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Health Risk Assessment of Metal Contamination in Four Green Leafy Vegetables from Various Open Markets in Benghazi

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ABSTRACT

This study analysed aluminium, essential metals (chromium, manganese, iron, nickel, copper and zinc), and toxic metals (lead and cadmium) in green leafy vegetables (arugula, mint, parsley, and spinach) obtained from various markets in Benghazi city. A flame Atomic Absorption Spectrometer was used to determine the metal contents. Hazard quotient (HQ) and hazard index (HI) calculations were employed to assess non-carcinogenic and carcinogenic risks associated with the metals in the vegetables. Results showed that aluminium was detected in only 50% of the samples. All vegetable samples contained manganese, iron, copper and zinc within the ranges of 1.072-6.87mg, 26.71-298.4mg, 1.00-3.45mg and 3.61-9.33mg per 1kg of vegetable dry weight, respectively. Nickel and chromium were found in 33% and 50% of the samples, respectively. Cadmium and lead were present in all samples except for two spinach and two parsley samples. The levels of essential metals in the samples were below the maximum allowable limits set by FAO-WHO regulations. However, the contents of nickel, cadmium and lead in some vegetable samples exceeded the maximum limits. The metal contents in the collected vegetables were mostly lower than reported results from previous international studies. The HQ and HI values for the analysed metals were all below unity, suggesting that the consumption of these selected vegetables as part of a daily diet poses no threat to human health.

تقييم المخاطر الصحية لبعض المعادن عن طريق استهلاك أربعة أنواع من الخضروات الورقية الخضراء التي تم جمعها من أسواق مفتوحة مختلفة في بنغازي

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الملخص

في هذه الدراسة تم تحليل الألمنيوم والمعادن الأساسية (الكروم والمنغنيز والحديد والنيكل والنحاس والزنك) والمعادن الثقيلة الضارة (الرصاص والكادميوم) في الخضروات الورقية الخضراء (الجرجير والنعناع والبقدونس والسبانخ) التي تم جمعها من أسواق مختلفة في مدينة بنغازي. واستخدام جهاز الامتصاص الذري باللهب لتحديد محتوى المعادن. كما تم استخدام حسابات حاصل الخطر (HQ) ومؤشر الخطر (HI) لتقييم المخاطر غير المسرطنة والمسرطنة المرتبطة بالمعادن الموجودة في الخضروات. أظهرت النتائج أن عنصر الألمنيوم تواجد في 50٪

الكلمات المفتاحية:
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فقط من العينات. واحتوت جميع عينات الخضروات علي المنجنيز والحديد والنحاس والزنك في النطاقات من 6.87-1.072 ملجم و 298.4-26.71 ملجم و 3.45-1.00 ملجم و 9.33-3.61 ملجم لكل كجم من وزن الخضار الجاف، على التوالي. بينما عنصري النيكل والكروم فقد تواجدا في 33٪ و 50٪ من العينات، على التوالي. أما الكادميوم والرصاص فكانا موجودين في جميع العينات باستثناء عينتي سبانخ وعينتي بقدونس. كانت مستويات المعادن الأساسية في العينات أقل من الحدود المسموح بها المحددة من قبل لوائح منظمة الأغذية والزراعة ومنظمة الصحة العالمية. ومع ذلك، كانت محتويات النيكل والكادميوم والرصاص في بعض عينات الخضروات تتجاوز الحدود القصوى المسموح بها. ومن هذه الدراسة وجد أن كل المحتويات المعادن التي تم تحليلها في عينات الخضروات الورقية في الغالب أقل من النتائج التي توصلت إليها الدراسات الدولية السابقة. كما كانت قيم HQ و HI للمعادن المحللة جميعها أقل من الوحدة، مما يشير إلى أن تناول الخضروات المختارة كجزء من النظام الغذائي اليومي لا يشكل تهديدًا لصحة الإنسان.

مؤشر الخطر.

1. Introduction

Vegetables are an essential part of a healthy diet, which offer a diverse array of nutrients that are important for human health, such as; vitamins, antioxidants, dietary fiber, and essential minerals. Moreover, vegetables are low in calories and fat, making them a healthy choice for various dietary plans. The World Health Organization (WHO) recommends consuming 400g of fruits and vegetables daily to fulfil the body's vitamin and mineral requirements [1-3]. In Libyan cuisine, vegetables play a significant role and are integral ingredients in various traditional dishes such as; couscous, soups, salads, and side dishes. Libyan people recognize the importance of including vegetables in their diet as a way to enhance flavour, nutritional value, and overall well-being [4].

Vegetables can be classified in various ways, taking into consideration different factors such as; botanical classification, nutritional composition, and culinary usage. Botanical classification is one approach to categorizing vegetables, resulting in different groups such as leafy, stem, root, bulb, fruit, and flower vegetables [5]. Different categories of vegetables are obtained based on botanical classification. Among these categories, green leafy vegetables (GLV) stand out. Examples of GLVs include spinach, arugula, parsley, kale, and collard greens. These vegetables are rich in chlorophyll and are often consumed fresh or cooked in stews. The leaves of GLVs primarily consist of cellulose, hemicellulose, and pectin, which contribute to their texture and firmness. Incorporating a vegetable-rich diet has been linked to a reduced risk of various chronic diseases, including heart disease, stroke, and certain types of cancer [1], [6],[7].

The GLVs serve also as a valuable source of essential minerals, including boron, potassium, calcium, iron, selenium, copper, molybdenum, nickel, zinc, and sulphur. These minerals play critical roles in various plant metabolic processes and are essential for human health. However, excessive exposure to these trace essential metals can have toxic effects on the human body [2]. Furthermore, it is worth noting that leafy vegetables have the potential to accumulate higher levels of non-essential toxic heavy metals, such as mercury, lead, cadmium, and arsenic, compared to other types of vegetables. These heavy metals originate from contaminated soil in the surrounding environment and can be transported to water sources, plants, animals, and ultimately humans [8]. In some cases, the use of contaminated water for irrigation can contribute to higher levels of heavy metals contamination in crops. Additionally, the application of pesticides and fertilizers containing metals can result in the entry of pollutants into the plant's tissues, including the edible parts such as fruits and leaves [9]. It is worth noting that GLVs have a greater tendency to accumulate heavy metals compared to other plants, primarily due to their larger leaf surface area and higher rates of translocation and transpiration [10]. Furthermore, during the transportation, marketing, and storage processes, the surfaces of leafy vegetables may be more susceptible to contamination from heavy metal deposition, particularly from vehicular and industrial emissions [8]. The presence of heavy metal contamination in GLVs poses a significant concern due to its potential impact on human health. These toxic metals not only disrupt the metabolic processes of plants, thereby affecting the nutritional value of vegetables, but they can also pose health risks to humans through the food chain. Moreover, these heavy metals have long biological half-lives, which means they can accumulate in various organs of the body and lead to undesirable side effects. It is important to be mindful of the potential risks associated with heavy metal contamination in leafy vegetables and to ensure their consumption from safe and uncontaminated sources. Consequently, the permissible limits of heavy metals in vegetables are regularly revised to ensure safety [10]. It is important to note that these safe limits for heavy metals in vegetables can vary among countries and organizations, with differing guidelines provided by various sources [11-14]. Therefore, it is crucial to monitor the concentrations of heavy metals in GLVs and take necessary ways to reduce their levels if they exceed the established safe limits

In recent times, numerous scientific studies have employed various analytical techniques to detect the metal content present in GLVs. There has also been an increased focus among researchers on assessing the health risks associated with the consumption of contaminated vegetables [10]. To evaluate the potential human health risks posed by consuming vegetables contaminated with heavy metals, different methods are utilized. These include determining the daily intake of metals (DIM), estimating the estimated daily intake (EDI), Average Daily Dose (ADD) and referring to health risk indices established by international organizations such as the United States Environmental Protection Agency (USEPA) and WHO [9].

In previous surveys conducted in Libya, only one study by Elbagermi et al. has evaluated the heavy metals contents in vegetables collected from Misurata [15]. Therefore, the present study aims to assess the concentrations of nine metals (aluminium, cadmium, copper, chromium, iron, lead, manganese, nickel, and zinc) in four types of GLVs obtained from the markets in Benghazi. The Flame Atomic Absorption Spectrophotometer (FAAS) will be employed to analysis the contents of metals. The collected vegetables are locally grown and commonly consumed both in raw and cooked forms by the local population. The results obtained from the analysis of our selected GLVs samples will be compared with findings from other research articles conducted in different countries. Furthermore, the study will also calculate the associated public health risks related to the analysed metals in the selected vegetables.

2. Materials and Method

2.1. Sampling

In this study, four commonly consumed GLV species were randomly purchased from three different open markets in Benghazi city. The data collection period spanned from February 22, 2023, to March 22, 2023. The selected GLVs included arugula (*Eruca sativa*), mint (*Mentha arvensis*), parsley (*Petroselinum sativum*), and spinach (*Spinacia oleracea*). To ensure representative samples, three replications from each market and a composite sample was prepared by mixing them together. The composite samples were then packaged in clean plastic bags and appropriately labelled. To eliminate any foreign dust particles adhering to the vegetable surfaces, the samples were thoroughly washed using distilled water. Excess water drops were removed by gently shaking the vegetables, followed by drying with a hair dryer to eliminate excess moisture. The edible parts of each dried fresh sample were chopped into small pieces. These samples were further dried to a constant weight by incubating them for 48 hours at approximately 60°C in a laboratory oven (Lammart). After drying, the vegetable samples were reweighed and crushed using a clean pestle and mortar to obtain homogenized samples. The ground samples were then stored in airtight sealed plastic bags at room temperature until the analysis was conducted [16].

2.2. Preparation of Vegetables Samples for Metal Analysis

To prepare the vegetable samples for analysis using FAAS, an acid-based digestion method was employed [17], [18]. Two grams of plant powder were weighed in duplicate and placed in small beakers. Then, 10 mL of a concentrated acid mixture (consisting of 5:1 ratio of nitric acid to perchloric acid) was added to each beaker. The mixture was allowed to sit overnight at room temperature. The following day, the beakers were heated on a hot plate, gradually increasing the temperature until reaching 100°C. As the mixture dissolved and brown fumes evolved, the samples were allowed to cool to room temperature. The digested solutions were then transferred to 100 mL volumetric flasks, and distilled water was added to reach the desired volume. These solutions were filtered and subsequently analysed for the presence of aluminium, chromium, manganese, iron, nickel, copper, zinc, lead, cadmium. The analysis was conducted by measuring the absorbance using a computer-controlled system equipped with GBC AA Avanta Software, version 1.33 FAAS. Blank solutions were prepared under the same conditions as the samples. Prior to analysing each element, the instrument was auto-zeroed using blank solution. The calibration plot method was employed for the analysis of metals in this study. The concentration of each metal (in mg/kg) in the vegetable samples was calculated using equation 1.

$$\mathrm{Conc(mg/kg)} = \frac{\mathrm{conc(mg/L)}}{\mathrm{sample\ weigh}} \times \mathrm{dilution\ factor} \quad \text{Equation 1}$$

2.3. Statistical analysis

Duplicate measurements were conducted for all metal analyses to ensure accuracy and reliability. The Statistical Package for Social Science (SPSS) program, version 22, was utilized to calculate the means, standard deviations, as well as the minimum and maximum values of metal concentrations in each sample. To assess the differences in metal concentrations among vegetable samples from different markets, a one-way analysis of variance (ANOVA) was performed. Furthermore, the Least Significant Difference (LSD) test was employed to determine significant differences at a significance level of $p < 0.05$.

2.4. Health Risk Assessment

To evaluate the potential health risks, both carcinogenic and non-carcinogenic, associated with the consumption of vegetables contaminated with heavy metals, the USEPA model was utilized [18], [19]. Specifically, the health risk assessment in this study focused on the ingestion pathway. The ADD, hazard quotient (HQ), and hazard index (HI) were calculated to quantify the health risks associated with heavy metals. The ADD (in mg/kg/day) of each metal was determined using equations 2.

$$\mathrm{ADD} = \frac{C_{metal} \times IR \times EF \times ED}{Bw \times AT} \quad \text{Equation 2}$$

In Equation 2, the calculation involves several parameters; include the average mean concentration of the metal in the selected vegetable in terms of dry weight (C_{metal}; mg/kg), the exposure frequency (EF; 365 days/year), the exposure duration (ED; 70 years, which corresponds to the average lifetime of the Libyan population), the consumer's body weight (Bw; 70 kg for adults), and the average time of consumption (AT; 25,550 days). Additionally, the ingestion rate of fresh leafy vegetables (IR) was considered in this study, with a value of 0.0022 kg/person/day. This value was reported by Ametepey *et al*., specifically for African citizens [18], [20].

The HQ is used to assess the non-carcinogenic risk associated with heavy metals in contaminated vegetables. In this study, the HQ was determined by comparing the ADD of the contaminated metals to the oral reference dose (RfD), equation 3 [19].

$$\mathrm{HQ} = \frac{\mathrm{ADD}}{\mathrm{RfD}} \quad \text{Equation 3}$$

The RfD is the level of metals have not caused any harmful health effect [8]. The RFD values of Al, Pb, Cd, Cu, Fe, Cr, Zn, Ni, Mn are 1.0, 0.0035, 0.001, 0.040, 0.7, 1.5, 0.3, 0.02 and 0.14 mg/kg/day, respectively [16],[19].

The HI provides an overall assessment of the potential health risks associated with the combined exposure to multiple metals. In this study, the HI was computed by summing the hazard quotients for each metal, as indicated by equation 4 [20].

$$\mathrm{HI} = HQ_{Al} + HQ_{Cr} + HQ_{Mn} + HQ_{Fe} + HQ_{Ni} + HQ_{Cu} + HQ_{Zn} + HQ_{Pb} + HQ_{Cd} \quad \text{Equation 4}$$

3. Results and Discussion

3.1. The Contents of Metals in Selected Green Leafy Vegetables

While vegetables are known for their nutritional value, it is important to note that they can also contain elevated levels of heavy metals [21]. Researchers have highlighted that vegetables can absorb heavy metals from polluted air, water and soil. Leafy vegetables, in particular, have a tendency to accumulate higher concentrations of heavy metals in their leaves. Consequently, these heavy metals can enter the food chain [22]. In this study, various GLVs (arugula, mint, parsley, and spinach) were collected from different markets in Benghazi city, and their metals contents, including chromium, manganese, iron, nickel, zinc, aluminium, lead and cadmium, were determined. The concentrations of these metals were measured based on the dry weight (DW) of the selected GLVs. The results, presented in Table 1 for essential heavy metals, and Table 2 for aluminium and the two toxic heavy metals, are reported as mean (m) ±standard deviation (sd) and range. The detected metals exhibited diverse levels, with some present in significant quantities, others in trace amounts, and a few not detected in specific vegetable samples.

Table 1: The content of some essential heavy metals in GLVs collected from three markets in Benghazi.

Vegetable Sample	Concentrations of Metals (mg/kg DW)					
	Cr	Mn	Fe	Ni	Cu	Zn
Samples collected from Market 1						
Arugula1	0.040	1.072	200.55	<DL	2.01	5.90
Mint1	<DL	5.17	131.74	<DL	2.81	7.18
Parsley1	<DL	1.59	71.66	<DL	1.63	4.02
Spinach1	0.11	4.66	39.70	*0.2*	1.00	5.95
Samples collected from Market 2						
Arugula2	<DL	5.26	26.71	<DL	2.052	4.52
Mint2	0.062	4.20	171.91	<DL	1.44	7.11
Parsley2	0.033	6.87	298.4	<DL	1.99	3.61
Spinach2	<DL	4.24	179.2	0.11	3.24	9.33
Samples collected from Market 3						
Arugula3	<DL	3.76	187.0	<DL	1.23	5.34
Mint3	0.059	6.51	277.0	<DL	3.45	4.95
Parsley3	0.21	2.79	137.1	0.11	2.77	5.01
Spinach3	<DL	6.41	224.2	0.77	2.98	6.10
Min	<DL	1.072	26.71	<DL	1.00	3.61
Max	0.062	6.87	298.4	0.77	3.45	7.18

Each value is the average of two measurements.

<DL= below the Detection Limit. DL is 0.002mg/kg for Cr and 0.005mg/kg for Ni.

The samples with numbered codes indicated the market of collection. In this investigation, the parsley samples collected from market 3 exhibited the highest level of chromium (0.21 ±0.009mg/kg), while the lowest level (0.033 ±0.003mg/kg) was observed in samples from market 2. However, it is worth noting that the maximum concentration of chromium in our samples was ten times lower than the safe permission limit for vegetables set by FAO/WHO (2.3mg/kg) [11-14]. The concentration ranges of chromium in our samples (0.033-0.21mg/kg) were lower compared to the corresponding vegetables collected from Palestine (<DL-0.88mg/kg for spinach) (<DL-2.51 mg/kg for arugula) (<DL-0.66 mg/kg for parsley) [23], Bangladesh (1.40-3.30mg/kg for spinach) (1.40-16.70mg/kg for mint) [17], and UAE (3.3-3.6mg/kg for spinach) [24].

Manganese and iron are essential nutrients for human health, and GLVs are recognized as important dietary sources of both metals. Manganese plays a crucial role as a component of enzyme systems, including those involved in oxygen handling. It also contributes to the formation of connective tissue, bones, blood-clotting factors, and sex hormones [25]. The concentration range of manganese in the vegetable samples was ranged between 1.027-6.87mg/kg. On the other hand, iron is a vital component of myoglobin [25]. According to Ali and Al-Qahtani, leafy vegetables generally have higher iron contents compared to other types of vegetables. This can be attributed to the fact that leaves are considered the food-making factories in plants, which promote iron uptake and accumulation in the leaves [26]. In this study, significant levels of iron were detected in all vegetable samples. The concentration of iron ranged from 26.71 to 200.55mg/kg in arugula samples, 131.74 to 277.0mg/kg in mint samples, 71.66 to 298.4mg/kg in parsley samples, and 39.70 to 224.2mg/kg in spinach samples, as shown in Table 1. In our study, the highest concentrations of manganese (6.87 ±0.39mg/kg) and iron (298.4 ±2.0mg/kg) in parsley samples were found to be lower than the highest concentrations of manganese and iron detected in parsley samples from Palestine (Mn:57.82mg/kg, Fe:569.61mg/kg) (Mn:117.37mg/kg, Fe:4433.04mg/kg) [23], Saudi Arabia (Mn 61.3 mg/kg, Fe 389.6 mg/kg) [8], and Turkey (Mn 51.64 ±11.9mg/kg) [9]. Although our results were lower compared to some published findings on manganese and iron contents in leafy vegetables, they were also significantly lower than the safe permission limits set by FAO/WHO for manganese (500mg/kg) and iron (425 mg/kg) [11-14].

Nickel is naturally present in various foods, and certain vegetables and fruits, such as bananas and pears, contain moderate amounts of nickel [19]. This metal plays a role in protein structure and function, acting as a cofactor in the activation of specific enzymes involved in glucose breakdown or utilization [25]. In our study, nickel was detected in all spinach samples obtained from different markets, with concentrations ranging from 0.11 to 0.77mg/kg. Additionally, nickel was identified in a parsley sample collected from market 3, with a concentration of 0.11 mg/kg. The concentrations of nickel in parsley and spinach samples collected from market2 and market 3, respectively, were similar to the permission limit set by FAO/WHO (0.1mg/kg) [11-14]. However, the concentrations of nickel in the other two spinach samples were exceeded the safe limit. Furthermore, the maximum concentration of nickel in our samples was higher than the mean concentration of nickel found in spinach samples collected from West Libya (Misurata) (0.26 ±0.065mg/kg) [15]. Conversely, the maximum concentration of nickel in spinach samples from Bangladesh (0-1.7mg/kg) [17] and spinach and parsley samples from Turkey (1.56±0.37mg/kg for spinach, and 2.47±0.68mg/kg for parsley) were higher than our results [9].

Significant amounts of copper and zinc were detected in all the GLVs collected in this study. The highest mean concentration of copper metal was detected at 3.45 ±0.3mg/kg in mint samples, while the lowest was detected at 1.23 ±0.2mg/kg in arugula and 1.00±0.1mg/kg in spinach. Copper metal is essential constituent of several enzymes, it involved in many metabolic reactions. Copper is necessary for the hematologic and neurologic systems. It is also necessary for the growth and formation of bone, and myelin sheaths in the nervous systems [25]**.** The highest concentration of copper in our samples was lower than copper concentrations in GVLs samples collected from Misurata/Libya (5.32mg/kg) [15], Palestine (0.8-14.3mg/kg) [23], Saudi Arabia (21.6mg/kg) [8], Turkey (6.59±1.53mg/kg) [9] and Bangladesh (4.0-14.4mg/kg) [17]. However, the concentration range of copper in spinach samples (1.00-3.24mg/kg) was higher than the concentration range of spinach samples collected from UAE (0.90-0.94mg/kg) [24].

Zinc metal plays an essential role in numerous biochemical pathways and is particularly important for maintaining healthy skin, supporting a strong immune system, and promoting resistance to infections. It is involved in insulin activity, the metabolism of the ovaries and testes, and liver function. Zinc deficiency can lead to various negative effects on health. This includes impaired cognitive function, compromised immune function, behavioural issues, memory impairment, difficulties with spatial learning, and neuronal atrophy [25]. In our study. zinc contents in the vegetable samples varied across the different types, with range varied between 3.61 ±0.07mg/kg in parsley samples and 9.33 ±0.3mg/kg in spinach samples collected from market 2, Table 1. Comparatively, the mean concentration of zinc in spinach samples collected from Misurata, Libya (16.83 ± 2.82 mg/kg), was higher than the concentration range observed in our spinach samples (5.95-9.33mg/kg) [15]. Furthermore, the zinc concentration range in vegetable samples collected from Palestine (2.79-106.5mg/kg) [23], Bangladesh (4.0-14.4 mg/kg) [17], Saudi Arabia (9.6-21.6 mg/kg) [8], and Turkey (31.30-36.28 mg/kg) [9] fall within the zinc concentration range observed in our samples.

Copper and zinc contents in the collected samples were significantly below the safe permission limits set by FAO/WHO for both metals in vegetables [11-14].

Table 2: The content of aluminium, Lead and Cadmium metals in GLVs collected from Three markets in Benghazi

Market	Vegetable Sample	Metals Concentrations (mg/kg DW)		
		Al	Pb	Cd
Market 1	Arugula1	<DL	0.31 ±0.001	0.2 ±0.006
	Mint1	<DL	0.41 ±0.001	0.21 ±0.002
	Parsley1	0.018±0.002	0.11 ±0.001	0.19 ±0.004
	Spinach1	0.012±0.001	0.11±0.007	0.79 ±0.013
Market 2	Arugula2	<DL	0.54 ±0.05	0.36±0.01
	Mint2	<DL	0.59 ±0.01	0.094±0.009
	Parsley2	0.013±0.001	<DL	0.078 ±0.004
	Spinach2	0.026±0.001	<DL	<DL
Market 3	Arugula3	0.036±0.002	0.38±0.005	0.11±0.005
	Mint3	0.022±0.001	0.22±0.02	0.15±0.008
	Parsley3	<DL	0.030±0.0007	<DL
	Spinach3	<DL	0.33±0.04	0.18±0.02
	Min	<DL	<DL	<DL
	mix	0.036	0.59	0.79

DL is 0.003mg/kg for Pb and 0.005mg/kg for Cd.

Aluminium is a naturally occurring metal that can be found in soil, water, and food. It is commonly present in vegetables, especially those grown in acidic soils. Aluminium does not have any biological function in the human body [27]. Exposure to aluminium has been associated with adverse health effects such as anemia, bone disease, kidney damage, and dialysis encephalopathy. Overexposure to aluminium has also been linked to an increased risk of neurological disorders, including Alzheimer's and Parkinson's diseases [19],[27]. To address the potential health risks associated with aluminium exposure, a provisional tolerable weekly intake (PTWI) was established at 2.0 mg/kg body weight. The PTWI is a concept used by regulatory agencies to estimate the amount of a substance that can be ingested weekly over a lifetime without significant health risks [28]. In our study, aluminium metal was detected in only 50% of the vegetable samples. low aluminium concentrations were observed in one spinach sample collected from market 1 (0.012 ±0.001mg/kg) and two parsley samples collected from market 1 and market 2 (0.018 ±0.002mg/kg and 0.013 ± 0.001mg/kg, respectively). On the other hand, accumulated aluminium was found in the arugula and mint samples collected from market 3, as well as one spinach sample collected from market 2, with concentrations of 0.036 ±0.002mg/kg, 0.022 ±0.0007mg/kg, and 0.026 ±0.001mg/kg, respectively, Table 2. Our results regarding aluminium concentrations were lower than the levels detected in leaf vegetables, including spinach, salad, and chard, collected from Italy (1.921-4.0667mg/kg) [29]. Furthermore, our result revealed significantly lower aluminium concentrations compared to the concentrations in certain vegetables collected from Jamaica (4.25-93.12mg/kg) [20].

Lead and cadmium were detected in all vegetable samples, except for three samples where their concentrations were below the detection limits of the spectrophotometric method of analysis. These samples included a spinach sample from market 2, and two parsley samples collected from market 2 and market 3. The lead content in the vegetable samples ranged from 0.11 mg/kg to 0.59 mg/kg, while the cadmium content ranged between 0.078 mg/kg and 0.79 mg/kg, Table 2. It was found that all arugula, mint, and one spinach sample contained lead concentrations higher than the maximum authorized limits set by FAO/WHO for toxic metals in vegetables. Similarly, the concentrations of cadmium in two arugula samples, one mint sample, and one spinach sample exceeded the permitted limit set by FAO/WHO for toxic metals in vegetables. Additionally, spinach samples from market1 contained cadmium concentrations approximately four times higher than the maximum limit set by FAO/WHO for cadmium in vegetables [11-14]. However, the level of cadmium in the spinach sample was lower than the maximum concentration of cadmium detected in spinach samples collected from Palestine (<DL-1.62mg/kg) [23], Bangladesh (0.00-0.9mg/kg) [17], and Saudi Arabia (1.03mg/kg) [8]. The concentrations of lead in arugula, spinach, and mint samples were lower than the maximum levels of lead found in the same vegetables collected from Bangladesh (spinach 0.00-2.00mg/kg; mint 0.00-6.00mg/kg) [17] and Saudi Arabia (spinach 1.94mg/kg) [8]. In contrast, the concentration ranges of both lead and cadmium in various vegetable samples collected from Libya (Misurata) (lead:0.32 ±0.02mg/kg; cadmium:0.27 ±0.03mg/kg) [15], Iran (<DL-0.23 mg/kg; cadmium <DL-0.03mg/kg) [30], and Turkey (lead 0.26 ±0.06mg/kg; cadmium 0.03 ±0.004mg/kg) [9] were lower than the concentration ranges observed in the collected vegetable samples in our study.

Our findings indicate that there were variations in metal concentrations among the same vegetables collected from different markets in Benghazi, with the exception of nickel in arugula samples. These variations in metal concentrations within vegetables from the same market can be attributed to differences in their morphology and physiology, which affect processes such as metal uptake, exclusion, accumulation, and retention [18]. On the other hand, variations in metal contents of the same vegetables from different markets may be influenced by environmental factors, including the soil in which they were grown and the irrigation water used during planting [31].

3.1. Health Risk Assessment of Metals in Green Leafy Vegetables

The health risk assessment of metals in vegetables involving evaluating the dietary risks to human health associated with the oral consumption of GLVs containing certain levels of metal contaminants. One of important parameters in this assessment is the ADD which represents the estimated daily intake of a metal through a specific exposure route. In this study, the ADD values of aluminium and heavy metals in the selected GLVs were calculated and presented in Table 3. The results revealed that the lowest ADD values were detected for nickel and aluminium after consumption of arugula, mint and parsley. However, after the consumption of spinach, the ADD for aluminium and chromium were the lowest in adult. Furthermore, the ADD values for iron were the highest in mint followed by parsley and spinach, and zinc and manganese were the second and the third most abundant metals consumed by different vegetables. On the other hand. The ADD values indicated that the highest contribution for lead and cadmium intakes come from arugula and spinach. In fact, he ADD values for all analysed metals were lower than PMTDI values. Additionally, the ADD values for both lead and cadmium metals (Table 3) were significantly lower than the values recorded in the previous study of the same vegetables collected from Turkey [9], Bangladesh [17] and India [18].

Table 3: Average Daily Dose of the analysed metals in the selected GLVs

Metal	Leafy Green Vegetables Average Daily Dose (mg/kg/day)			
	Arugula	**Mint**	**Parsley**	**Spinach**
Cr	4.09×10^{-7}	1.26×10^{-6}	2.55×10^{-6}	1.16×10^{-6}
Mn	1.06×10^{-4}	1.66×10^{-4}	1.18×10^{-4}	1.60×10^{-4}
Fe	4.34×10^{-3}	6.09×10^{-3}	5.32×10^{-3}	4.64×10^{-3}
Ni	0	0	1.16×10^{-6}	1.13×10^{-5}
Cu	5.53×10^{-5}	8.08×10^{-5}	6.69×10^{-5}	7.57×10^{-5}
Zn	1.65×10^{-4}	2.01×10^{-4}	1.32×10^{-4}	2.24×10^{-4}
Al	3.77×10^{-7}	2.29×10^{-8}	3.14×10^{-7}	4.09×10^{-7}
Pb	1.29×10^{-5}	1.28×10^{-5}	1.47×10^{-6}	4.61×10^{-6}
Cd	7.70×10^{-6}	4.81×10^{-6}	2.76×10^{-6}	1.02×10^{-5}

The evaluation of the ingestion exposure of heavy metals in human body by consumption food involve the calculation of both HQ and HI indexes values to determine the possibility of non-carcinogenic and carcinogenic health risk of the vegetables` consumers [8],[17]. The non-carcinogenic risk and cancer risk values of aluminium and heavy metals in the selected GLVs were presented in Table 4. According to the HQ values, the most hazardous metals were determined as cadmium (1.54×10^{-2})>iron (6.20×10^{-3})> lead (3.58×10^{-3}) >copper (1.38×10^{-3})> chromium (1.36×10^{-3}) in augural, cadmium (9.62×10^{-3}) >iron (8.69×10^{-3})> chromium (4.19×10^{-3})> lead (3.55×10^{-3})> copper (2.02×10^{-3}) in mint, chromium (8.49×10^{-3})>iron (7.59×10^{-3})> cadmium (5.51×10^{-3})>copper (1.67×10^{-3})> manganese (8.42×10^{-4}) in parsley and cadmium (2.05×10^{-2})>iron (6.63×10^{-3})> chromium (3.88×10^{-3})> copper (1.89×10^{-3})> lead (1.28×10^{-3}) in spinach samples. In our study, it is notable that the individual HQ values of the analysed metals in the four vegetables, using oral RfD for adults, were lower than 1. This result indicted that the consumption of the selected vegetables as daily diet might have no undue risk of non-carcinogenic health effects [20]. The HI values for consumption of the selected vegetables, were recorded in Table 4 at 0.0366, 0.0299, 0.0292 and 0.0250 for spinach, mint, arugula and parsley, respectively. Our results, revealed that spinach contributing about 30% to the total HI of analysed vegetables. Furthermore, the value of total HI is 0.1207 (Table 4), which is the summation of the four HI values of individual vegetables, which is also less than 1.

Table 4: The quotient and hazard index values of the analysed metals among the four types of GLVs

HQ_{metal}	Green Leafy Vegetables			
	Arugula	**Mint**	**Parsley**	**Spinach**
HQ_{Cr}	1.36×10^{-3}	4.19×10^{-3}	8.49×10^{-3}	3.88×10^{-3}
HQ_{Mn}	7.54×10^{-4}	1.19×10^{-3}	8.42×10^{-4}	1.15×10^{-3}
HQ_{Fe}	6.20×10^{-3}	8.69×10^{-3}	7.59×10^{-3}	6.63×10^{-3}
HQ_{Ni}	0	0	5.81×10^{-5}	5.66×10^{-4}
HQ_{Cu}	1.38×10^{-3}	2.02×10^{-3}	1.67×10^{-3}	1.89×10^{-3}
HQ_{Zn}	5.50×10^{-4}	6.72×10^{-4}	4.41×10^{-4}	7.47×10^{-4}
HQ_{Al}	3.77×10^{-7}	2.29×10^{-8}	3.14×10^{-7}	4.09×10^{-7}
HQ_{Pb}	3.58×10^{-3}	3.55×10^{-3}	4.08×10^{-4}	1.28×10^{-3}
HQ_{Cd}	1.54×10^{-2}	9.62×10^{-3}	5.51×10^{-3}	2.05×10^{-2}
HI=$\sum HQ_{metal}$	0.0292	0.0299	0.0250	0.0366
Total Hazard Index (THI) =0.1207				

4. Conclusion

Compared Leafy vegetables demonstrate a pronounced capacity for accumulating heavy metals compared to other types of fruits and vegetables. Our elemental analysis identified zinc, copper, iron, and manganese in all selected samples, with chromium and nickel present in 50% and 33% of samples, respectively. Concentrations followed the order: iron < zinc < manganese < copper < chromium < nickel. However, nickel concentrations exceeded permissible limits established by the FAO/WHO. Conversely, toxic metals such as cadmium and lead were found in 83% of samples, surpassing FAO/WHO limits in 25% and 58% of samples, respectively, while aluminum was detected in 50% of samples below 0.05 mg/kg. Metal contents varied and occasionally aligned with previous studies within Libya and internationally [8, 9, 15, 17, 20-24, 29, 30].

Risk assessments indicated low ADD and HQ values for all analysed metals, with HI values suggesting a low hazard level (HI < 1.0) in spinach > mint > arugula > parsley. Hence, daily consumption of these vegetables is unlikely to pose significant health risks.

Based on our findings, minimizing potential health risks associated with heavy metal contamination in GLVs can be achieved by:

- Thoroughly washing vegetables to remove surface contaminants.
- Identifying sources of heavy metal contamination through soil and irrigation water testing.
- Implementing regular monitoring and analysis of heavy metals in vegetables, coupled with ongoing risk assessments to evaluate long-term health impacts.
- Contributing to ongoing research efforts aimed at addressing heavy metal contamination issues effectively.

5. References

[1]- Arasaretnam, S., Kiruthika, A., Mahendran, T., (2018). Nutritional and mineral composition of selected green leafy vegetables. Ceylon J. Sci, **47**(1), 35-41. DOI: http://doi.org/10.4038/cjs.v47i1.7484.

[2]- Kumar, D., Kumar, S. and Shekhar, C., (2020), Nutritional components in green leafy vegetables: A review. J. Pharmacogn. Phytochem., **9**(5),2498-2502.www.phytojournal.com.

[3]- Bojar, I., Owoc, A., Humeniuk, E., Wierzba, W. and Fronczak, A., (2012), Inappropriate consumption of vitamins and minerals by pregnant women in Poland. Ann. Agric. Environ. Med., **19**(2), 263-266. www.aaem.pl.

[4]- EL-Tellawy F.M., EL-Nawawy M.A., Mahmoud K.W., El-Zorkani A.A., Widad M. E., Widad A., (2018), Determination some of heavy metals in traditional food in north Africa Libya and Egypt. J. Environ. Sci, **42**(3), 37-56.

[5]- V.E. Rubatzky and M. Yamaguchi, World vegetables: principles, production, and nutritive values, Chapter 3: vegetable classification, Springer Science & Business Media, 2nd edition, 2012, pp. 29-31.

[6]- Otitoju, G.T.O., Ene-Obong, H.N. and Otitoju, O., (2014), Macro and micro nutrient composition of some indigenous green leafy vegetables in south-east zone Nigeria. J Food Process Technol, **5**(11), 5-11. http://dx.doi.org/10.4172/2157-7110.1000389.

[7]- Ezeilo, C.A., Okonkwo, S.I., Chibuzor, C., Onuorah, A.L.C. and Ugwunnadi, N.E., (2020), Determination of heavy metals in some fruits and vegetables from selected markets in Anambra state, ACTA Sci. Nutr. Heal, **4**, 163-171.

[8]- Alturiqi, A.S., Albedair, L.A. and Ali, M.H., (2020), Health risk assessment of heavy metals in irrigation water, soil and vegetables from different farms in Riyadh district, Saudi Arabia. J.Elem., **25**(4),1269-1289. DOI:10.5601/jelem.2020.25.3.2016.

[9]- Leblebici, Z., Kar, M. and Başaran, L., (2020), Assessment of the heavy metal accumulation of various green vegetables grown in Nevşehir and their risks human health. Environ. Monit. Assess., **192**, 1-8. https://doi.org/10.1007/s10661-020-08459-z.

[10]- Khan, S., Farooq, R., Shahbaz, S., Khan, M.A. and Sadique, M., (2009), Health risk assessment of heavy metals for population via consumption of vegetables. World Appl Sci J, **6**(12), 1602-1606.

[11]- FAO/WHO, (2015), Joint FAO/WHO Expert Committee on Food additives (2015) "Summary and Conclusion of the 61st Meeting Joint FAO/WHO Expert Committee on Food Additives" Rome Italy.

[12]- FAO/WHO, (2011), Joint FAO/WHO Food Standards Programme Codex Committee on Contaminants in Foods, 21-25.

[13]- Khan, M.N., Aslam, M.A., Muhsinah, A.B. and Uddin, J., (2023), Heavy Metals in Vegetables: Screening Health Risks of Irrigation with Wastewater in Peri-Urban Areas of Bhakkar, Pakistan. Toxics, **11**(5), 460-478.

https://doi.org/10.3390/toxics11050460

[14]- Aftab, K., Iqbal, S., Khan, M.R., Busquets, R., Noreen, R., Ahmad, N., Kazimi, S.G.T., Karami, A.M., Al Suliman, N.M.S., Ouladsmane, M., (2023), Wastewater-irrigated vegetables are a significant source of heavy metal contaminants: toxicity and health risks. *Molecules*, ***28***(3), 1371-1382. https://doi.org/10.3390/molecules28031371.

[15]- Elbagermi, M.A., Edwards, H.G.M. and Alajtal, A.I., (2012), Monitoring of heavy metal content in fruits and vegetables collected from production and market sites in the Misurata area of Libya. Int. Scholarly Res. Not., 2012, 1-5. doi:10.5402/2012/827645

[16]- Alain, T.K., Luc, B.T. and Ali, D., (2021), Assessment of heavy metal concentration and evaluation of health risk of some vegetables cultivated in Loumbila Farmland, Burkina Faso. J. Environ. Prot., **10**, 1019-1032.

DOI:10.4236/jep.2021.1212060.

[17]- Sultana, R., Tanvir, R.U., Hussain, K.A., Chamon, A.S. and Mondol, M.N., 2022. Heavy metals in commonly consumed root and leafy vegetables in Dhaka city, Bangladesh, and assessment of associated public health risks. Environmental Systems Research, **11**(1),15-27.

https://doi.org/10.1186/s40068-022-00261-9

[18]- Gaurav, V.K., Kumar, D., Sharma, C., (2018), Assessment of metal accumulation in the vegetables and associated health risk in the upper-most Ganga-Yamuna Doab Region, India. Am. J. Plant Sci., **9**(12), 2347-2358. https://doi.org/10.4236/ajps.2018.912170.

[19]- Ametepey, S.T., Cobbina, S.J., Akpabey, F.J., Duwiejuah, A.B. and Abuntori, Z.N., (2018), Health risk assessment and heavy metal contamination levels in vegetables from Tamale Metropolis, Ghana. International Journal of Food Contamination, **5**(1), 1-8. https://doi.org/10.1186/s40550-018-0067-0.

[20]- Antoine, J.M., Fung, L.A.H. and Grant, C.N., (2017), Assessment of the potential health risks associated with the aluminium, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. Toxicol. Rep., **4**, 181-187. https://doi.org/10.1016/j.toxrep.2017.03.006.

[21]- Olfat M. Nassar, O.M., Nasr, H.A., El-Sayed, M.H. and Kobisi, A.E.N., (2018), Heavy metal levels in some popular vegetables from some selected markets in Saudi Arabia. Egypt. J. Bot., **58**(3), 627-638.

DOI: 10.21608/ejbo.2018.4548.1192.

[22]- Omeje, K.O., Ezema, B.O., Okonkwo, F., Onyishi, N.C., Ozioko, J., Rasaq, W.A., Sardo, G. and Okpala, C.O.R., (2021), Quantification of heavy metals and pesticide residues in widely consumed Nigerian food crops using atomic absorption spectroscopy (AAS) and gas chromatography (GC). Toxins, **13**(12), 870-887.

https://doi.org/10.3390/toxins13120870.

[23]- AlKhatib, M., Qutob, A., Kattan, E., Malassa, H. and Qutob, M., (2022), Heavy Metals Concentrations in Leafy Vegetables in Palestine, Case Study: Jenin and Bethlehem Districts. J. Environ. Prot., **13**(1), 97-111. DOI: 10.4236/jep.2022.131006

[24]- Hussain, M.I., Qureshi, A.S., (2020), Health risks of heavy metal exposure and microbial contamination through consumption of vegetables irrigated with treated wastewater at Dubai, UAE. Environ. Sci. Pollut. Res., **27,** 11213-11226. https://doi.org/10.1007/s11356-019-07522-8.

[25]- Al-Fartusie, F.S., Mohssan, S.N., (2017), Essential trace elements and their vital roles in human body. Indian J Adv Chem Sci, **5**(3), 127-136. DOI: 10.22607/IJACS.2017.503003

[26]- Ali, M.H., Al-Qahtani, K.M., (2012), Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets. Egyte. J of Aqutic research, **38**(1), 31-37. https://doi.org/10.1016/j.ejar.2012.08.002.

[27]- Filippini, T., Tancredi, S., Malagoli, C., Cilloni, S., Malavolti, M., Violi, F., Vescovi, L., Bargellini, A. and Vinceti, M., (2019), Aluminum and tin: Food contamination and dietary intake in an Italian population. J. Trace Elem. Med. Biol., **52**, 293-301. https://doi.org/10.1016/j.jtemb.2019.01.012.

[28]- Sato, K., Suzuki, I., Kubota, H., Furusho, N., Inoue, T., Yasukouchi, Y. and Akiyama, H., (2014), Estimation of daily aluminum intake in Japan based on food consumption inspection results: impact of food additives. Food Science & Nutrition, **2**(4), 389-397. https://doi.org/10.1002/fsn3.114.

[29]- Collado-López, S., Betanzos-Robledo, L., Téllez-Rojo, M.M., Lamadrid-Figueroa, H., Reyes, M., Ríos, C., Cantoral, A., (2022), Heavy metals in unprocessed or minimally processed foods consumed by humans worldwide: a scoping review. Int. J. Environ. Res. Public Health, **19**(14), 8651-8676. https://doi.org/10.3390/ijerph19148651.

[30]- Tajdar-Oranj, B., Javanmardi, F., Parastouei, K., Taghdir, M., Fathi, M. and Abbaszadeh, S., (2024), Health Risk Assessment of Lead, Cadmium, and Arsenic in Leafy Vegetables in Tehran, Iran: the Concentration Data Study. Biol. Trace Elem. Res., **202**(2), 800-810. https://doi.org/10.1007/s12011-023-03707-y.

[31]- Isiuku, B.O., Enyoh, C.E., (2020), Monitoring and modeling of heavy metal contents in vegetables collected from markets in Imo State, Nigeria. Environ Anal Health Toxicol., **35**(1), 1-13. doi: 10.5620/eaht.e2020003.