.029	0.001	0.060	0.001
D4	0.029	0.001	0.361	0.001
D5	0.007	0.001	0.010	0.001
D6	0.065	0.001	0.089	0.053
D7	0.051	0.001	0.647	0.001
D8	0.015	0.001	0.044	0.001
D9	0.029	0.001	0.111	0.018
D10	0.058	0.001	0.498	0.193
D11	0.130	0.001	0.726	0.130
WHO standard	3.6µg/day/kg	1μg/day/kg	20.5 mg/day	11 mg/day

EDI = Cm x Cf x Df/Bw; Cm = Metal concentration, Cf = conversion factor (0.085), Df = Daily intake of drinks (30cl), Bw = Average body (65 kg)

Sample	Lead (Pd)	Cadmium (Cd)	Iron (Fe)	Zinc (Zn)
D1	0.008	5.70E-05	0.022	0.005
D2	0.004	5.70E-05	0.027	0.000
D3	0.002	5.70E-05	0.005	0.000
D4	0.002	5.70E-05	0.028	0.000
D5	0.001	5.70E-05	0.001	0.000
D6	0.005	5.70E-05	0.007	0.004
D7	0.004	5.70E-05	0.050	0.000
D8	0.001	5.70E-05	0.003	0.000
D9	0.002	5.70E-05	0.008	0.001
D10	0.004	5.70E-05	0.038	0.015
D11	0.010	5.70E-05	0.056	0.010

CDI= Cm x Edi x EFi x IR/Bw x AT; Cm =Metal concentration, ED = Exposure duration (24 y), EFi = Exposure frequency (365 d/y), IR = Intake rate (0.03 kg/person/day), AT =Average time (365 d x number of exposure years (24 y) [8,760]).

Table 4: Target health quotient (THQ) of heavy metals in selected energy drinks

Sample	Lead (Pd)	Cadmium (Cd)	Iron (Fe)	Zinc (Zn)
D1	0.229	1.1E-01	3.143	0.0167
D2	0.114	1.1E-01	3.857	0
D3	0.057	1.1E-01	0.714	0
D4	0.057	1.1E-01	4.000	0
D5	0.029	1.1E-01	0.143	0
D6	0.143	1.1E-01	1.000	0.013
D7	0.114	1.1E-01	7.143	0
D8	0.029	1.1E-01	0.429	0
D9	0.057	1.1E-01	1.143	0.003
D10	0.114	1.1E-01	5.429	0.05
D11	0.286	1.1E-01	8.000	0.033

THQ =CDI/RFD; CDI =Chronic Daily Intake, RFD = Reference Oral Dose.

### DISCUSSION

The concentration of Pd, Cd, Fe, and Zn was recorded in selected energy drinks and analyzed using an Atomic Absorption Spectrophotometer as listed in table 1. The concentration of Lead for all the samples of energy drinks was between 0.018 to 0.276 mg/l. This level exceeds the maximum permissible level of Pd stated by the WHO (0.01 mg/l). This result is related to another report where Pd was found in high concentrations of up to 0.447 mg/l in non-alcoholic drinks [18]. Pd causes toxicity and poisoning when body tissues are exposed to it, which can lead to high blood pressure and anemia [19]. The levels of Pd seen in the samples of energy drinks is of great interest as it can lead to toxicity of Pd in the body and other disease associated with Pd intoxication.

Spectroscopic methods are useful in the quantitation of concentrations in test samples [20]. From the AAS analysis, Cd concentration in the samples was<0.002 mg/l, below the acceptable limit of 0.003 mg/l. This concentration of Cd is not likely to cause kidney damage in consumers following long-term exposure. Low levels or high of Zinc in the body are associated with the development of prostate enlargement and human cancer [21]. The concentration of Zinc in the samples was between<0.002 to 0.493 mg/l, below the permissible limit of 5.00 mg/l stated by WHO, hence not of great concern as its health benefits outweigh its toxic effects. Iron (Fe) concentration ranged from 0.026 to 1.85 mg/l, also below the acceptable limit of 3.0 mg/l as stated by WHO, hence will not have a deleterious effect on human health following long-term use by consumers.

The EDI of Pd, Cd, Fe, and Zn was estimated for all sampled energy drinks. Pd had the highest and lowest EDI of 0.130~mg/l and 0.015~mg/l respectively across the samples, which is below the WHO standard. This suggests that Pd found in the analyzed samples does not pose any threat to the health of consumers. Cd EDI was 0.001~mg/l which is below the WHO standard of  $1\mu g/day/kg$ , implying that the analyzed samples are free from Cd intoxication and therefore safe for consumption. For Fe, the EDI was between 0.010~to~0.726~mg/l, below the WHO standard, while zinc was between 0.001~to~0.193~mg/l, also below the WHO standard of EDI given as 11~mg/day. Indicating that the samples are safe for consumption.

The CDI of Pd, Cd, Fe, and Zn were estimated to be 0.001 to 0.010,  $5.7 \times 10^{-5}$ , 0.001 to 0.050, and 0.0001 to 0.010 mg/l respectively. All heavy metal concentrations analyzed were within acceptable WHO standards [22].

The THQ (Target Health Quotient) is defined as the ratio of Chronic Daily Intake to toxic elements and the Reference Oral Dose which is the highest level at which no adverse health effects are expected from the consumption of food and related products. The Reference dose is specific to the trace elements being assessed. THQ is a standard parameter that describes the non-carcinogenic health risk posed by exposure to the respective toxic element. If the THQ is<1, then non-carcinogenic health effects are not expected. If, However, a THQ value of>1, suggests the possibility that adverse health effects could be experienced [23]. All the samples had THQ<1. Another similar study was conducted in Southwest Nigeria, where different heavy metals, including Mg, Zn, Pb, As, and Al; of which all the heavy metal but Aluminum was above the recommended concentration [24].

### CONCLUSION

The THQ values obtained from the comprehensive study for the selected heavy metals (Pd, Cd, Fe, and Zn) were below 1, which is within the standard acceptable THQ levels, implying that there are no carcinogenic health hazards associated with the intake of the examined energy drinks. Because some chemicals have been linked to carcinogenic disposition after prolonged exposure, more eleborate nano-based quantification with larger sample sizes and hyphenated techniques should be performed before, during, and after the production of energy drinks to reduce health-related risks.

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#### **AUTHORS CONTRIBUTIONS**

Samuel J. Bunu: laboratory demonstrations, supervision, data analysis, manuscript draft, and review; Dorathy George: laboratory/field work, data generation, initial write-ups; Deghinmotei Alfred-Ugbenbo: data analysis, manuscript review; Benjamin U. Ebeshi: conception, supervision, manuscript review, and approval.

#### CONFLICT OF INTERESTS

There is no conflict of interest among the authors

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