Determination of Some Physicochemical Properties, Heavy Metals and Micronutrients of Some Energy Drinks Available in Nigeria

Hamza Abubakar Hamza*, Abubakar Danjuma Bajoga, Yusuf Mohammed Auwal and Hankouraou Seydou

Received: 18 January 2025/Accepted: 02 March 2025/Published: 14 March 2025 https://dx.doi.org/10.4314/cps.v12i3.19

Abstract: This study investigates the concentrations of selected elements in energy drinks and assesses their compliance with international safety standards. Energy drinks have gained widespread popularity, but concerns regarding the presence of heavy metals and their potential health risks necessitate rigorous scientific evaluation. This study aimed to determine the levels of copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and chromium (Cr) in commercially available energy drinks and evaluate their potential health implications. A total of 30 energy drink analyzed samples were using atomic absorption spectrophotometry (AAS) and energy dispersive x-ray fluorescent (EDXRF). Statistical analyses, including correlation analysis, chi-square tests, and multiple regression, were performed to identify significant relationships between elements and deviations from World Health Organization (WHO) permissible limits. The results showed that Mn had a strong positive correlation with Cu (r = 0.686, p < 0.01), while Fe and Zn exhibited no significant influence on Mn concentrations. The chi-square test revealed that some elements exceeded WHOrecommended limits, with Cu and Mn concentrations in certain samples posing potential health risks. Multiple regression analysis indicated that Cu significantly predicted Mn concentration ($\beta = 0.686$, p =0.001), explaining 48.8% of the variance ($R^2 =$ 0.488). In contrast, the regression model for Zn showed no significant predictive power ($R^2 =$ 0.018, p = 0.936), indicating weak associations between Zn and other elements in the energy drinks. These findings highlight the need for

stricter regulatory monitoring and enforcement to ensure that elemental concentrations in energy drinks remain within safe limits. Manufacturers should enhance quality control measures to prevent contamination and safeguard consumer health. Further research is recommended to explore additional factors influencing heavy metal accumulation in energy drinks, including ingredient sourcing, production methods, and storage conditions.

Keywords: Energy drinks, heavy metals,

copper, manganese, statistical analysis, health risk assessment

Hamza Abubakar Hamza

Department of pure and applied Physics, Gombe State University, P.M.B. 127, Gombe, Nigeria

Email: <u>hamzadurbi@gsu.edu.ng</u>

Orcid id: <u>https://orcid.org/0009-0005-9958-5508</u>

Abubakar Danjuma Bajoga

Department of pure and applied Physics, Gombe State University, P.M.B. 127, Gombe, Nigeria

Email: a.bajoga@gsu.edu.ng

Yusuf Mohammed Auwal

Department of Physics, Yobe State University, Damaturu, Nigeria Email: auwalgamajan@ysu.edu.ng

Hankouraou Seydou

Department of pure and applied Physics, Gombe State University, P.M.B. 127, Gombe, Nigeria

Email: seydou5k@yahoo.com

1.0 Introduction

Food is an essential component of life, providing necessary nutrients for growth, development, and overall well-being (Iweala et al., 2014). Food sources primarily originate from plants, such as fruits, vegetables, cereals, and tubers, as well as from animal products (Izah et al., 2016). Based on their preparation and consumption, foods can be broadly classified into two categories: ready-to-eat foods, which require minimal or no preparation, and foods that need further processing before consumption. Ready-to-eat foods include fruits, fruit juices, nutritional drinks, snacks, and beverages (Orutugu et al., 2015).

Beverages, a widely consumed category of ready-to-eat food products, are integral to daily dietary habits. They are sold in supermarkets, restaurants, bars, and convenience stores and are frequently served at social gatherings such as festivals, weddings, and family events. Among various beverage types, energy drinks have gained significant popularity due to their purported ability to enhance physical and mental performance.

Energy drinks are non-alcoholic beverages formulated to provide an instant energy boost and enhance cognitive alertness. They typically contain caffeine, sugar, amino acids, herbal extracts, and B vitamins, all of which are believed to contribute to increased endurance, improved concentration, and enhanced reaction time (Alford et al., 2001). The history of energy drinks dates back to 1987 when Red Bull was first introduced in Austria, later gaining widespread global acceptance in the 1990s following its entry into the United States market. Since then, the energy drink industry has expanded rapidly, with sales increasing by 80% in 2006 alone (Foran et al., 2012). This growth is driven by aggressive marketing strategies that position these beverages as essential for combating fatigue and enhancing



physical performance (Van den Eynde et al., 2008).

In Nigeria, energy drinks have become increasingly popular, particularly among young adults and adolescents. The proliferation of various brands in the market has been accompanied by claims of enhanced energy and improved mental levels alertness. However, concerns have been raised regarding the safety of these products, particularly their caffeine content, sugar levels, and the presence of potentially harmful substances such as heavy metals (Clauson et al., 2008; Kelle et al., 2022; Ogoko, 2017). Studies have reported adverse health effects associated with excessive energy consumption, including insomnia, drink nervousness, headaches. and tachycardia (Chelben et al., 2008). Additionally, some reports have linked excessive consumption to more severe health risks, such as seizures and hospitalization due to underlying mental health conditions (Ivadurai & Chung, 2007).

Despite the widespread consumption of energy drinks in Nigeria, limited studies have been conducted to evaluate their physicochemical properties, heavy metal contamination, and micronutrient composition. Previous research on energy drinks has predominantly focused on their caffeine content, sugar levels, and shortterm physiological effects. However, the potential health implications of prolonged exposure to heavy metals, such as lead (Pb), cadmium (Cd), chromium (Cr), cobalt (Co), and nickel (Ni), remain largely unexplored in the Nigerian context. Heavy metals, even at trace levels, can accumulate in the human body over time. leading to serious health complications such as neurotoxicity, organ damage, and metabolic disorders (Jomova & Valko, 2011). Additionally, micronutrients such as iron (Fe), zinc (Zn), copper (Cu), and magnesium (Mg) play crucial roles in human metabolism, and their imbalance can have detrimental effects on health (Prashanth et al., 2015).

Given these concerns, this study aims to assess the physicochemical properties, heavy metal content, and micronutrient composition of selected energy drinks available in Nigeria. The findings of this study will provide valuable insights into the safety and quality of these beverages, informing regulatory authorities and consumers about potential health risks. By bridging the existing knowledge gap, this research will contribute to ongoing discussions regarding the formulation, regulation, and public health implications of energy drinks in Nigeria and beyond.

2.0 Materials and Methods

2.1 Sample Collection

Thirty (30) brands of energy drinks, including twenty-three (23) liquid and seven (7) powdered samples, were purchased from local markets for analysis. Upon purchase, all samples were stored in a refrigerator at 4°C prior to analysis to prevent degradation.

2.2 Sample Preparation and Analysis

2.2.1 Sample Preparation for Atomic Absorption Spectroscopy (AAS)

The liquid energy drink samples were digested using a mixture of concentrated nitric acid (HNO₃) and hydrochloric acid (HCl) to decompose organic matter and release metal ions. A 10 mL aliquot of each sample was placed in an oven at 105°C to remove moisture content. The dried samples were then finely ground using a pestle and mortar to increase the surface area for digestion.

A digestion mixture of HCl and HNO₃ in a 3:1 ratio (aqua regia) was prepared. Precisely 1 g of each ground sample was weighed using an analytical balance and transferred into a digestion flask. Twenty (20) mL of the acid mixture was added to each digestion flask inside a fume hood. The digestion process was carried out on a Kjeldahl heater for 4 to 5 hours with continuous addition of acid until complete decomposition of organic matter, indicated by a pale yellow solution. After digestion, the samples were diluted with deionized water and filtered to remove particulates. The final volume was adjusted to 100 mL with deionized water. The prepared solutions were allowed to settle before analysis using a Bulk 205 Atomic *Absorption Spectrophotometer (AAS)*.

2.2.2 Sample Preparation for X-Ray Fluorescence (XRF)

For the powdered energy drink samples, 3 g of each sample was placed into a 25 mm diameter pellet mold. A transparent X-ray foil cover (polypropylene, 6 µm thick) was used to seal the pellet mold. The samples were compressed into pellets using an automatic hydraulic press. The prepared pellets were loaded into the X-ray excitation chamber of the XRF spectrometer using an automated sample changer system. A time-based irradiation program, controlled by dedicated software, was used to analyze both real samples and standard reference materials. The acquired X-ray spectra were recorded and stored for subsequent quantitative elemental analysis.

2.3 Analysis of Physical Parameters

pH Determination: The pH of the energy drink samples was measured using a digital pH meter (JENWAY 3505). Prior to measurement, the instrument was calibrated using standard buffer solutions (pH 4.0, 7.0, and 10.0).

Conductivity Determination: Conductivity was measured using a digital conductivity meter (HACH Sension 5). The meter was calibrated using a potassium chloride (KCl) standard solution before sample analysis.

Turbidity Measurement: Turbidity was determined using a digital turbidity meter (HACH DR/890 Colorimeter). The instrument was calibrated using standard turbidity solutions before measurement.

TotalDissolvedSolids(TDS)Determination:The total dissolved solids(TDS)content was measured using a digitalTDS/conductivitymeter(HACH Sension 5).



Calibration was performed using a standard solution before analysis.

3.0 Results and Discussion

3.1 Physicochemical Properties of Energy Drinks

The physicochemical properties of the analyzed energy drink samples, including pH,

turbidity, total dissolved solids (TDS), and conductivity, are presented in Table 1. These parameters were assessed to evaluate the quality and potential health implications of the selected energy drinks in comparison with WHO standards (2004).

•		-		
Table 1:	Physicoche	mical Proper	rties of Energy	y Drinks

S/N	Sample	Sample	pН	Turbidity	TDS	Conductivity
	Code	Туре		(NTU)	(mg/L)	(µS/cm)
1	SY	Liquid	4.53	132	492	1036
2	RB	Liquid	4.69	85	974	1983
3	PH	Liquid	4.73	95	786	1587
4	PW	Liquid	3.06	111	293	416
5	XC	Liquid	5.03	87	688	1420
6	HS	Liquid	2.92	110	306	440
7	3H	Liquid	4.52	107	502	718
8	WB	Liquid	4.06	81	386	543
9	BR	Liquid	3.01	68	362	521
10	HD	Liquid	3.97	108	763	1092
11	BH	Liquid	2.96	112	412	582
12	OR	Liquid	4.02	53	546	853
13	SD	Liquid	2.96	96	336	347
14	BS	Liquid	3.68	121	582	536
15	ME	Liquid	4.28	74	460	738
16	VE	Liquid	3.53	58	524	552
17	FL	Liquid	3.25	98	625	721
18	PR	Liquid	4.99	54	354	472
19	SK	Liquid	4.20	72	462	664
20	IP	Liquid	3.87	81	483	735
21	MP	Liquid	3.06	45	825	592
22	AR	Liquid	3.50	55	471	746
23	CX	Liquid	3.42	38	352	425
24	EJ	Powder	4.25	74	620	1648
25	KR	Powder	5.36	82	546	825
26	KK	Powder	4.84	88	538	748
27	PS	Powder	5.28	64	830	2170
28	PE	Powder	5.86	58	1072	2230
29	AL	Powder	4.64	8	420	887
30	ES	Powder	5.53	14	843	1752
WHO	-	-	6.5 -	≤ 5	≤ 500	≤ 400
Standard			8.5			
(2004)						



The pH of the analyzed energy drinks ranged from 2.92 to 5.86, which is significantly below the WHO standard (6.5-8.5) for potable water. This indicates that all the tested energy drinks are acidic, with some samples (HS: 2.92, BH: 2.96, BR: 3.01) being extremely acidic. The low pH values could contribute to dental erosion, gastrointestinal irritation. and metabolic imbalances when consumed excessively.

Turbidity levels varied widely among the samples, ranging from 8 NTU to 132 NTU, far exceeding the WHO limit of 5 NTU. The highest turbidity was recorded in SY (132 NTU), while the lowest was in AL (8 NTU). High turbidity in liquid samples suggests suspended particulates or undissolved compounds, which may indicate poor filtration or the presence of artificial additives.

TDS values ranged from 293 mg/L to 1072 mg/L, with 43% of samples exceeding the WHO-recommended limit of 500 mg/L. Powdered samples generally had higher TDS levels compared to liquid ones, with PE (1072 mg/L) and PS (830 mg/L) showing significantly high values. High TDS can alter taste, contribute to mineral buildup in the body, and indicate the presence of inorganic contaminants.

Conductivity, which correlates with ionic concentration, ranged from 347 μ S/cm to 2230 μ S/cm, surpassing the WHO-recommended limit (400 μ S/cm) in all cases. The highest conductivity was recorded in PE (2230 μ S/cm) and PS (2170 μ S/cm), likely due to high mineral content. Elevated conductivity in beverages suggests a high presence of dissolved ions, which could impact electrolyte balance in consumers.

The low pH values observed in the energy drink samples indicate potential health risks, including tooth enamel erosion and gastrointestinal irritation. To mitigate these risks, it is recommended that manufacturers adjust their formulations to reduce acidity.

The high turbidity levels detected in some samples may suggest the presence of suspended particles or inadequate ingredient dissolution, which could negatively impact consumer perception. Improving filtration processes could enhance clarity and help ensure compliance with WHO standards.

The elevated levels of total dissolved solids (TDS) and conductivity observed in several samples indicate a high concentration of dissolved salts and possible contamination with inorganic substances. Continuous monitoring and reformulation are necessary to maintain an appropriate mineral balance and ensure consumer safety.

Overall, the analyzed energy drink samples exhibited low pH, high turbidity, excessive TDS, and elevated conductivity, all of which deviate from WHO standards. These findings emphasize the need for improved formulation strategies and stricter regulatory controls to enhance the safety and quality of energy drinks. The concentration of heavy metals in energy drinks was analyzed to assess potential contamination and associated health risks. Table 2 presents the levels of cobalt (Co), chromium (Cr), cadmium (Cd), arsenic (As), nickel (Ni), and lead (Pb) detected in both liquid and powdered energy drink samples. These results provide insight into the safety and quality of energy drinks concerning heavy metal content.

S/N	Sample Code	Sample Type	Со	Cr	Cd	As	Ni	Pb
1	SY	Liquid	0.0215	0.0641	ND	0.0023	ND	0.1393
2	RB	Liquid	ND	0.0463	0.0127	0.0004	0.0875	0.0451

Table 2: Concentration of Heavy Metals in Energy Drinks (mg/L)



3	PH	Liquid	0.0054	ND	0.0055	ND	0.0136	ND
4	PW	Liquid	ND	0.0175	0.0131	ND	0.0652	ND
5	XC	Liquid	ND	ND	ND	ND	ND	0.0545
6	HS	Liquid	ND	0.0983	0.0155	0.0002	0.0156	ND
7	3H	Liquid	0.0053	0.0125	0.0125	0.0016	0.0557	0.0825
8	WB	Liquid	ND	0.0253	0.0116	ND	0.0982	ND
9	BR	Liquid	ND	0.0493	0.0162	ND	0.0365	ND
10	HD	Liquid	ND	0.0473	0.0084	0.0037	0.0625	ND
11	BH	Liquid	0.0052	0.2563	0.0182	ND	0.0478	ND
12	OR	Liquid	ND	0.0263	0.0198	ND	0.0984	ND
13	SD	Liquid	0.0084	0.0672	0.0282	ND	0.0794	ND
14	BS	Liquid	0.0182	0.0854	0.0145	0.0042	0.0432	0.0615
15	ME	Liquid	0.0029	0.0323	0.0074	0.0028	0.0608	ND
16	VE	Liquid	0.0165	ND	ND	0.0011	0.0064	ND
17	FL	Liquid	0.0027	0.0063	ND	0.0021	0.0942	ND
18	PR	Liquid	0.0826	0.0113	0.0106	ND	0.0451	0.0451
19	SK	Liquid	ND	0.0046	ND	0.0012	0.0075	ND
20	IP	Liquid	0.0162	0.0296	0.0015	ND	0.0516	ND
21	MP	Liquid	0.0017	0.4159	0.0566	0.0056	0.0062	ND
22	AR	Liquid	ND	0.0291	0.0037	0.0014	0.0015	ND
23	CX	Liquid	0.0126	ND	ND	0.0036	0.0183	ND
24	EJ	Powder	0.0835	ND	0.0128	0.0451	0.0624	0.2092
25	KR	Powder	0.0534	0.3764	ND	0.0316	0.0046	0.1754
26	KK	Powder	0.0175	0.0265	0.0052	ND	0.0249	0.0154
27	PS	Powder	0.0263	ND	0.0074	ND	0.0573	ND
28	PE	Powder	ND	0.0034	ND	0.0012	0.0632	0.1225
29	AL	Powder	0.0041	0.0025	ND	0.0025	0.0432	0.0832
30	ES	Powder	ND	0.2501	0.0183	0.0063	0.0473	ND
WHO	-	-	0.05	0.05	0.003	0.01	0.07	0.01
Limit								

**Results are expressed as Mean. ND: Not Detected

The presence of heavy metals in energy drinks raises concerns regarding consumer safety. Cobalt was detected in multiple samples, with the highest concentration found in the powdered sample EJ (0.0835 mg/L), exceeding the WHO limit of 0.05 mg/L. Chromium was present in several samples, with particularly high levels in MP (0.4159 mg/L) and KR (0.3764 exceeding mg/L), far the recommended limit of 0.05 mg/L. While chromium (III) is essential for human metabolism, excessive levels, especially of chromium (VI), pose carcinogenic risks.

Cadmium, which is known for its toxicity and bioaccumulation potential, was detected in several samples, with the highest level recorded in MP (0.0566 mg/L), surpassing the permissible limit of 0.003 mg/L. Long-term cadmium exposure can cause kidney damage and skeletal disorders. Arsenic was found in multiple samples, with EJ (0.0451 mg/L) and KR (0.0316 mg/L) showing the highest levels, close to or exceeding the WHO limit of 0.01 mg/L. Chronic arsenic exposure is linked to cardiovascular diseases and cancer.

Nickel was detected in most samples, with concentrations ranging from 0.0015 mg/L (AR)



to 0.0942 mg/L (FL). While nickel is an essential trace element, excessive intake can result in allergic reactions and respiratory issues. Lead, a highly toxic heavy metal, was detected in several samples, with the highest concentration found in EJ (0.2092 mg/L) and KR (0.1754 mg/L), far exceeding the WHO permissible limit of 0.01 mg/L. Lead exposure, particularly in children, is associated with neurological impairments, reduced cognitive function, and cardiovascular diseases.

These results indicate significant contamination of certain energy drink samples with heavy metals, raising concerns about their potential health impacts. Similar studies have reported varying levels of heavy metals in energy drinks, with some exceeding regulatory limits. The contamination could arise from raw materials, processing equipment, or packaging Regulatory materials. agencies must implement stricter monitoring and control measures to ensure compliance with safety standards.

Overall, these findings emphasize the need for manufacturers to adopt better quality control practices, including sourcing raw materials from safer sources and implementing efficient filtration processes. Routine monitoring of

heavy metal content in energy drinks is essentialm to ensure consumer safety and prevent long-term health risks associated with heavy metal exposure.

3.3 Concentration of Micronutrients in Energy Drinks

Table 3 presents the concentration of essential micronutrients (Cu, Fe, Mn, and Zn) in various energy drinks analyzed. These micronutrients play vital roles in human metabolism, enzymatic functions, and overall physiological well-being. The results are expressed in mg/L, and any non-detected (ND) values indicate concentrations below the detection limit of the analytical method employed.

S/N	Sample Code	Sample Type	Cu	Fe	Mn	Zn
1	SY	Liquid	0.0720	1.9616	ND	13.8875
2	RB	Liquid	0.0845	0.9812	0.0195	0.0527
3	PH	Liquid	0.0175	0.0096	0.0245	0.0117
4	PW	Liquid	0.0613	5.7042	0.0426	0.0785
5	XC	Liquid	0.0027	0.4183	ND	0.0492
6	HS	Liquid	0.0421	1.5666	0.0327	6.5448
7	3H	Liquid	0.0852	1.8725	0.0213	0.0725
8	WB	Liquid	0.1036	1.3252	0.0257	0.4542
9	BR	Liquid	0.0723	2.0897	0.0354	0.0618
10	HD	Liquid	0.0832	1.0625	0.0393	2.7614
11	BH	Liquid	0.0659	2.2791	0.0274	0.0713
12	OR	Liquid	0.1038	3.1862	0.0284	0.4547
13	SD	Liquid	0.0825	4.5159	0.0252	0.2351
14	BS	Liquid	0.0736	1.5756	0.0163	0.0492
15	ME	Liquid	0.7338	0.0622	0.5432	0.0471
16	VE	Liquid	0.0212	0.0176	0.0043	0.0165
17	FL	Liquid	0.0818	0.0155	ND	0.0245
18	PR	Liquid	0.2161	0.3043	0.8442	0.0109

 Table 3: Concentration of micronutrients in energy drinks (mg/L)



19	SK	Liquid	0.0818	0.0133	0.0727	0.0104	
20	IP	Liquid	0.7931	0.5275	0.2649	0.0933	
21	MP	Liquid	0.5013	0.4725	0.6333	0.0726	
22	AR	Liquid	0.0174	0.0532	0.0408	0.0186	
23	CX	Liquid	0.0045	0.0126	0.0114	0.0329	
24	EJ	Powder	0.1027	0.5442	0.0245	0.3523	
25	KR	Powder	0.5841	0.3861	0.6218	0.7421	
26	KK	Powder	0.3105	0.0671	0.4126	0.0561	
27	PS	Powder	0.0514	0.1642	0.4662	0.1754	
28	PE	Powder	0.0181	0.3288	0.0157	0.6437	
29	AL	Powder	0.0415	0.7991	0.0073	0.1726	
30	ES	Powder	0.0652	2.2797	0.0272	0.0718	

The results indicate varying concentrations of micronutrients in the energy drinks analyzed. Copper (Cu) concentrations range from 0.0027 to 0.7931 mg/L, with IP showing the highest concentration. This is within the permissible limit of 2 mg/L set by WHO for drinking water. Iron (Fe) levels fluctuate significantly, with PW having the highest content (5.7042 mg/L). Some values exceed the WHO guideline limit of 2 mg/L, indicating a potential concern for iron overload in certain samples.

Manganese (Mn) is present in most samples except SY, XC, and FL, with the highest concentration in PR (0.8442)mg/L). Manganese intake above 0.4 mg/L may pose health risks, especially for individuals with compromised liver function. Zinc (Zn) concentrations are highest in SY (13.8875 mg/L), surpassing the WHO-recommended limit of 5 mg/L for drinking water. The elevated Zn levels in certain samples may pose particularly excessive health risks, in consumption.

Compared to literature values, similar studies on energy drinks report wide variations in metal content depending on brand formulation, water sources, and fortification strategies. Some of the elevated concentrations observed in this study may be due to ingredient sourcing, contamination from processing equipment, or intentional fortification. This study highlights the significant variability in micronutrient concentrations in energy drinks. While most values remain within safe limits, elevated Fe and Zn in some samples could have implications for long-term consumption. control Regular monitoring and quality are recommended to measures ensure compliance with health and safety standards.

3.4 Statistical analysis

Table 4 presenting the results of the independent t-test for the heavy metal and micronutrient concentrations in liquid and powder energy drink samples.

Element	t-value	p-value	Interpretation
Cobalt (Co)	-1.42	0.197	No significant difference between liquid
			and powder samples
Chromium (Cr)	-0.59	0.571	No significant difference between liquid
			and powder samples
Copper (Cu)	-0.22	0.832	No significant difference between liquid
			and powder samples

 Table 4: Independent t-test Results for Heavy Metals and Micronutrients in Energy Drinks



Iron (Fe)	1.53	0.140	No significant difference between liquid
			and powder samples

The statistical analysis reveals that there is no significant difference (p > 0.05) in the concentrations of cobalt, chromium, copper, and iron between liquid and powder energy drink samples. This suggests that both formulations contain similar levels of these elements, potentially due to consistent ingredient sourcing and manufacturing processes.

Comparing with literature, heavy metal concentrations in beverages have been linked to raw material quality, packaging, and processing methods. Studies have shown that excessive levels of these metals can pose health risks, particularly lead and cadmium, which were also analyzed in the study. The absence of significant differences between the two sample types implies that regulatory compliance and formulation consistency may be maintained across different energy drink types. However, continuous monitoring is necessary to ensure that all metal concentrations remain within permissible limits set by WHO and other regulatory bodies.



Fig. 2: Bar Chart of Average Iron (Fe) Concentration in Energy Drinks





Fig. 3: Bar Chart of Average chromium concentration in Energy Drinks



Fig. 4: Bar Chart of Average manganese Concentration in Energy Drinks



Fig. 5: Bar Chart of Average zinc Concentration in Energy Drinks



The boxplot for copper (Cu) concentration in energy drinks (Fig. 1) provides an overview of the distribution of Cu levels across the analyzed samples. The interquartile range (IQR) represents the middle 50% of values, while the whiskers extend to the minimum and maximum detected concentrations. Outliers, if present, indicate samples with significantly higher or lower Cu levels. The variability in Cu content among different samples suggests differences in ingredient composition and manufacturing processes. Comparing these levels with regulatory standards will help assess compliance and potential health risks.

The bar chart of Fe concentration illustrates the mean values across different energy drink samples (Fig. 2). Iron is an essential micronutrient, but excessive levels can pose health risks, including oxidative stress. Variations in Fe content may be due to the source of raw materials or fortification practices. The comparison with literature and standard values will indicate whether the detected levels are within the permissible limits set by regulatory bodies.

Chromium (Cr) is an essential trace element that supports glucose metabolism but is toxic in high concentrations. The bar chart (Fig. 3) shows the average Cr concentration in different energy drink samples, revealing variations among brands. Some samples exhibit nondetectable Cr levels, while others have measurable amounts. The presence of Cr might originate from contamination during processing or ingredient sources. The comparison with standard values will determine if the levels are safe for consumption.

Manganese (Mn) is another vital micronutrient, crucial for enzyme function and bone development. The bar chart (Fig. 4) illustrates the differences in Mn concentrations among energy drink samples. While some brands contain minimal amounts, others have significant levels. Elevated Mn intake can lead



to neurotoxic effects, making it essential to compare detected values with regulatory limits. The observed differences might stem from variations in ingredient composition or production methods.

Zinc (Zn) is a critical nutrient involved in immune function and metabolism. The bar chart (Fig. 5) shows the mean Zn levels in energy drinks, highlighting the variation in Zn content across different samples. Some energy drinks appear to be good Zn sources, while others contain minimal or negligible amounts. The variation could result from differences in fortification practices or ingredient composition. Assessing the Zn levels against standard dietary recommendations will help determine if these drinks contribute significantly to daily intake.

The statistical visualizations highlight the disparities in heavy metal and micronutrient concentrations among energy drinks. Some elements are present in trace amounts, while others show substantial variations. Comparing these findings with literature and standard values is essential for evaluating their safety and nutritional significance. Further statistical tests, such as ANOVA, correlation analysis, or regression models, could provide deeper insights into the factors influencing these variations.

The correlation matrix presented in Fig. 6 provides insights into the relationships between different heavy metals and micronutrients in energy drinks. A strong positive correlation is observed between iron (Fe) and manganese (Mn), suggesting that these elements may originate from similar sources or undergo similar processing pathways in the formulation of the beverages. Copper (Cu) and zinc (Zn) also exhibit a moderate positive correlation, which could be linked to their shared roles as essential trace elements and common presence in dietary supplements. Conversely, a negative correlation is noted between lead (Pb) and some essential micronutrients, indicating that elevated levels of lead may be associated with lower concentrations of beneficial elements. This trend could be due to competitive absorption mechanisms or differential contamination sources. The correlation analysis provides valuable insights into the compositional patterns of energy drinks, supporting the need for regulatory monitoring and ensuring that heavy metal levels remain within permissible limits. Comparisons with literature values further highlight the importance of assessing the potential health risks associated with excessive exposure to toxic metals while maintaining adequate levels of essential micronutrients.



Fig. 6: Correlation matrix for the investigated metals

Chi-square analysis was also performed to compare the observed elemental contents with WHO expected standard level using the following equation

$$\chi^2 = \frac{(O-E)^2}{E}$$
(1)

where O is the observed value and E is the expected (WHO limit). Table 5 presents the chi-square test results comparing the observed mean concentrations of copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) in energy drinks with the expected values based on WHO permissible limits. The chi-square test assesses whether the observed values significantly deviate from the expected values, providing

insights into potential risks associated with the consumption of these drinks.

Table	5:	Chi	-Square	Test	Resi	ılts	for
Micror	nutri	ient	Concent	rations	in	En	ergy
Drinks							

Element	Observed Mean (mg/L)	Expected (WHO Limit mg/L)
Cu	0.1422	2.0
Fe	1.3583	0.3
Mn	0.1213	0.4
Zn	1.0037	3.0

The chi-square value obtained from the analysis is 6.98, with a corresponding p-value of 0.0725. This indicates that the difference



between the observed and expected values is not statistically significant at the conventional 0.05 level, though the deviation suggests a potential trend at a less stringent confidence level of 90%. Iron shows the highest deviation, as the observed mean of 1.3583 mg/L is considerably higher than the WHO permissible limit of 0.3 mg/L. This suggests that some energy drinks may contain iron levels that exceed recommended safety limits, which could have implications for consumers with conditions such as hemochromatosis or those who already have high dietary iron intake. Copper and manganese exhibit concentrations well below the WHO threshold, indicating no immediate concern for excessive intake. Zinc levels are also below the expected limit, but the difference is not as pronounced as in the case of Cu and Mn.

The findings suggest that while most micronutrient levels in the energy drinks analyzed do not pose a significant health risk. the elevated iron concentrations warrant further investigation. Long-term exposure to excessive iron intake through frequent consumption of these drinks could lead to potential health oxidative effects. including stress and Continuous gastrointestinal distress. monitoring and regulation of micronutrient levels in commercially available energy drinks remain essential to ensure consumer safety.

regression The multiple analysis was conducted using Iron (Fe) concentration as the dependent variable and Copper (Cu). Manganese (Mn), and Zinc (Zn) concentrations as independent variables. The model yielded an R-squared value of 0.122, indicating that only 12.2% of the variability in Fe concentration is explained by the independent variables.

The regression equation is:

Fe = 1.5205 - 0.0921(Cu) -

 $1.9697(Mn) + 0.0059(Zn) \tag{2}$

The p-values for Cu (0.957), Mn (0.213), and Zn (0.978) suggest that none of the independent variables significantly predict Fe concentration



Snapshot 1 shows results obtained from multiple regression analysis. The multiple analysis regression was performed to determine the relationship between Manganese (Mn) concentration in energy drinks and the independent variables Copper (Cu), Iron (Fe), and Zinc (Zn) concentrations. The model yielded an R-squared value of 0.488, indicating that approximately 48.8% of the variability in Mn concentration can be explained by the combined influence of Cu, Fe, and Zn. The adjusted R-squared (0.422)suggests moderate model fit after accounting for the number of predictors.

The F-statistic (7.317) and its associated p-value (0.00129) indicate that the regression model is statistically significant, meaning that at least one of the predictors has a significant impact on Mn concentration.

Examining individual coefficients, Cu (β = 0.6861, p = 0.001) shows a strong, significant positive relationship with Mn, suggesting that as Cu concentration increases, Mn concentration also increases. However, Fe (β = -0.0338, p = 0.213) and Zn (β = -0.0088, p = 0.756) do not show significant relationships with Mn, implying that their variations do not significantly influence Mn concentration in energy drinks.

The Durbin-Watson statistic (2.273) suggests no serious autocorrelation issues in the residuals. However, the Jarque-Bera test (p < 0.0000001) indicates that the residuals deviate from normality, which could suggest the need for further model improvements, such as



transformation of variables or inclusion of additional predictors.

The East Format View Help				
	OLS Regres	ssion Results		
				=====
Dep. Variable:	Mn	R-squared:		0.488
Model:	OLS	Adj. R-squared:		0.422
Method:	Least Squares	F-statistic:		7.317
Date:	Fri. 28 Mar 2025	<pre>Prob (F-statistic):</pre>	0.	00129
Time:	20:30:58	log-likelihood:	g	. 0682
No. Observations:	27	ATC:	_	10.14
Df Residuals:	23	BIC	-	4 953
Df Model:		510.		4.555
Covariance Type:	nonrobust			
coef	std err	t P> t	[0.025 0	.9751
const 0.0927	0.061	1.513 0.144	-0.034	0.219
Cu 0.6861	0.172	3.994 0.001	0.331	1.041
Fe -0.0338	· 0.026 -	-1.281 0.213	-0.088	0.021
Zn -0.0088	0.028 -	-0.315 0.756	-0.067	0.049
				=====
Omnibus:	21.254	Durbin-Watson:		2.273
Prob(Omnibus):	0.000	Jarque-Bera (JB):	3	3.082
Skew:	1.685	Prob(JB):	6.5	5e-08
Kurtosis:	7.249	Cond. No.		10.0

Snapshot 1

regression The multiple analysis (was conducted to examine the relationship between Zinc (Zn) concentration in energy drinks and the independent variables Copper (Cu), Iron (Fe), and Manganese (Mn) concentrations. The model yielded an R-squared value of 0.018, meaning that only 1.8% of the variability in Zn concentration is explained by the combined influence of Cu, Fe, and Mn. The adjusted Rsquared (-0.110) suggests that the model does not adequately fit the data, indicating that the inclusion of these predictors does not meaningfully explain variations in Zn concentration.

The F-statistic (0.1392) and its associated pvalue (0.936) indicate that the overall regression model is not statistically significant, implying that none of the independent variables have a strong effect on Zn concentration.

Examining the individual coefficients, Cu (β = -0.3096, p = 0.854), Fe (β = 0.0056, p = 0.978),

and Mn (β = -0.4878, p = 0.756) all have high p-values, indicating that their relationships with Zn are not statistically significant. This suggests that variations in Cu, Fe, and Mn do not significantly predict Zn concentration in energy drinks.

The Durbin-Watson statistic (2.285) indicates no significant autocorrelation in the residuals. However, the Jarque-Bera test (p < 2.28e-64) shows a severe departure from normality, with high skewness (3.805) and kurtosis (17.234). This suggests that the residuals are not normally distributed, which may indicate issues with the model, such as outliers or a nonlinear relationship.

Overall, these results suggest that Zn concentration in energy drinks is not significantly influenced by Cu, Fe, or Mn. The low R-squared value and high p-values indicate that the selected predictors do not explain Zn variability. This suggests that other factors, such as the source of ingredients, production



processes, or contamination pathways, may have a more substantial impact on Zn levels in energy drinks. A different set of predictors or a non-linear modeling approach may be needed

for better understanding the factors affecting Zn concentration.

		OLS	Regre	ssion R	esults		
Dep. Variable: Model: Method: Date: Time: No. Observation Df Residuals: Df Model:		Least S Fri, 28 Ma 20	Zn OLS quares r 2025 :30:58 27 23 3	R-sq Adj. F-st Prob Log- AIC: BIC:	uared: R-squared: atistic: (F-statistic Likelihood:	.):	0.018 -0.110 0.1392 0.936 -45.118 98.24 103.4
Covariance Type	e:	non	robust				
	coef	std er	r	t	P> t	[0.025	0.975]
const Cu Fe Mn	0.6171 -0.3096 0.0056 -0.4878	0.46 1.66 0.20 1.54	0 2 3 8	1.340 -0.186 0.028 -0.315	0.193 0.854 0.978 0.756	-0.335 -3.748 -0.415 -3.690	1.570 3.129 0.426 2.715
Omnibus: Prob(Omnibus): Skew: Kurtosis:			52.627 0.000 3.805 17.234	Durb Jarqı Prob Cond	in-Watson: ue-Bera (JB): (JB): . No.		2.285 293.079 2.28e-64 15.2

Notes:

[1] Standard Errors assume that the covariance matrix of the errors is correctly \le Snapshot 2

5.0 Conclusion

The findings from this study reveal that the concentrations of various elements in energy drinks vary significantly, with certain elements showing statistically significant relationships. The regression analysis indicates that copper has a strong positive effect on manganese concentration, while iron and zinc do not significantly influence manganese levels. The model explains a moderate proportion of the variance, suggesting that additional factors may contribute to manganese concentration beyond the variables considered. The chisquare analysis comparing observed element concentrations with WHO standards shows that some elements exceed permissible limits, indicating potential health risks associated with consumption. Correlation analysis highlights significant associations between certain elements, suggesting possible interactions in the composition of the energy drinks.

The study concludes elemental that concentrations in energy drinks should be monitored to ensure consumer safety. The presence of elements exceeding WHO standards suggests the need for stricter regulatory oversight and quality control measures in the production and distribution of these beverages. The findings also underscore the importance of understanding how different elements interact within these products, as such interactions could influence their overall safety and nutritional value.

Based on the results, it is recommended that regulatory agencies enforce stricter monitoring of heavy metal concentrations in energy drinks to ensure compliance with international safety standards. Manufacturers should implement improved quality control measures to prevent contamination and ensure that element concentrations remain within safe limits. Further research should explore additional



factors influencing element concentrations, such as ingredient sources, processing methods, and storage conditions, to provide a more comprehensive understanding of their impact on consumer health.

5.0 References

- Abou-Arab, A. A. K, Abou -Arab M. A., & Abu-Salam F. M. (2017). Metal content in energy drinks: A review and analysis. *Journal of food and Nutrition Research*, 5, 2, pp. 1-13.
- Adeleke, R. O., & Abiodun, O. A. (2010).
 Physico-chemical Properties of Commercial Local Beverages in Osun State, Nigeria. *Pakistan Journal of Nutrition* 9, 9., pp. 853-855.
- Anna M. T., Rachel A B., & Michael P. W. (2019). Assessment of heavy metal contamination in energy drinks. *Food Additives & Contaminants*: Part B, 12,1, pp. 34-41.
- Ashurst, P. R. (2005). Chemistry and Technology of Soft drinks and Fruit Juices.
 2nd Edn., Vol. III (4) Blackwell Scientific Publication, London, pp: 433.
- Alford C., Cox H., & Wescott R. (2001). The Effects of Red Bull Energy Drink on Human Performance and Mood", *Amino Acids*, 21, pp. 139-150. <u>http://dx.doi.org/</u> 10.1007/s007260170021.
- Al-Mayaly K. I. (2013). Determination of heavy Metals in some Artificial Fruit juices in IraqiLocal Markets. *Intern. Journal. Of Research and Dev. In Pharm. And Life Sci.* 2, 4, pp. 507-510.
- Bassiouny, M. A. & Yang, J. (2005). Influence of drinking patterns of carbonated beverages on dental erosion. *General Dentistry*, 53, pp. 207-210.
- Bengol, M., Yentur, G., Er B., & Oktem, A. B. (2010). Determination of some heavy metal levels in soft drinks from Turkey using ICP/OES method. *Czech J. Food Sci.*, 28, pp. 213–216.

- Chelben, J., Piccone-Sapir, A., Ianco, I., Shoenfeld, N., Kotler, M. & Strous, R. D. (2008).Effects of amino acid energy drinks leading to hospitalization in individuals with mental illness. *Gen. Hosp. Psychiatry*, 30, 2, pp. 187–189.
- Clauson K. A., Shields K. M., McQueen C. E., & Persad N. (2008). Safety issues associated with commercially available energy drinks. J. Am. Pharm. Assoc. 48, 3, pp. 55–63.
- David J. C., Mark A. B. & Jennifer A. S., (2020). Heavy metal contamination in energy drinks: An analysis of 100 products. *Journal of Environmental Health*, 82, 2, pp. 8-15.
- Duffus, J. H. (2002). Heavy metall a meaningless term? *Pure Appl Chem* 74, pp. 793–807.
- Foran, M., Strickland F., Perkins, K. & Smith, J. A. (2012). Excessive Intraoperative bleeding with Chronic Energy Drink Consumption. *Journal of Oral & Maxillofacial Surgery*, 70, 6, pp. 1439-1441.
- Goyal S. K., Gupta V. K., and Singh A. K., (2023). Assessment of heavy metals in energy drinks marked in India. *Journal of Environmental Science and Health*, part B, 58, pp. 151-158.
- Institute of Medicine (2001). Food and Nutrition board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, DC: National Academy Press. Pp 7-30.
- Iweala, E. E. J., Olugbuyiro, J. A. O., Durodola, B. M., Fubara-Manuel, D. R., & Okoli, A.O. (2014). Metal contamination of foods and drinks consumed in Ota, Nigeria. *Res. J. Environ. Toxicol*, 8, pp. 92–97.



- Iyadurai, S. J. & Chung, S. S. (2007). Newonset seizures in adults: possible association with consumption of popular energy drinks. *Epilepsy Behav.*, 10, 3, pp. 504–508.
- Izah, S. C., Kigigha, L. T. & Anene, E.K. (2016). Bacteriological Quality Assessment of Malus domestica Borkh and Cucumis sativus L. in Yenagoa Metropolis, Bayelsa state, Nigeria. *Br. J. Appl. Res.*, 1, pp. 5–7.
- Izah, S.C., Orutugu, L.A., Kigigha, L.T. (2015). A review of the quality assessment of zobo drink consumed in Nigeria. ASIO J. Microbiol. Food Sci. Biotechnol. Innov. 1, pp. 34–44.
- Jain A. K., Jain S. A., & Sharma R. K. (2023) Heavy metal contamination in energy drinks: A comparative study of different brands. *Journal of Food and Beverage* toxicology, 5, 2, pp. 2: 1-8.
- Kannan S. S., Kuamr S., & Rathore R. S. (2015). Determination of heavy metals in energy drinks by inductively coupled plasma mass spectrometry. *Journal of Food Science and Technology*, 52, pp. 4, pp. 2314-2320.
- Kelle, H. I., Ogoko, E. C., Nduka, J. K., Udeozo, P. I., & Ubani, M. C. (2022). Health risk assessment of heavy metal exposures through edible clay from southeastern and south-southern Nigeria. *Pacific Journal of Science and Technology*, 23, 1, pp. 113-123.
- Kim J. M., Lee S. H., & Kim Y. C., (2024). Heavy metal analysis of energy drinks consumed in the United State. *Food and Chemical Toxicology*, 149, pp. 113-231.
- Krejpcio, Z., Sionkowski, S., & Bartela, J. (2005). Safety of fresh fruits and juices available on the Polish market as determined by heavy metal residues. *Polish Journal of Environmental Studies*, 14, pp. 877–81.

- Maduabuchi, J. M. U., Nzegwu, C. N., Adıgba,
 E. O., Aloke, R. U., Ezomike, C. N.,
 Okocha, C. E., Obi, E., & Orisakwe, O. E.
 (2006). Lead and cadmium exposures from
 canned and non-canned beverages in
 Nigeria: A public health concern. *Science Total of Environment*, 366, pp. 621–626.
- Maurice, S. M. (2010). *The soft drinks companion*: A technical handbook for the beverage industry, CRC Press. p. 45.
- Marschner, H. (2015). *Mineral nutrition of higher plants*. Oxford University Press, London. Pp. 13-20.
- Ministry of Agriculture, Fisheries and Food (MAFF) (2017). *Metals and other elements in beverages*. Food Surveillance Information Sheet. Geneva. vol. 165.
- Mohamed A. E., Ahmad M. A., & Hoda A. E. (2020). Assessment of heavy metals in energy drinks and their potential health risks. *Journal of Food and Nutrition Research*, 8, 2, pp. 1-9.
- Mohammed, S. G., Al-Hashimi, A. G. & Al-Hussainy, K. S. (2012). Determination of Caffeine and Trace Minerals Contents in Soft and Energy Drinks Available in Basrah Markets. *Pakistan Journal of Nutrition* 11, 9, pp. 747-750.
- Nurudeen, S. A. (2014). Evaluation of some chemical Constituents of selected energy drinksunpublished master's dissertation, Department of Chemistry Ahmadu Bello University, Zaria Nigeria.
- Obuzor, G. U. & Ajaezi, N. E. (2010). Nutritional content of popular malt drinks produced in Nigeria. *African Journal of Food Science* Vol. 4(9), pp. 585 – 590.
- Ogoko, E. C. (2017). Physicochemical properties and heavy metal concentration of groundwater in Owerri Metropolis, Nigeria. *Current Journal of Applied Science and Technology*, 23, 1, pp. 1-10.
- Onianwa, P. C., Adetol, I. G., Iwebue, C. M. A., Oto, M. F. & Tella, O. O. (1999). Trace



heavy metals composition of some Nigerian beverages and food drinks. *Food Chemistry*, 66, pp. 275-279.

- Orutugu, L.A., Izah, S. C., &Aseibai, E.R. (2015). Microbiological quality of Kunu drink sold in some major markets of Yenagoa Metropolis, Nigeria. *Cont. J. Biomed. Sci.* 9, pp.9–16.
- Pflaum, T., Hausler, T., Baumung, C., Ackermann, S., Kuballa, T., Rehm J., & Lachenmeier, D. W. (2016). Carcinogenic compounds in alcoholic beverages: An update. *Archives of Toxicology*, 90, 10, pp. 2349-2367. https://doi.org/10.1007/ s00204-016-1770-3.
- Prasad, A. S. (2022). Zinc: an overview. *Nutrition*; 11, pp. 93-99.
- Ryan-Harshman, M., & Aldoori, W. (2005). Health benefits of selected minerals, *Can. Fam Physician.* 51, 5, pp. 673–675.
- Salako, S.G., Adekoyeni, O. O., Adegbite, A.
 A., & Hammed, T. B. (2016).
 Determination of Metals Content of Alcohol and Non-alcoholic Canned Drinks Consumed at Idiroko Border Town Ogun State Nigeria. *Br. J. Appl. Sci. Technol.*, 12, pp. 1–8.
- Samuel J. B., Benjamin U. E., Hilda F. K., Adesegun J. K, Edebi N. V., & Chubiyojo B. I., (2023). Atomic Absorption Spectroscopic (AAS) Analysis of Heavy Metals and Health Risks Assessment of Common some Energy Drinks. Pharmacology and Toxicology of Natural Medicines. 3. 1. 1-10. pp. doi: 10.52406/ptnm.v1i1.55
- Sharma S. S., Sigh A. K., & Sharma R. K., (2024). Assessment of heavy metals in energy drinks and their impact on human health. *Journal of Food Science and Technology*, 61, 3, pp. 849-857.
- Sibel U., Ozlem D. & Mustafa S. (2018). Determination of heavy metals in energy dinks by ICP-MS. *Journal of Food Science and Technology*, 55, 4, pp. 1058-1064.

- Van den Eeden S., K., Koepsell, T.D., Longstreth, W.T.Jr., van Belle G., Daling J. R. & McKnight B. (2018). "Aspartame ingestion and headaches: A randomized crossover trial", *Neurology*, 44, pp. 1787– 1793.
- Victor F. M., Juan F. G., & Maria C. R. (2019). Heavy metal contamination in energy drinks: Apublic health concern *Journal of Environmental Health*, 81, 6, pp. 8-14.
- World Health Organization (WHO), (2011). *Guidelines for drinking water quality, 4th Edition.* World Health Organization, Geneva, 2011, pp. 564.

Compliance with Ethical Standards Declaration

- **Ethical Approval**
- Not Applicable
- **Competing interests**

The authors declare no known competing financial interests

Data Availability

Data shall be made available on request

Conflict of Interest

The authors declare no conflict of interest

Ethical Considerations

This research adhered to ethical guidelines, ensuring that all data collection and analysis procedures complied with environmental and scientific research standards.

Funding

The authors declared no external source of funding.

Authors' Contributions

The authors declare that the article was jointly written by the authors for the publication of this paper.

