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Evaluation the level of Cu, Zn, Fe, and Pb in instant coffee available in Iraqi market

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ABSTRACT

Coffee is one of the most consumed beverages in the world. It contains many micronutrients, such as zinc, manganese, copper, and iron, but may also contain toxic metals like cadmium and lead, which have harmful effects as a result of their accumulation. The aim of this study is to evaluate the levels of some trace elements (Cu, Zn, Fe, and Pb) in instant coffee available in Iraqi markets and to assess the potential health risks associated with their consumption. This study included sixteen commercial brands of instant coffee samples (one sample per brand, with three replicates per sample) collected from Iraqi markets between July and October 2025. Element levels were estimated using a Shimadzu AA-646 atomic absorption spectrophotometer (AAS) . All samples were prepared in triplicate and results are reported as mean ± standard deviation. The levels of Cu, Zn, Fe, and Pb ranged between (1.00–1.9) µg/g, (7.81–10.3) µg/g, (37.8–44.6) µg/g, and (0.008–0.018) µg/g, respectively. All concentrations of these elements, except iron, were within the permissible limits established by Iraqi Standard Specifications (No. 1447, 1999) and international guidelines (WHO/FAO). The estimated daily intake (EDI), target hazard quotient (THQ), and cancer risk (CR) for Pb indicated no significant non-carcinogenic or carcinogenic risks for consumers at typical consumption levels. However, the elevated iron levels in all samples warrant further investigation.

1-Introduction

Coffee is one of the most consumed beverages around the world, due to its taste and flavor [1]. Coffee also contains nutrients and antioxidants, including fats, carbohydrates, vitamins, minerals, caffeine, etc. [2]. Conventionally, coffee is shipped for use as either whole beans or ground coffee [3,4]. Instant coffee is made by the extraction, evaporation, and drying of ground coffee to produce a solid, dry substance [5,6]. Numerous studies indicated that consuming approximately 2–4 cups of coffee daily leads to a reduced risk of death, liver damage, colorectal cancer, cirrhosis, type 2 diabetes, and cardiovascular disease [7,8]. Food contamination with metals is a global health concern, especially in agriculturally polluted areas. Estimates indicate that more than 13% of arable land and 40% of freshwater bodies are contaminated with metals such as cadmium, zinc, copper, nickel, cobalt, chromium, arsenic, and lead [9]. This contamination results from the overuse of phosphate fertilizers and inadequate irrigation [10]. Even small concentrations of these metals can accumulate in body tissues, leading to neurotoxicity, kidney damage, and an increased risk of cancer [11,12]. In addition, instant coffee contains higher concentrations of minerals such as zinc, calcium, magnesium, manganese, iron, chromium, aluminum, and nickel, often due to production methods [13,14]. The levels of these elements depend on the coffee bean origin, soil composition, agricultural practices, processing conditions, and packaging materials. For public health, the population should be educated about the benefits and risks of instant coffee. Further research on the effects of instant coffee on human health is essential to safeguarding public health and maximizing the health benefits of coffee. The aim of this study is to evaluate the levels of some trace elements (Cu, Zn, Fe, Pb) in instant coffee available in the Iraqi market and to assess the potential health risks associated with their consumption.

2-Materials and Methods

Sixteen commercial brands of instant coffee were collected between July and October 2025 from various markets in Baghdad and other governorates of Iraq. All samples were collected in their original sealed packaging to ensure integrity. Their country of manufacture and coded data are listed in Table (1). For each brand, one sample package was purchased, and three independent sub-samples were taken from the same package for analysis, each analyzed in triplicate (total n=9 measurements per brand).

Sample Preparation and Digestion: A wet digestion method was employed according to [15] with slight modifications. After sample collection, 0.5 g of each instant coffee sample was accurately weighed into a 100 mL borosilicate glass beaker. The sample was digested by adding 5 mL of concentrated nitric acid (65% HNO_3) and heating the beaker on a hot plate at 90–100°C until brown oxides evaporated. Then, 5 mL of concentrated hydrochloric acid (37% HCl) was added, and the mixture was heated until the volume was reduced to approximately half. The digested sample was allowed to cool, filtered through Whatman No. 42 filter paper, and quantitatively transferred to a 100 mL volumetric flask, made up to volume with deionized distilled water. The digested samples were stored in acid-washed polyethylene bottles at 4°C until analysis. A blank solution was prepared using the same digestion procedure without the coffee sample.

Instrumentation and Analytical Conditions: Cu, Zn, Fe, and Pb were analyzed using a Shimadzu AA-646 atomic absorption spectrophotometer (AAS) (Japan) equipped with hollow cathode lamps specific to each element. The wavelengths used for Cu, Zn, Fe, and Pb were 324.8 nm, 213.9 nm, 248.3 nm, and 217.0 nm, respectively. The detection limits (LOD) and quantification limits (LOQ) for each element were determined from the calibration curves and were as follows: Cu (LOD: 0.01 µg/g, LOQ: 0.03 µg/g), Zn (LOD: 0.005 µg/g, LOQ: 0.015 µg/g), Fe (LOD: 0.02 µg/g, LOQ: 0.06 µg/g), and Pb (LOD: 0.002 µg/g, LOQ: 0.006 µg/g). Calibration curves were prepared using standard solutions of each element (Merck, Germany) with concentrations ranging from 0.1 to 10 µg/mL, with correlation coefficients (R^2) > 0.995 for all elements.

Quality Control and Validation: Certified reference material (CRM) IAEA-336 (Lichen) was used to validate the digestion and analytical procedures. Recovery percentages for Cu, Zn, Fe, and Pb were in the range of 92–105%, which is within the acceptable range (80–120%) for trace element analysis. All sample preparations and measurements were performed in triplicate, and results are expressed as mean ± standard deviation (SD).

Health Risk Assessment: The estimated daily intake (EDI) of each metal was calculated based on the average consumption of instant coffee (approximately 2 cups per day, equivalent to 4 g of instant coffee powder). The EDI (μg/kg body weight/day) was calculated using the formula: EDI = (C × IR)/BW, where C is the metal concentration (μg/g), IR is the ingestion rate (g/person/day), and BW is the average body weight (70 kg for an adult). The target hazard quotient (THQ) for non-carcinogenic risk was calculated as THQ = EDI/RfD, where RfD is the oral reference dose (Cu: 40 μg/kg/day, Zn: 300 μg/kg/day, Fe: 700 μg/kg/day, Pb: 3.6 μg/kg/day). A THQ value < 1 indicates no significant non-carcinogenic risk. The cancer risk (CR) for lead was calculated as CR = EDI × CSF, where CSF is the cancer slope factor for Pb $(0.0085\ mg/kg/day)^{-1}$. A CR value $< 1.0 \times 10^{-6}$ indicates acceptable cancer risk, while values between 1.0×10^{-6} and 1.0×10^{-4} indicate possible risk.

Statistical Analysis: All data were analyzed using SPSS Statistics software (version 26). Results are expressed as mean ± standard deviation (SD) for three independent replicates. One-way ANOVA was performed to test for significant differences between brands, followed by Duncan's multiple range test for post-hoc comparisons at $P < 0.05$. Pearson's correlation analysis was conducted to evaluate the relationships between different metal concentrations. Differences with $P < 0.05$ were considered statistically significant.

Table (1): types of instant coffee manufacture country and coded.

no.	type of instant coffee	made in	sample code
1	Torabika premium (2 in 1)	Indonesia	A
2	café crown	Istanbul	B
3	cobizco	Malaysia	C
4	cobizco (3 in 1)	Malaysia	D
5	santos	Indonesia	E
6	coffee break	Jordan	F
7	top cappuccino	Indonesia	G
8	Alicafe (black cold)	Malaysia	H
9	nescafe (gold)	Switzerland	I
10	nescafe (glassic)	Switzerland	J
11	nescafe (3 in 1)	Switzerland	K
12	mahmood coffee (3 in 1)	Turkey	L
13	good day	Indonesia	M
14	al dhiafa coffee	Iraq	N
15	Brazilian class	Jordan	O
16	al ameed (2 in 1)	Jordan	P

3-Results and Discussion

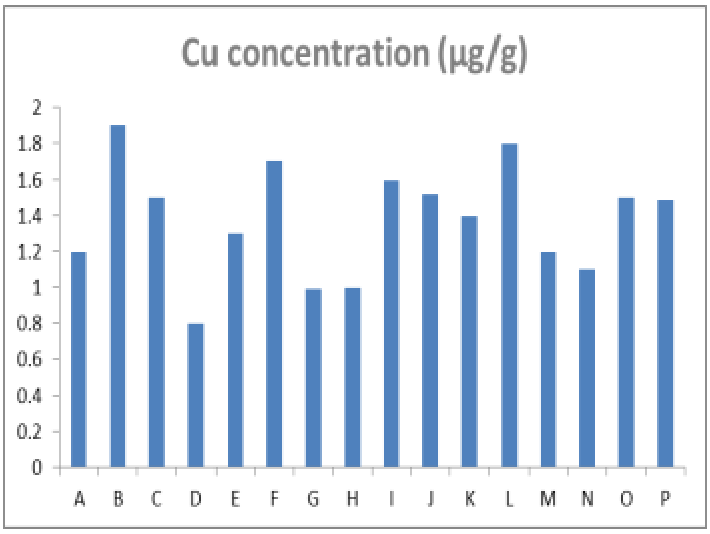
The concentrations (mean ± SD) of the analyzed elements (Cu, Zn, Fe, and Pb) are shown in Table (2). One-way ANOVA revealed significant differences ($P < 0.05$) between brands for all elements analyzed.

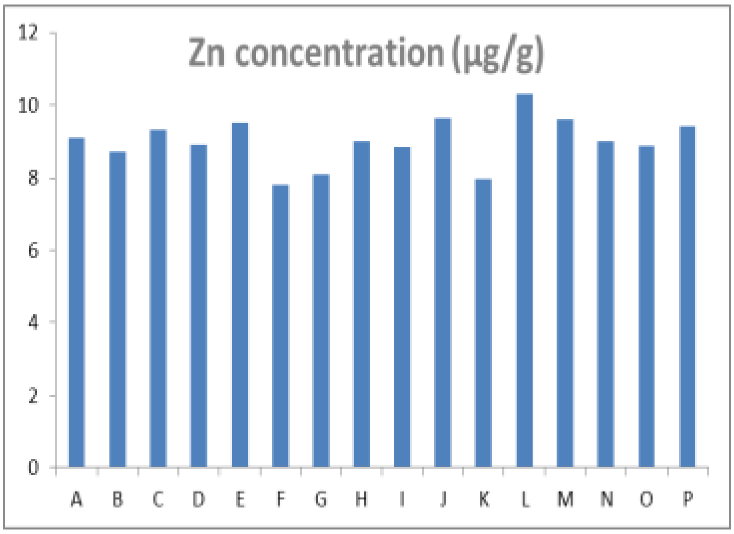
Table (2): Concentration of Cu, Zn, Fe, and Pb (mean ± SD) in instant coffee samples (μg/g, n=3).

Sample Code	Cu (μg/g)	Zn (μg/g)	Fe (μg/g)	Pb (μg/g)

A	1.2 ± 0.1 ^{cd}	9.1 ± 0.7 ^{bcd}	40.2 ± 2.5 ^{bcd}	0.014 ± 0.002 ^{ab}
B	1.9 ± 0.2 ^{a}	8.7 ± 0.4 ^{cde}	41.5 ± 2.6 ^{abc}	0.009 ± 0.001 ^{cde}
C	1.5 ± 0.2 ^{b}	9.3 ± 0.8 ^{abc}	40.7 ± 1.9 ^{bcd}	0.010 ± 0.003 ^{bcd}
D	0.8 ± 0.1 ^{f}	8.9 ± 0.6 ^{bcde}	39.8 ± 1.8 ^{cd}	0.008 ± 0.002 ^{de}
E	1.3 ± 0.2 ^{c}	9.5 ± 0.8 ^{ab}	42.1 ± 2.3 ^{abc}	0.010 ± 0.003 ^{bcd}
F	1.7 ± 0.2 ^{ab}	7.8 ± 0.3 ^{f}	38.9 ± 1.7 ^{d}	0.010 ± 0.004 ^{bcd}
G	0.99 ± 0.2 ^{de}	8.1 ± 0.1 ^{ef}	43.1 ± 3.1 ^{ab}	0.012 ± 0.002 ^{abc}
H	1.00 ± 0.2 ^{de}	9.0 ± 0.7 ^{bcd}	42.8 ± 2.6 ^{ab}	0.015 ± 0.003 ^{a}
I	1.6 ± 0.2 ^{ab}	8.8 ± 0.4 ^{bcde}	41.3 ± 2.1 ^{abc}	0.017 ± 0.001 ^{a}
J	1.5 ± 0.2 ^{b}	9.6 ± 0.9 ^{ab}	42.5 ± 2.5 ^{ab}	0.010 ± 0.002 ^{bcd}
K	1.4 ± 0.2 ^{bc}	7.9 ± 0.3 ^{ef}	42.8 ± 2.6 ^{ab}	0.018 ± 0.003 ^{a}
L	1.8 ± 0.2 ^{a}	10.3 ± 0.9 ^{a}	40.7 ± 1.9 ^{bcd}	0.015 ± 0.001 ^{a}
M	1.2 ± 0.2 ^{cd}	9.6 ± 0.9 ^{ab}	37.8 ± 1.9 ^{d}	0.011 ± 0.003 ^{bcd}
N	1.10 ± 0.1 ^{cde}	8.9 ± 0.8 ^{bcde}	43.2 ± 2.8 ^{ab}	0.009 ± 0.004 ^{cde}
O	1.50 ± 0.2 ^{b}	8.8 ± 0.7 ^{bcde}	44.6 ± 2.9 ^{a}	0.010 ± 0.010 ^{bcd}
P	1.49 ± 0.2 ^{b}	9.4 ± 0.8 ^{abc}	43.3 ± 2.9 ^{ab}	0.010 ± 0.002 ^{bcd}
Iraqi Standard Limit	**≤ 2.0**	**≤ 15.0**	**≤ 15.0**	**≤ 0.02**
P-value (ANOVA)	**<0.05**	**<0.05**	**<0.05**	**<0.05**

Different letters (a-f) in the same column indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.





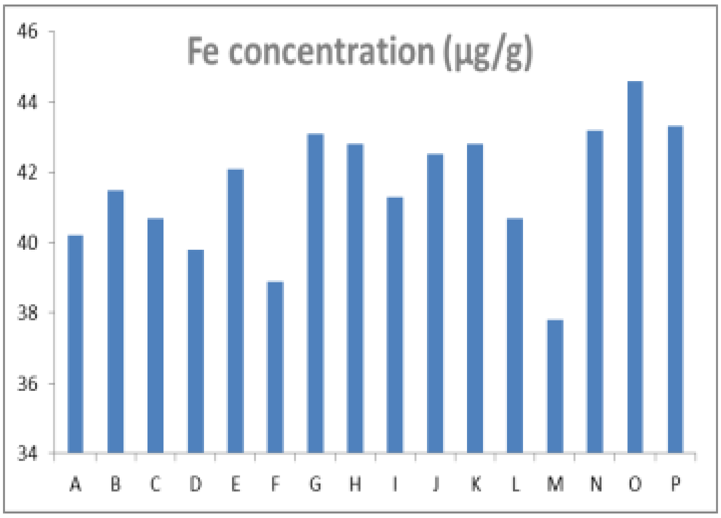


Figure (3): concentration of Fe in instant coffee

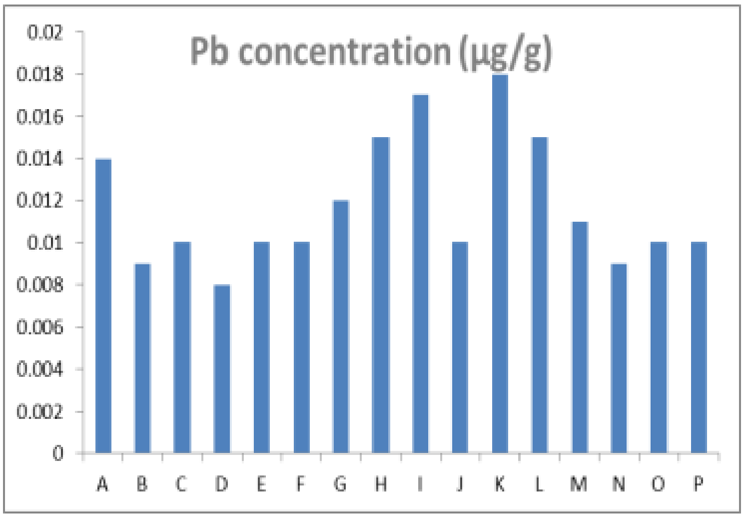


Figure (4): concentration of Pb in instant coffee

Copper (Cu): The level of copper ranged between (1.00–1.9) µg/g as shown in Table (2) and Fig (1). Copper is essential for enzyme function, energy production, maintaining healthy blood vessels, bone formation, iron fixation, noradrenaline production, and adrenaline formation [16]. Considering these concentration levels, it can be said that instant coffee B (Café Crown) is the richest in copper (1.9 ± 0.2 µg/g), while instant coffee D (Cobizco 3 in 1) is the poorest in copper concentration (0.8 ± 0.1 µg/g). These results fall within the Iraqi Standard Specifications No. 1447 (1999) [17] (≤ 2.0 µg/g) and also comply with WHO/FAO guidelines. The variation in copper levels between brands may be attributed to differences in coffee bean origin, soil composition, and processing conditions.

Zinc (Zn): Zinc is important in maintaining the immune and reproductive systems health [18]. From Table (2) and Fig (2), the highest concentration of Zn was in brand L (Mahmood Coffee, 10.3 ± 0.9 µg/g) while the lowest was in instant coffee F (Coffee Break, 7.81 ± 0.34 µg/g). These results fall within Iraqi Standard Specifications No. 1447 (1999) (≤ 15.0 µg/g). The observed variations may be due to differences in the zinc content of the soils where coffee beans were cultivated, as zinc availability in soil is influenced by pH, organic matter, and microbial activity.

Iron (Fe): Iron is a microelement involved in the process of binding, transporting, and releasing oxygen [19]. Iron deficiency can cause anemia and other health problems, while excess iron can cause vomiting, poisoning, stomach discomfort, and nausea [20]. The iron concentration ranged between (37.8–44.6) µg/g. The richest instant coffee with iron was brand O (Brazilian Class, 44.6 ± 2.9 µg/g) while the lowest one was brand M (Good Day, 37.8 ± 1.9 µg/g), as shown in Table (2) and Fig (3). All iron concentrations were significantly higher than the permissible limit of Iraqi Standard Specifications (≤ 15.0 µg/g). The wide distribution of Fe in the soil and air pollution with this metal may be the reason for the variation in Fe content. Additionally, iron contamination may arise from processing equipment, packaging materials, or the water used in the instant coffee manufacturing process. It should be noted that iron is an essential nutrient, and the WHO/FAO does not have a specific maximum limit for iron in coffee, as it is not considered a toxic element at the levels found in this study. However, the high levels compared to the Iraqi standard warrant further investigation.

Lead (Pb): Lead is a heavy metal characterized by its toxicity [21]. The concentration of Pb in coffee ranged between 0.008 and 0.018 µg/g as shown in Table (2) and Fig (4). All lead concentrations in instant coffee samples were well below the permissible limit of Iraqi Standard Specifications (≤ 0.02 µg/g) and also comply with European Commission regulations (EC No. 1881/2006, which sets a maximum limit of 0.02 µg/g for lead in coffee). The production process for instant coffee differs slightly from that of ground coffee. After roasting, extraction, and grinding, the next step is concentration to obtain a dry powder. Therefore, the concentration of the elements present depends on the initial composition of the coffee beans. Additionally, some minerals are more water-soluble than others, and some form compounds with the matrix, reducing their extractability [22]. The level of elements in the final instant coffee beverage is affected by the same factors that influence the coffee beans themselves, such as the water used, the brewing method, the coffee-to-water ratio, and the roasting and grinding processes [23]. Research indicates that lead concentrations are low in all types of instant coffee, and consuming it in moderation does not significantly affect health.

Comparison with Other Studies: The results of this study are consistent with findings from other countries. For example, studies from Turkey [10] reported lead levels in instant coffee ranging from 0.005–0.015 µg/g, similar to our findings. Studies from Brazil [20] and Iran [12] reported zinc and copper levels within similar ranges. However, the iron levels observed in our study (37.8–44.6 µg/g) are higher than those reported in some European studies [22,23], which may be attributed to differences in soil composition, processing methods, or potential contamination during packaging. These comparisons highlight the need for region-specific monitoring of trace elements in food products.

Health Risk Assessment:

The estimated daily intake (EDI), target hazard quotient (THQ), and cancer risk (CR) for lead were calculated based on an average consumption of 4 g of instant coffee powder per day (equivalent to approximately 2 cups). The results are presented in Table (3).

Table (3): Health risk assessment parameters for the analyzed elements in instant coffee.

Element	EDI (µg/kg/day)	RfD (µg/kg/day)	THQ	CR
Cu	**0.057–0.108**	**40**	**0.0014–0.0027**	—
Zn	**0.446–0.589**	**300**	**0.0015–0.0020**	—
Fe	**2.160–2.549**	**700**	**0.0031–0.0036**	—
Pb	**0.00046–0.00103**	**3.6**	**0.00013–0.00029**	**3.9×10^{-9}–8.7×10^{-9}**

The EDI values for all elements were well below their respective oral reference doses (RfD). The THQ values for all elements were less than 1.0, indicating no significant non-carcinogenic risk from the consumption of instant coffee at typical levels. The cancer risk for lead ranged from 3.9×10^{-9} to 8.7×10^{-9}, which is well below the acceptable threshold of 1.0×10^{-6}, indicating no significant carcinogenic risk from lead exposure through instant coffee consumption.

Correlation Analysis: Pearson's correlation analysis (Table 4) revealed significant positive correlations between Cu and Pb ($r = 0.52$, $P < 0.05$), and between Zn and Pb ($r = 0.48$, $P < 0.05$), suggesting that these metals may originate from similar sources or have similar geochemical behavior. No significant correlations were found between Fe and other elements, indicating that iron contamination may arise from different sources, such as processing equipment or packaging.

Table (4): Pearson's correlation coefficients between metal concentrations in instant coffee samples.

Element	Cu	Zn	Fe	Pb
Cu	1.00			
Zn	**0.32**	1.00		
Fe	**0.18**	**0.22**	1.00	
Pb	**0.52***	**0.48***	**0.15**	1.00

Correlation is significant at $P < 0.05$ (two-tailed).

4-Conclusion

This study evaluated the levels of some trace elements (Cu, Zn, Fe, and Pb) in sixteen commercial brands of instant coffee gathered from Iraqi markets. All the concentrations analyzed were within permissible limits except for iron, which exceeded the Iraqi Standard Specifications limit ($\leq$ 15.0 µg/g) in all samples. The estimated daily intake (EDI), target hazard quotient (THQ), and cancer risk (CR) calculations indicated that the consumption of instant coffee at typical levels does not pose significant non-carcinogenic or carcinogenic health risks to Iraqi consumers. However, the elevated iron levels in all brands suggest a need for further investigation into the sources of iron contamination in instant coffee production and processing. It is recommended that Iraqi regulatory authorities establish up-to-date maximum permissible limits for essential trace elements in instant coffee, based on international guidelines and regional consumption patterns. Future research should include larger sample sizes representing different batches and production dates, the use of more advanced techniques such as ICP-MS for multi-element analysis, and assessment of the bioavailability of these trace elements from coffee consumption. Additionally, regular monitoring programs should be implemented to ensure the safety and quality of instant coffee products available in the Iraqi market.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

All data supporting the findings of this study are available within the manuscript.

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