



Determination of Heavy Metals in Selected Types of Local and Imported Tea from Iraqi Markets

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Keywords: Heavy Metals; Toxicity; Black Tea; Green Tea; EDX; Atomic Absorption.

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Abstract:

The presence of heavy metals in plants, including tea plants, is influenced by factors such as the plant's origin, geographic location, soil geochemistry, and environmental contaminants in soil, water, and air. Elevated concentrations of heavy metals pose serious health risks to humans, including the potential for various cancers and damage to vital organs such as the liver, kidneys, and brain. In this study, seven tea samples were collected from the Iraqi market to examine their safety for consumption, indicated as T1-T7. The selection of the samples is based on a questionnaire answered by 140 individuals; T1-T6 samples are black tea, and T7 is green tea. Although the primary objective of this research is to determine the heavy metal content and toxicity of all the tea samples, the secondary one is to assess whether the drying process affects the levels of heavy metals by comparing green and black tea. The analysis is conducted on the samples via the Energy-Dispersive X-ray (EDX) and atomic absorption spectroscopy (AAS). The EDX results showed the presence of S, K, Al, and P in the T1 sample, while K, Al, and Mg

were identified in the T2 sample, S, K, Al, and P in T3, only K in T4, K and Fe in T5, K, P, and S in T6, and Only K in T7. The results obtained from the AAS showed that all samples have normal concentrations of Fe and Zn but not for Ni (in samples T1, T2, and T3) and manganese (in samples T1, T2, T4, T5, T6, and T7). Those detected concentrations are higher than the permissible levels, and their levels are within the toxic limits according to the standard limits set by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). However, the other heavy metals (Cd, Cu, Pb, Cr, and Co) were either not detected or were below the detection limits of AAS, indicating that these samples are free from the mentioned toxic heavy metals.

Keywords: Heavy Metals; Toxicity; Black Tea; Green Tea; EDX; Atomic Absorption

(Immediately after the abstract, provide 5-7 keywords and arrange them alphabetically, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes).

تحديد العناصر الثقيلة في أنواع مختارة من الشاي المحلي والمستورد من الأسواق العراقية

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الخلاصة:

يتأثر وجود المعادن الثقيلة في النباتات، بما في ذلك نباتات الشاي، بعوامل عدة مثل أصل النبات، والموقع الجغرافي، والكيمياء الجيولوجية للتربة، والملوثات البيئية في التربة والمياه والهواء. وتشكل التراكيز المرتفعة من هذه المعادن مخاطر صحية جسيمة على الإنسان، بما في ذلك احتمال الإصابة بأنواع مختلفة من السرطان وتلف الأعضاء الحيوية مثل الكبد والكلى والدماغ. في هذه الدراسة، تم جمع سبع عينات من الشاي من السوق العراقية لفحص مدى سلامتها للاستهلاك، أُشير إليها بالرموز T1 إلى T7، وتم اختيار العينات بناءً على استبيان شمل ١٤٠ فردًا، حيث كانت العينات T1-T6 من الشاي الأسود، بينما كانت العينة T7 من الشاي الأخضر. يهدف البحث إلى تحديد محتوى المعادن الثقيلة وسمية جميع العينات،

مع تقييم تأثير عملية التجفيف على مستويات المعادن الثقيلة من خلال مقارنة الشاي الأسود بالشاي الأخضر. تم تحليل العينات باستخدام تقنية الأشعة السينية للطاقة المشتتة (EDX) وتقنية مطيافية الامتصاص الذري (AAS)، حيث أظهرت نتائج تحليل EDX وجود عناصر مثل الكبريت والبوتاسيوم والألمنيوم والفوسفور في العينة T1، وعناصر البوتاسيوم والألمنيوم والمغنيسيوم في العينة T2، وعناصر الكبريت والبوتاسيوم والألمنيوم والفوسفور في العينة T3، والبوتاسيوم فقط في العينة T4، وعناصر البوتاسيوم والحديد في العينة T5، وعناصر البوتاسيوم والفوسفور والكبريت في العينة T6، والبوتاسيوم فقط في العينة T7. أما نتائج تحليل AAS، فقد أظهرت أن جميع العينات تحتوي على تراكيز طبيعية من الحديد والزنك، بينما تجاوزت تراكيز النيكل في العينات T1 وT2 وT3، وتراكيز المنغنيز في العينات T1 وT2 وT4 وT5 وT6 وT7 الحدود المسموح بها وفقاً للمعايير التي وضعتها منظمة الصحة العالمية (WHO) ومنظمة الأغذية والزراعة (FAO)، مما يشير إلى وجود مستويات سامة من هذه المعادن. ومع ذلك، لم تُكشف المعادن الثقيلة الأخرى (الكاديوم، النحاس، الرصاص، الكروم، الكوبالت) أو كانت تراكيزها أقل من حدود الكشف باستخدام تقنية AAS، مما يدل على أن هذه العينات خالية من المعادن الثقيلة السامة المذكورة.

الكلمات المفتاحية: المعادن الثقيلة، السمية، الشاي الأسود، الشاي الأخضر، EDX، الامتصاص الذري.

1. Introduction:

Camellia Sinensis, globally renowned as the most popular botanical source of tea, is cherished for both its flavor and reputed therapeutic benefits [1]. Tea drinking has deep historical roots, dating back approximately five millennia, as evidenced by ancient tales from China and India [2]. In Iraq, tea holds significant cultural significance, being enthusiastically embraced and routinely savored across various occasions and gatherings. Particularly, Iraqi Kurds exhibit a remarkable fondness for tea; the annual consumption rate is around 1.5 kg per person, positioning the Kurdistan region among the world's top four areas, alongside Turkey, Ireland, and the United Kingdom [3, 4]. Traditionally, tea has been imbibed for its purported abilities to enhance circulation, detoxify the body, and bolster resistance against illnesses [5].

Tea originates from the leaves of the Camellia Sinensis shrub [6], which thrive in regions characterized by high humidity, moderate temperatures, acidic soils, and spanning from sea level to high-altitude mountains [7,8]. While both black and green teas are derived from the same plant, their distinct appearance, flavor, and aroma are remarkably different. This is attributed to the variations in the dryness process during tea production. This process imbues teas with floral, spicy, or fruity notes, resulting in discernible differences between black and green teas based on their processing methods and oxidation levels [9]. Nonetheless, it's important to note that tea leaves are prone to absorbing heavy metals (HMs) throughout their lifecycle, from cultivation to packaging and transportation, potentially leading to increased metal exposure in consumers [10]. Among the common heavy metals that tea leaves may

accumulate are Cadmium (Cd), Copper (Cu), Lead (Pb), Chromium (Cr), Nickel (Ni), Iron (Fe), Manganese (Mn), mercury (Hg), Zinc (Zn), and Cobalt (Co). [11].

Heavy metals are natural elements characterized by their relatively high atomic mass and density; they play integral roles in the food chain, various human activities, and consumption patterns. Heavy metals are characterized by their relatively high atomic mass and density, occurring naturally in varying concentrations across the Earth's crust. Typically defined by a density of at least 5 g/cm³, HMs can also be distinguished by an atomic mass greater than 23 or an atomic number surpassing 20 [12]. In biological systems, heavy metals are classified as essential or nonessential. In contrast, essential heavy metals, such as Mn, Fe, Cu, and Zn, are vital for living organisms and are required in trace amounts. Contrarily, nonessential heavy metals, like Cd, Pb, and Hg, are toxic and lack biological significance [13-15]. The accumulation of HMs in tea plants is influenced by various factors, including the plant's origin, geographic location, soil geochemistry, and environmental contaminants present in soil, water, and air [16, 17]. Soil contamination sources, such as industrial activities, pesticide and fertilizer usage, and untreated wastewater, also contribute to elevated levels of HMs. Consequently, tea plants absorb these metals from the soil, potentially leading to high concentrations in edible parts, such as leaves [18-20]. While naturally occurring in soil, mining and smelting activities can exacerbate contamination levels. Here, tea plants, with their ability to uptake HMs from the soil, may transfer certain amounts to the leaves that are ultimately used in tea production [21-23].

2. Material and methods:

2-1 Sample Selection:

The sample selection method was based on the availability of products in the Iraqi market. However, three critical factors were considered while selecting the samples. Firstly, the product's popularity was considered, which ensured that only commonly used products were included in the study. Thus, a questionnaire was created and distributed online to individuals from varying economic backgrounds, and over 140 responses were obtained. This approach helped to establish a clear understanding of the popularity of the products within the target population and aided in the selection of products for the study. **Figure 1** shows the results of the public questionnaire.

The second factor was the geographical location, which played a crucial role in ensuring a diverse range of products was collected. Finally, the price of the product was also considered to ensure that a range of products, from low to high prices, were included in the study. The combination of these three factors helped to provide a diverse range of samples that were

representative of the Iraqi market. Furthermore, one of the samples was green tea instead of black tea for broader exploration.

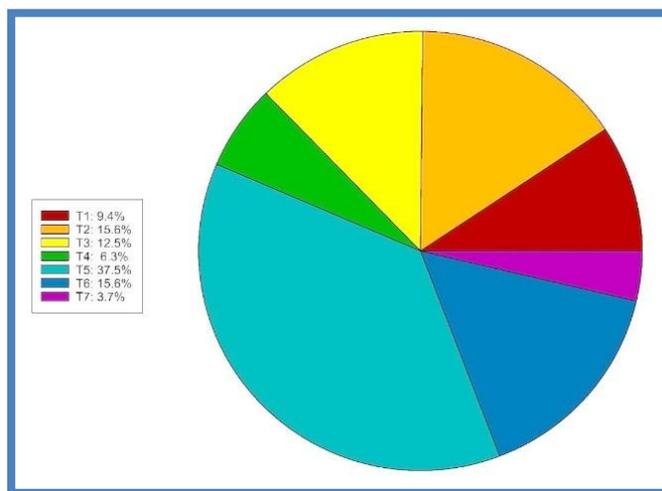


Figure 1: The public questionnaire results of selected samples.

2-2 Reagents and Chemicals

2-2-1 Reagents Used in Acid Digestion: Sample preparation involved analytical-grade nitric acid (65%, Sigma Aldrich) and hydrochloric acid (37%, Sigma Aldrich) [24, 25].

2-2-2 Reagents Used in Atomic Absorption: All the solutions were prepared using deionized water [26, 27]. Standard solutions for calibration of Mg, Zn, K, Cu, Cr, Ni, Co, Cd, Pb, and Fe were prepared from 1000 mg/L (ppm) Standard Stock Solution of GFS Fishers' AAS Reference Standard. Dilution correction was applied for samples diluted or concentrated during analysis.

2-3 Samples Drying

The tea samples, each weighing 1 g, were individually placed in watch glasses. Subsequently, they were dried in an electrical oven at 100°C overnight. Hence, they were ensured to be completely dry and ready for use in acid digestion and for Energy-Dispersive X-ray (EDX) spectroscopy analysis.

2-4 EDX Analysis

An EDX of Bruker model XFlash6110 was used to analyze the chemical characterization/elemental analysis of materials.

2-5 Acid Digestion Method

A 0.5 g of the selected dry sample was placed separately into 200 ml beakers, then 15 ml of 65% HNO₃ and 10 mL of 37% HCl were added [24, 25, 28]. The contents were mixed by stirring thoroughly and heated on a hot plate. The heating continued until the dissolution of the content, and the digested sample was then filtered using Whatman filter paper No.41. The

filtration solution was diluted with deionized water to 100 ml. The resulting solution was used for the spectrophotometric determination of various metals using atomic absorption spectroscopy (AAS). In accordance with chemical laboratory safety rules, this procedure was conducted in a fume hood.

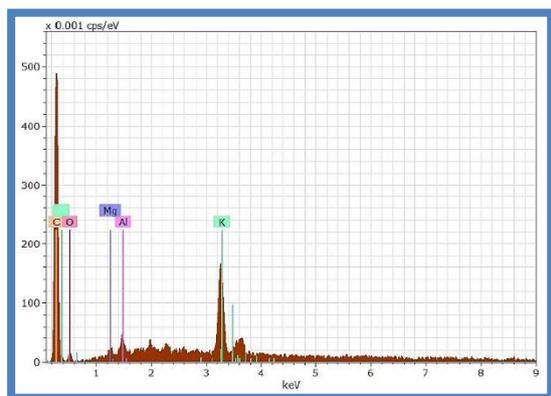
2-6 Atomic absorption Spectroscopy (AAS) Analysis

An Agilent AAS of the FS240 model was used to determine the content of heavy metals and their concentrations in the previously acid-digested prepared samples. To determine the concentration of heavy metals (HMs) in the samples, the results were multiplied by a calculation factor obtained by dividing 100 mL (the volume used for sample dilution following digestion) by 0.5 g (the quantity of tea sample used for the analysis).

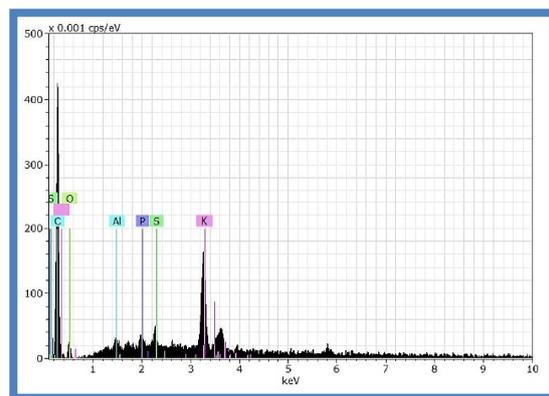
3. Results and Discussion:

3-1 Energy-Dispersive X-ray (EDX) Analysis

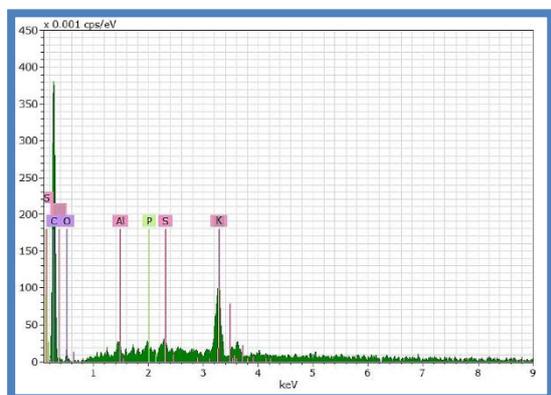
The six black tea samples (T1-T6) and the green tea sample (T7) selected from Iraqi markets were tested via EDX spectroscopy for elemental determination. The normal scan results obtained for this test were illustrated in **Figures 2**, a-g, respectively. The elements shown in the figures were in concentrations higher than 0.003 cps/ev.



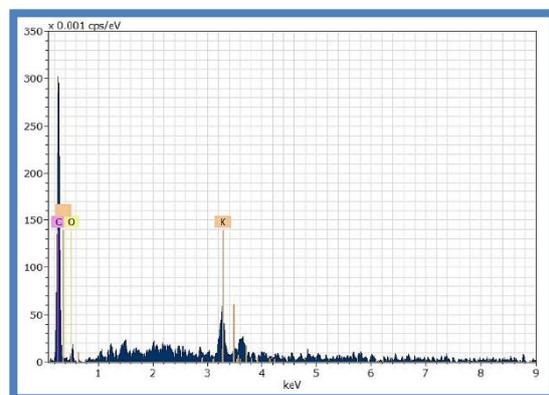
(a)



(b)



(c)



(d)

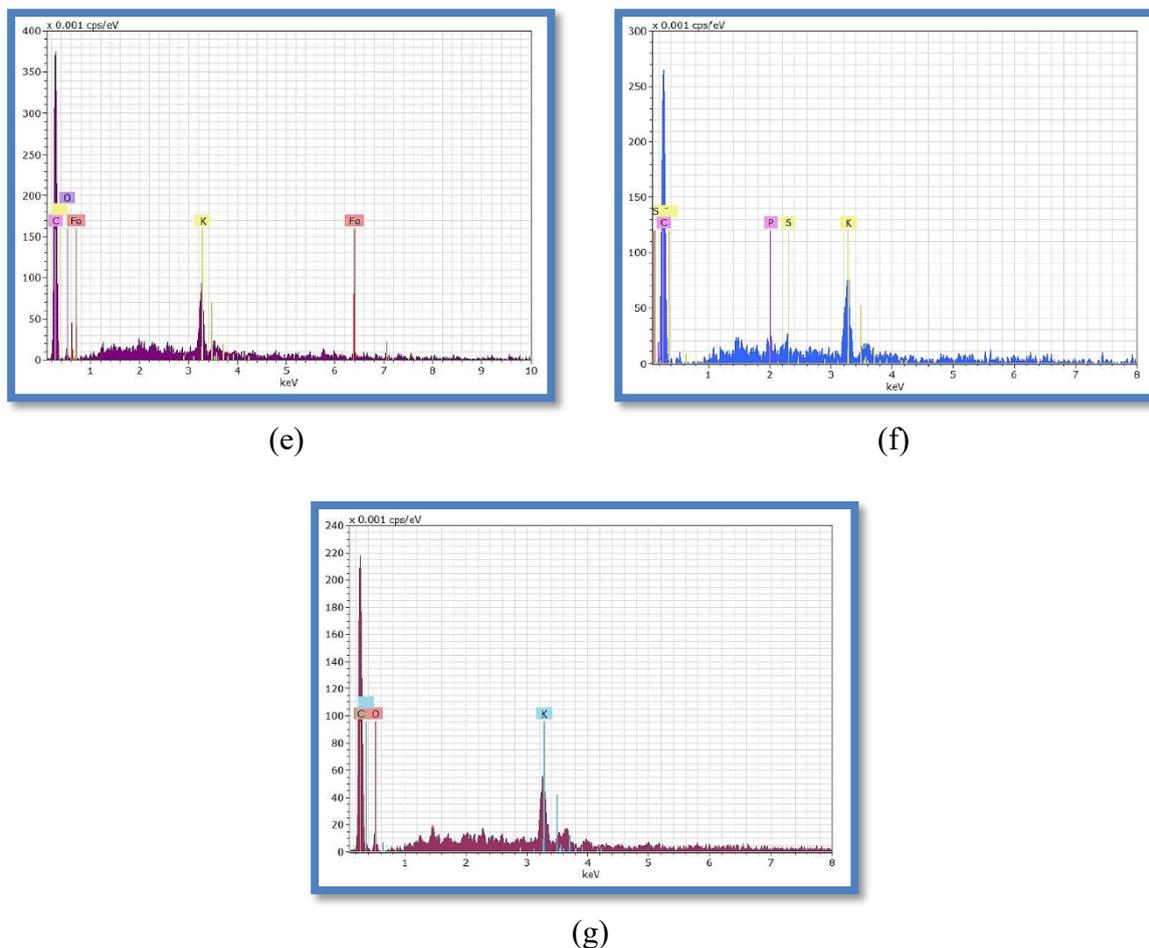
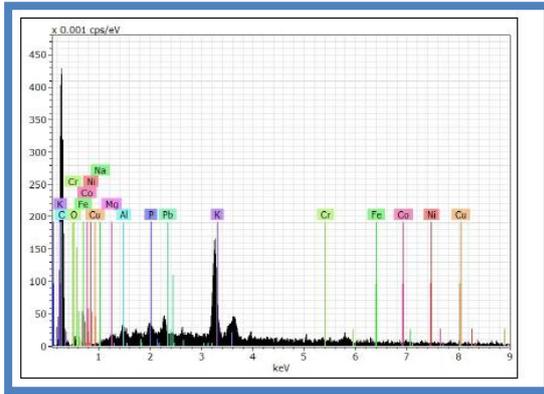


Figure 2: EDX Analysis for Samples (a) T1, (b) T2, (c) T3, (d) T4, (e) T5, (f) T6, and (g) T7.

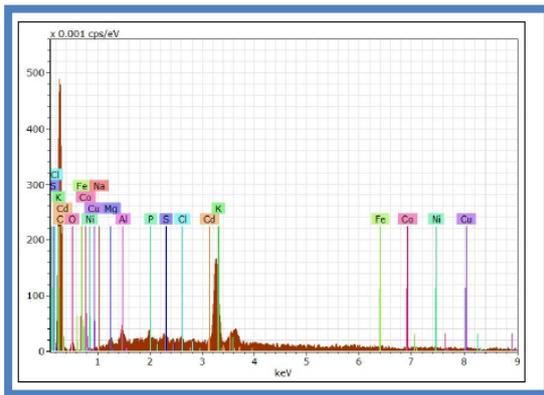
A normal scan of EDX analysis shows the presence of S, K, Al, and P in T1, while K, Al, and Mg were found in T2. Furthermore, S, K, Al, and P were found in T3, but only K was detected in T4. K and Fe were detected in T5, K, and P, and only S was identified in T6, and only K was found in T7. All of the elements that were diagnosed above and detected are considered essential elements, which are necessary for living organisms and may be required in the body in low concentrations. However, the other elements were detected to be within the acceptable range (concentrations lower than 0.003 cps/ev) as shown in **Figure 3** a and b for T1, c and d for T2, e and f for T3, g and h for T4, i and j for T5, k and l for T6, and m and n for T7. To determine the exact concentrations of elements identified by the EDX device, along with those indicated by other research studies but possibly present in a concentration too low to be detected by the EDX due to its sensitivity limits, a digestion process was conducted on the selected samples. Acid digestion using a mixture of 65% HNO₃ and 37% HCl was performed to release HMs from the organic components in tea samples. The resulting solutions were then tested using an atomic absorption instrument to detect and determine the concentrations of the metals.



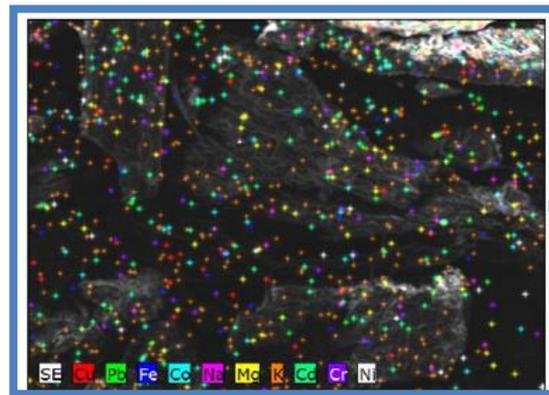
(a): Accurate EDX Analysis for T1.



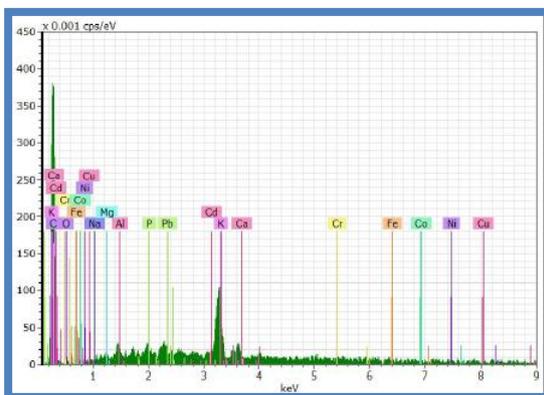
(b): Accurate EDX Analysis for T1.



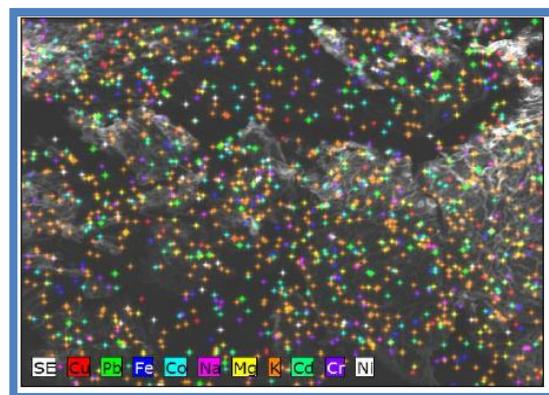
(c): Accurate EDX Analysis for T2.



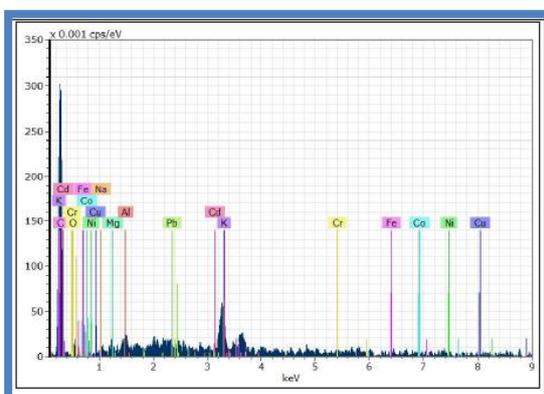
(d): Elements distribution in T2.



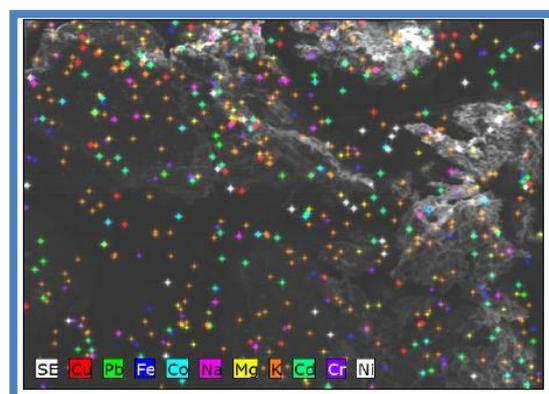
(e): Accurate EDX Analysis for T3.



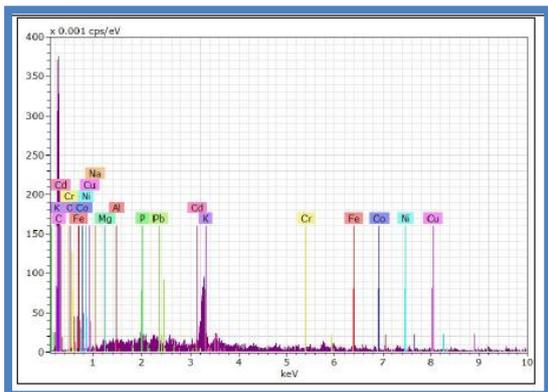
(f): Accurate EDX Analysis for T3.



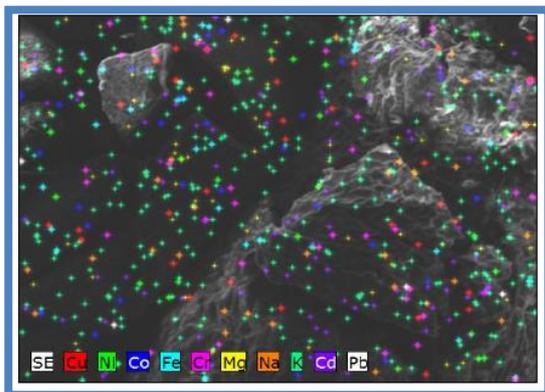
(g): Accurate EDX Analysis for T4.



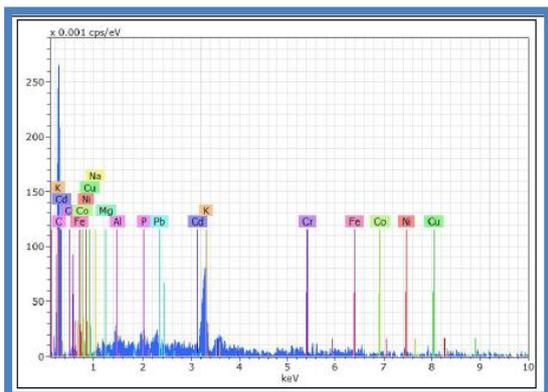
(h): Accurate EDX Analysis for T4.



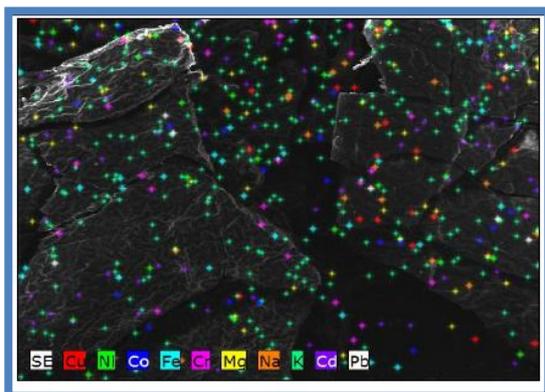
(i): Accurate EDX Analysis for T5.



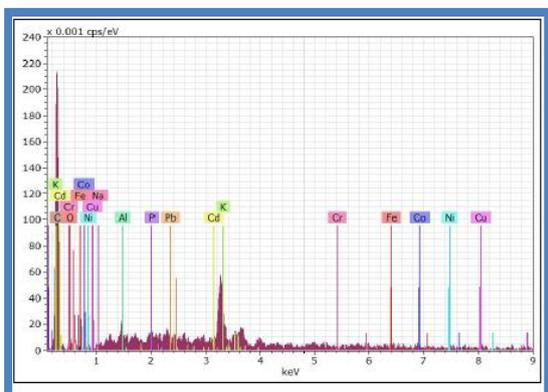
(j): Accurate EDX Analysis for T5.



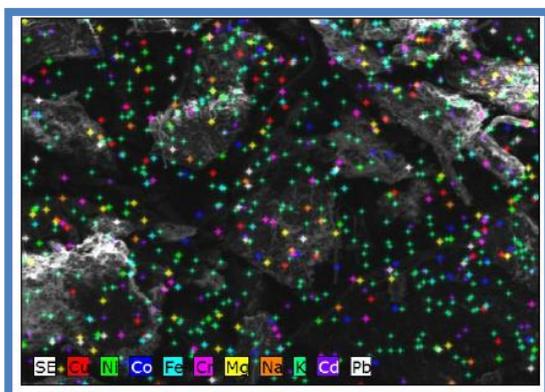
(k): Accurate EDX Analysis for T6.



(l): Accurate EDX Analysis for T6.



(m): Accurate EDX Analysis for T7.



(n): Accurate EDX Analysis for T7.

Figure 3: Accurate EDX Analysis for Samples a and b for T1, c and d for T2, e and f for T3, g and h for T4, i and j for T5, k and l for T6, and m and n for T7.

3-2 AAS Analysis

In order to establish a calibration curve for a specific metal within the detection limits of a device, four standard solutions with low concentrations (in ppm) of nine HMs and one essential element were prepared. Subsequently, solutions derived from the digestion of seven tea samples were compared with these standards. **Table 1** shows the final results for the selected elements in tea samples, while the ratios of metal are illustrated in **Table 2**.

Table 1: AAS Analysis results in ppm

Sample Element	T1	T2	T3	T4	T5	T6	T7
K	14000	14630	10178	6602	14000	12460	8260
Cd	ND	ND	ND	ND	ND	ND	ND
Cu	ND	ND	ND	ND	ND	ND	ND
Pb	ND	ND	ND	ND	ND	ND	ND
Cr	ND	ND	ND	ND	ND	ND	ND
Ni	46	46	66	ND	ND	ND	ND
Fe	500	500	376	376	376	ND	300
Mn	1866	666	332	932	566	466	1000
Zn	54	10	22	66	4	10	ND
Co	ND	ND	ND	ND	ND	ND	ND

ND= Not detected

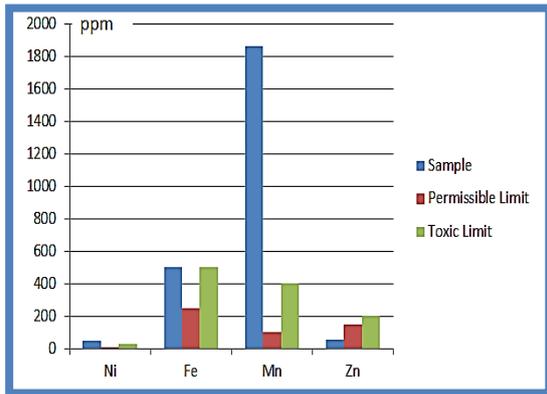
Table 2: The percentages (%) of HMs and potassium in tea samples

Sample Element	T1	T2	T3	T4	T5	T6	T7
K	1.4	1.46	1.01	0.66	1.4	1.24	0.82
Ni	0.0046	0.0046	0.0066	ND	ND	ND	ND
Fe	0.05	0.05	0.037	0.037	0.037	ND	0.03
Mn	0.18	0.06	0.03	0.09	0.06	0.05	0.1
Zn	0.005	0.001	0.002	0.007	0.0004	0.001	ND

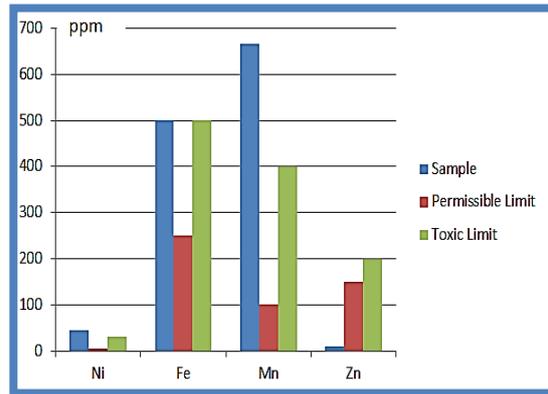
According to the standard limits given by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) (as presented in **Table 3**) and the results obtained from the AAS, all samples (6 black tea and the seventh green tea) had normal concentrations of Iron (it exceeded the permissible limit but did not exceed the toxic level). Also, Zinc and nickel (in samples T1, T2, and T3) and manganese (in samples T1, T2, T4, T5, T6, and T7) were higher than the permissible levels, and their concentrations were within the limits of toxic concentrations. The other HMs (Cd, Cu, Pb, Cr, and Co) were either not detected or were less than the detection limits of these metals in atomic absorption, which makes these samples free from highly toxic HMs. Metal concentrations for samples (T1-T7) compared with permissible and toxic concentrations are illustrated in **Figure 4** (a-g).

Table 3: Normal and Toxic concentrations of heavy metals given by WHO and FAO [29, 30].

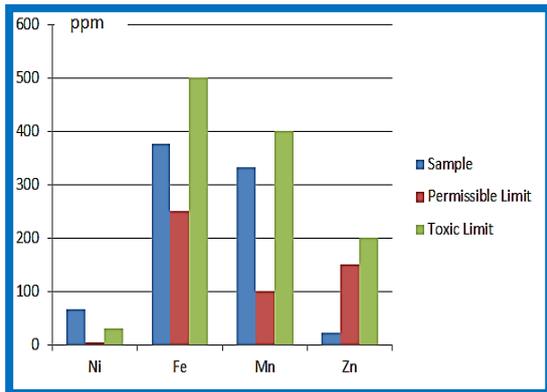
Element	Normal concentrations (mg kg ⁻¹)	Toxic concentration (mg kg ⁻¹)
Cu	3–15	20
Ni	0.1–5	30
Pb	1–5	20
Hg	<0.1–0.5	5
Cr	<0.1–1	2
Mn	15–100	400
Zn	15–150	200
Mo	0.1–0.5	10–50
Co	0.05–0.5	30–40
Fe	50–250	(>500)
As	10–60	<2
Sb	<2–29	5–10 g



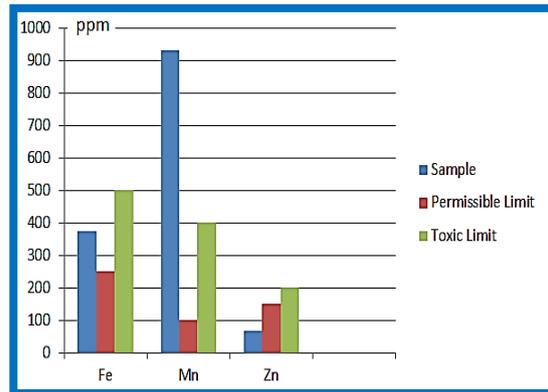
(a): Metal concentration of the sample(T1)



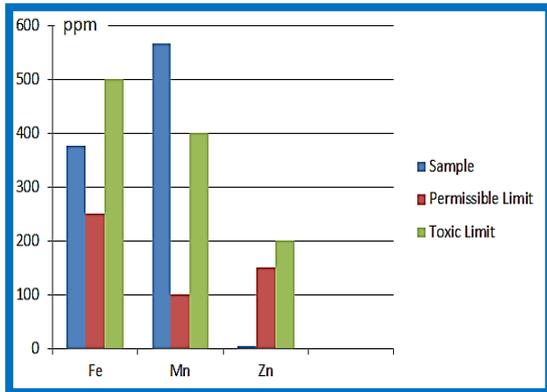
(b): Metal concentration of the sample(T2)



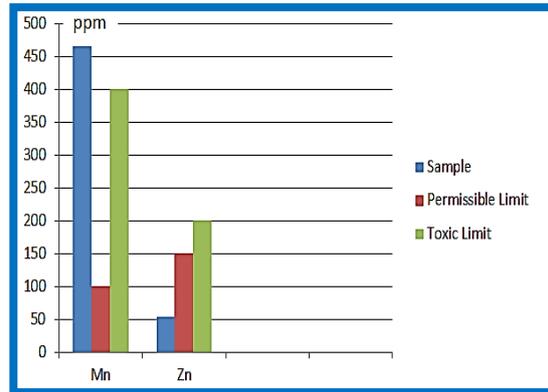
(c): Metal concentration of the sample(T3)



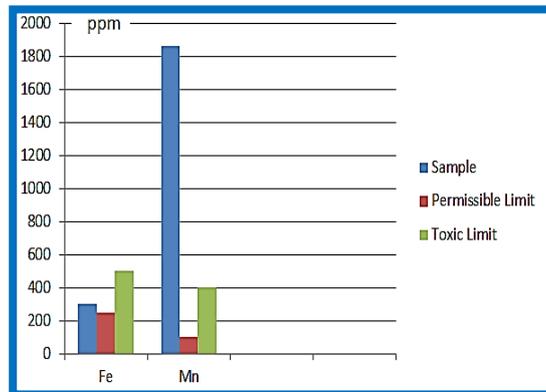
(d): Metal concentration of the sample(T4)



(e): Metal concentration of the sample(T5)



(f): Metal concentration of the sample(T6)



(g): Metal concentration of the sample(T7)

Figure 4: Metal concentrations for samples (a) T1, (b) T2, (c) T3, (d) T4, (e) T5, (f) T6, and (g) T7 compared with permissible and toxic concentrations.

Potassium is the third most important plant nutrient, and it's the second most important nutrient after nitrogen for tea. Tea requires a comparatively higher quantity of K for better production and quality of the product. Here, K plays a vital role in enzyme activation, water relation, translocation, energy relations, and translocation of assimilated photosynthesis and protein and starch synthesis. Potassium's concentrations are clearly higher than the other elements, but potassium is considered an electrolyte mineral that helps regulate heartbeat and balance the body's fluid levels. It can help offset the adverse effects of consuming too much sodium [31].

According to research published by the University of Maryland Medical Center, a cup of green tea supplies lower potassium concentrations than black tea [32]. In our work, the results proved that the potassium concentration of sample 7 (the green tea) had a lower potassium level than 83.3% of the black tea samples. From the EDX Analysis, sulfur, phosphorus, aluminum, and magnesium were detected in too-low concentrations that were placed within the safe range. Additionally, it is essential to mention that the selected samples did not contain HMs like Cd, Pb, and Hg, which are toxic and are regarded as biologically non-essential in remarkable quantities.

4. Conclusions

Tea is considered one of the foodstuffs commonly used globally and locally in Iraq, and the high consumption rates make it at the forefront of foodstuffs that require knowledge of its suitability for human consumption. Some consumer concerns are generated about the potential risks of consuming tea, especially with poor control over imported food. To dispel doubts or prove them, data were obtained in this work to determine the validity of the food product concerning its containment of HMs within the permissible limits according to international health and safety standards. Here, seven samples of tea types commonly used in the Iraqi market were selected. Processes of preparing the samples for specialized examinations of HMs (EDX and AAS) were conducted according to the scientific protocols related to them. Results of these tests show that all the selected samples had high concentrations of nickel (in samples T1, T2, and T3) and manganese (in samples T1, T2, T4, T5, T6, and T7). The other HMs (Cd, Cu, Pb, Cr, and Co) were not detected or found in levels less than the detection limits of the used AAS, which makes these samples free from highly toxic HMs. Researchers recommend studying the chemicals in tea and discussing their toxicity. We also recommend the necessity of examining imported materials to ensure that they are free of toxic materials or materials that affect human health.

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5. References

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