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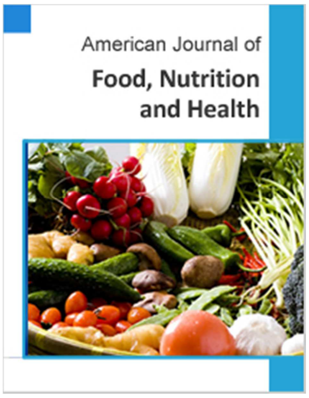


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# Quantitative Determination of Heavy Metal Concentrations in Herbal Teas Marketed in Various Countries including Libya

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## Abstract
This study presents the determination of the amount of some heavy metals (Cu, Cr, Ni, Cd, Mn, Fe, Mg and Pb) present in commercial brand herbal tea samples purchased from local markets in Misurata, Libya, by atomic absorption spectroscopy. The validity of the analytical procedure was monitored by analysing certified reference materials obtained from the Food and Drugs Control Centre, Libya. The concentration of Cu, Cr, Ni, Cd, Mn, Fe, Mg and Pb in all the tea leaf samples ranged from 5.141 to 17.1, 0.890 to 3.4, 0.0833 to 2.349, 0.035 to 0.38, 32.01 to 89.46, 79.01-167, 91.98 to 213.83 and 0.463 to 0.901 $\mu g\ g^{-1}$, respectively. The concentration of heavy metals in the tea leaves can be arranged in the following order, Mg > Fe > Mn > Cu > Cr > Ni > Pb > Cd. Moreover, it is observed that the concentrations of all the toxic elements tested in the investigated herbal plants are found below the permitted levels specified by the international regulatory standards for the medicinal plants.

## 1. Introduction

Tea (Camellia sinensis L.) is a perennial shrub which is grown commercially in about 30 countries. The most important tea exporting countries of the world are Kenya, China, India, Indonesia and Sri Lanka [1]. Tea is the most widely consumed beverage because of its taste, aroma and health benefits. Some 75% of the estimated 2.5 million metric tons of dried tea manufactured annually, is processed as black tea which is widely consumed, Where Tea is used in folk medicine for headache, digestion, diuresis, enhancement of immune defence, as an energizer and to prolong life [2-8]. Tea is considered to be an important source of elements such as manganese and potassium that could be beneficial for hypertensive patients. However, the intake of food contaminated by heavy metals is harmful to human health and several countries have imposed food laws to restrict the presence of heavy metal concentration in food and beverages. Heavy metals accumulation can be derived naturally by soil contamination, use of pesticides and fertilizers, also it comes from manufacturing processes [9, 10]. Metallic constituents of tea leaves differ according to the type of tea (green or black) and its geological source [11]. Various reports have discussed the potential health implications of metals in tea, particularly where tea bushes are known to accumulate trace metals [12].

Tea leaves are a source of mineral elements such as manganese, copper, zinc, iron, magnesium, aluminium, strontium, bromine, sodium, potassium, phosphorous, iodine and fluorine. Tea infusions contain very little vitamins, protein and carbohydrates but may be a source of essential dietary metals and metal binding polyphenols [13].

Several attempts have been made to assess tea quality by chemical analysis. However, to date, little work has been performed to determine the metal content of teas due to the analytical difficulties associated with both the separation of the constituent components and their quantitative measurement [14].

Elements that plants need to survive are called "plant nutrients". In the analysis of plant tissues, it is possible to see almost all the elements found in nature. Although plants are generally selective about the intake of nutrient ions, but as the concentration of nutrient elements found in the growth medium increases, some heavy metals can pass into the body of plants by passive means and can then enter the food chain [15]. As a result, this can affect plant toxicity and compromise the humans and animals health who feed on these plants. However, 16 of these elements (C, H, O, N, P, K, S, Ca, Mg, Fe, Zn, Mn, Cu, B, Cr, Mo) are essential nutrients for all plants. Another six elements (Co, Al, Na, Si, Ni and V) are useful elements that are known to be necessary for only some of the plants or processes [16-18].

Al, Cu, Fe, Mn, Sr and Zn are elements which have major significance for human health [19]. Especially in recent years, teas derived from plant leaves or flowers have experienced an increased consumption in alternative medicine treatments, some popular teas are black tea, green tea and chamomile tea [20, 22]. However, the study reported here is actually rather rare.

The main objective of this study was set to determine the quantity of heavy metals (Cd, Pb, Ni, Fe, Cu, Zn, Mg, and Cr) in herbal teas marketed in Misurata-Libya, it is especially relevant to note that this area was very recently the front line in a civil war zone, where much contamination of crops and plants was evident through destructive deployment of advanced weaponry, from which abnormally high levels of heavy metal deposition is expected. Finally, the results are compared with the outcomes of other publications relating to food and beverage consumption.

# 2. Material and Method

## 2.1. Sample Collection

Seven marked brands of tea, which are normally consumed in Misurata city in Libya, were collected from local markets in November 2013. Tea brands include Zahrat tea (brand 1), Two Rams (brand 2), Budgerigar (brand 3), Super Thamunniy (brand 4), Elarosa Tea (brand 5), Lipton (brand 6) and Al- huseyni Tea (brand 7).

Three packs of each brand with different manufacture dates were obtained. Each sample was analysed to determine the quantity of heavy metals by atomic absorption spectrometry (AAS). About 3 grams of dried sample were taken for analysis in each experiment.

## 2.2. Sample Preparation

The glassware and polyethylene containers used for analysis were washed with tap water, then soaked overnight in 6 N $HNO_3$ solutions and rinsed several times with ultrapure water to eliminate absorbance due to detergent.

3 grams of each sample, accurately weighed, were taken for the analysis. The samples were digested using the recommended method described by AOAC. [23].

3 g of each tea herbs sample were digested using 100 ml of concentrated $HNO_3$ for 10 min. The mixture was heated using electric heater until nearly dried. The mixture was left to cool at room temperature. The digested sample was mixed with mixture of concentrated $HNO_3$ and $HClO_4$ (5:1 v/v). The mixture was heated on electric heater until the solution turned white and gives out the white fumes. The digest was transferred into 50 ml volumetric flask and the volume was adjusted to the mark using distilled water. Concentrations of heavy metals were determined using atomic absorption spectrophotometery. A reference sample for the background correction was prepared using the same procedure.

## 2.3. Analytical Procedure

Cu, Cr, Ni, Cd, Mn, Fe, Mg and Pb in the designated tea samples were analysed using atomic absorption spectrophotometer (AA Analyst 6650, SHIMADZU Atomic Absorption Spectrophotometer). The absorption wavelength for the determination of each metal together with its linear working range and correlation coefficient of calibration graphs are given in Table 1. Data were rounded off suitably according to the value of standard deviation obtained from measurements performed in triplicate.

## 2.4. Statistical Analysis

The results were expressed as the means values with standard deviations. The Pearson correlation coefficient was used for comparing the results between elements and the significance level was determined as $p < 0.01$. The analysis was performed using a software package IBM SPSS Statistics 20

***Table 1.*** *Analytical characteristics of the AAS and flame determination.*

| metal | $\lambda$ (nm) | Concentration $\mu g\ ml^{-1}$ | Correlation coefficient (r) |
|---|---|---|---|
| Cr | 357.9 | 0.05-5.0 | 0.999 |
| Cu | 324.8 | 0.04-5.0 | 0.994 |
| Cd | 228.8 | 0.005-0.5 | 0.998 |
| Ni | 232.0 | 0.08-2.0 | 0.997 |
| Pb | 283.3 | 0.005-0.3 | 0.992 |
| Mn | 279.5 | 0.03-3.0 | 0.999 |
| Fe | 248.3 | 0.05-5.0 | 1 |
| Mg | 285.3 | 0.05-4.0 | 0.998 |

### 2.5. Quality Control

The quality of the analytical procedures was checked using standard Polish Certified Reference Material Tea Leaves (INCT-TL-1) from the Food and Drugs Control Centre, Libya. Each part of the (sample of dry powder) prepared for the study of recovery and analytical reproducibility was assessed using sample duplicates, blanks and certified standards. The analysis of certified reference material (CRM) allowed an evaluation of accuracy and precision over a wide range of element concentrations. The results from the analysis of certified reference material were all found to lie within the 95% reliability limit. The results are given in Table 2

***Table 2.*** *Certificate for the values of the standard reference material and the results of tea leaves.*

| No | Heavy metal | Determined values (μg. $g^{-1}$) | Element Certified values (μg. $g^{-1}$) | Recovery (%) |
|---|---|---|---|---|
| 1 | Cr | 1.82±0.08 | 1.91± 0.22 | 95.29 |
| 2 | Cu | 20.2±1.5 | 20.4±1.5 | 99.019 |
| 3 | Cd | 0.027±0.01 | 0.030±0.004 | 90 |
| 4 | Ni | 5.99± 0.21 | 6.12±0.52 | 97.87 |
| 5 | Pb | 1.76±0.34 | 1.78±0.24 | 98.88 |
| 6 | Mn (%) | 0.152±0.092 | 0.157±0.011 | 96.81 |
| 7 | Fe | 431 | 432 | 99.77 |
| 8 | Mg (%) | 0.212±0.020 | 0.224±0.017 | 94.64 |

## 3. Results and Discussion

The concentrations of Cu, Cr, Ni, Cd, Mn, Fe, Mg and Pb in all samples are shown in Table 3. The accuracy of the method of analysis used was compared to standard reference materials.

Magnesium functions with calcium in the transmission of nerve impulses in the brain. Both elements give relief in patients having depression. [24] Figure 1 shows the distribution pattern for Mg in the tea leaf samples with a mean of 141.11 μg. $g^{-1}$, with a range of 91.98 to 213.83 μg. $g^{1}$. The result obtained for the magnesium determination compares favourably with the values reported by Kazi et al. [25]. Figure 2 shows the distribution pattern of iron, with a range of 79.01-167. μg.$g^{-1}$, and a mean value of 119.39 μg. $g^{-1}$. Fe was the highest in brand 2 and the lowest in brand 6. The bioavailability of this element is influenced by the polyphenols found in tea that can markedly inhibit the absorption of iron. [26]

The copper contents in the examined tea samples ranged from 5.141 to 17.1 μg. $g^{-1}$ with the mean of 10.13 μg. $g^{-1}$ (Figure 3). The results obtained showed higher values than those values reported by Muntean Nicoleta et al. [27] and Marbaniang et al. [28]. The lowest value of copper content was found in brand 1 tea and the highest in brand 2 tea samples. It was evident from this study that the Cu content of all the tea samples were less than 17.1 μg. $g^{-1}$, which is well below the allowable limit of 150 μg. $g^{-1}$ proscribed under the Prevention of Food Adulteration Act, 1954 (PFA), India. The difference of Cu content in the tea herbs could be attributed to different types, grade and production areas of the teas. Cu pollution could be ascribed to occur mainly from the rolling machine used in factory tea leaf production and to agricultural fungicides. [29]

***Table 3.*** *Concentrations of Elements in Tea Products.*

| Metal concentration (μg. $g^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Heavy metal | brand 1 (mean ± SD) | brand 2 (mean ± SD) | brand 3 (mean ± SD) | brand 4 (mean ± SD) | brand 5 (mean ± SD) | brand 6 (mean ± SD) | brand 7 (mean ± SD) |
| Cr | 3.4 ± 0.082 | 2.84 ± 0.09 | 1.476± 0.10 | 0.890± 0.17 | 1.850± 0.18 | 1.065± 0.11 | 2.090± 0.21 |
| Cu | 5.141± 0.10 | 17.1 ± 1.25 | 7.121± 0.70 | 12.211± 0.09 | 8.116± 0.10 | 6.140± 0.10 | 15.133± 0.19 |
| Cd | 0.05 ± 0.02 | 0.12 ± 0.07 | 0.161± 0.15 | 0.38 ± 0.15 | 0.113± 0.15 | 0.14 ± 0.06 | 0.035± 0.03 |
| Ni | 2.349± 0.31 | 1.1 ± 0.40 | 1.9 ± 0.44 | 1.5 ± 0.23 | 0.1982± 0.50 | 0.0833± 0.02 | 0.182± 0.104 |
| Pb | 0.58 ± 0.14 | 0.587± 0.22 | 0.531± 0.18 | 0.860± 0.18 | 0.647± 0.19 | 0.463± 0.13 | 0.901± 0.18 |
| Mn | 43.42± 1.37 | 75.27± 2.13 | 89.46± 2.32 | 54.39± 1.13 | 62.91± 2.26 | 32.01± 1.10 | 47.81± 2.38 |
| Fe | 125.5± 1.21 | 167.1± 1.13 | 156.31± 1.47 | 89.46± 0.97 | 131.83± 2.14 | 79.01± 1.11 | 92.63± 0.98 |
| Mg | 121.62± 1.59 | 185.65± 2.03 | 213.83± 1.34 | 94.89± 1.72 | 136.96±0.97 | 91.98± 1.18 | 142.87± 2.21 |

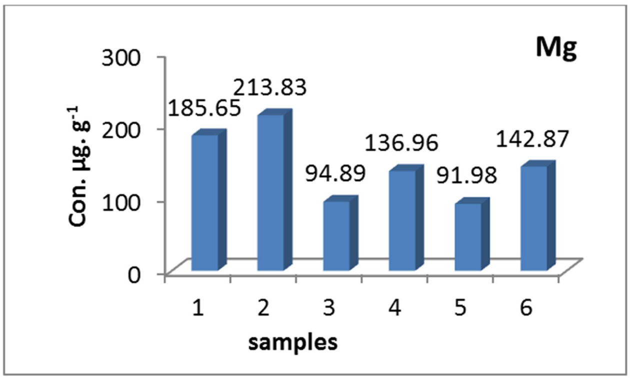


***Figure 1.*** *Distribution of Magnesium in Tea Leaves Sample.*

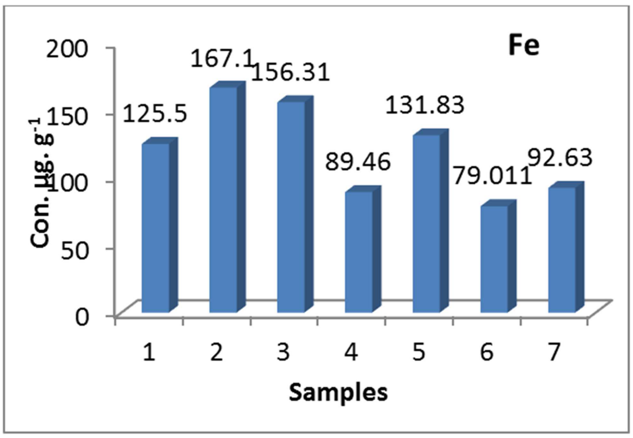


***Figure 2.*** *Distribution of irom in Tea Leaves Sample.*

The concentration of lead (Pb) in tea leaf samples is presented in Figure 4 with a mean of 0.652 μg. $g^{-1}$, and a range of 0.463 to 0.901 μg. $g^{-1}$. The main sources of Pb in tea samples could be ascribed to their growth media, such as soil.

Pb contamination in soil usually can be attributed to industrial activity, agricultural activities (application of insecticides) and urban activities (combustion of gasoline); here, in Misurata, the war zone could also be a contributory factor. Tea plants are normally grown in highly acidic soils where Pb is more bioavailable for root uptake; Deposits from polluted air into the leaves of the plant can be another source of Pb contamination of tea [29]. The results obtained showed higher values than those values reported by Muntean Nicoleta et al. [27].

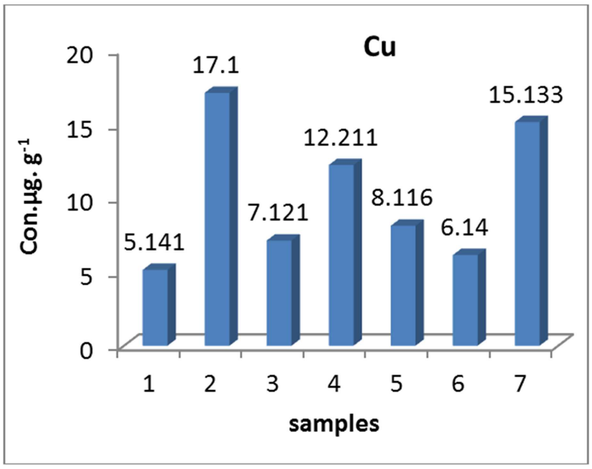


***Figure 3.*** *Distribution of copper in Tea Leaves Sample.*

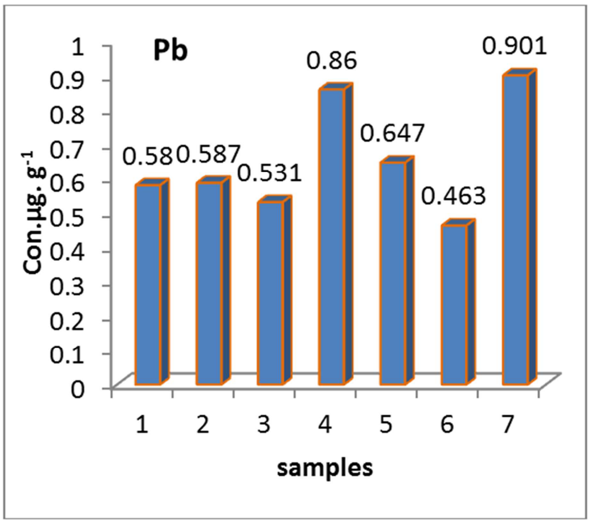


***Figure 4.*** *Distribution of Lead in Tea Leaves Sample.*

Cr in tea may have not only useful but also harmful effects on human health [30]. Cr(III) plays a crucial role in human physiology by stimulating glucose metabolism, controlling blood cholesterol levels, stimulating the synthesis of protein, increasing resistance to pain and suppressing hunger pain [30, 31]. Our study on the herbal tea leaves determined Cr levels 0.890 to 3.4 μg. $g^{-1}$ with a mean value of 1.944 μg. $g^{-1}$ (Figure 5), distinctly different reported values ranged from 0.33 to 2.43 μg. $g^{-1}$ Cr in herbal tea samples [32] and 0.45 to 0.99 μg. $g^{-1}$ in green tea [33] and a reported range from 2.95 to 7.6 μg/g in black tea samples from South India. Cr is normally considered as a local contaminant and is attributed mainly to contamination from the CTC rollers during the manufacturing of tea; the CTC rollers are comprised of gun metals which having only trace level of Cr content [34]. Yasmeen et al., [35] have reported 175 μg $g^{-1}$ Mn in black tea samples from Pakistan.

The levels of Mn in black tea samples from China have been reported by Xie et al., [36] as 607 ± 200 μg $g^{-1}$. Naithani and Kakkar [37] in their study of black tea samples in South India reported a mean Mn concentration of 140 ± 5.29 μg $g^{-1}$. Manganese is an important co-factor for many enzymes and plays an essential role in the body's functions [31].

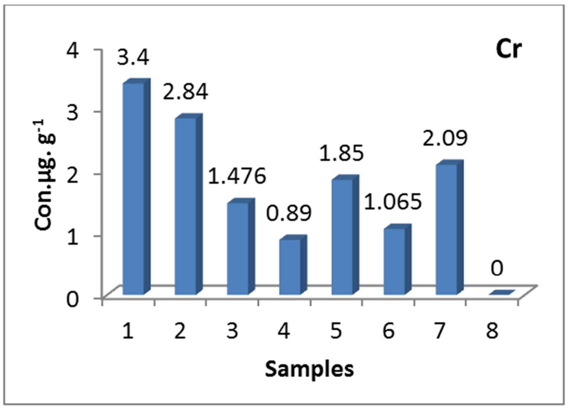


***Figure 5.*** *Distribution of Chromium in Tea Leaves Sample.*

However, high doses of dietary manganese can be associated with long-term toxicity. Therefore, an estimated safe and sufficient daily dietary intake is 2-5 mg [35 =38]. Our study on the herbal tea leaves found Mn levels in the range of 32.01 to 89.46 μg. $g^{-1}$ with a mean value of 57.89 μg. $g^{-1}$. The highest and lowest concentrations of Mn were found in brand 3 and brand 6 respectively (Figure 6).

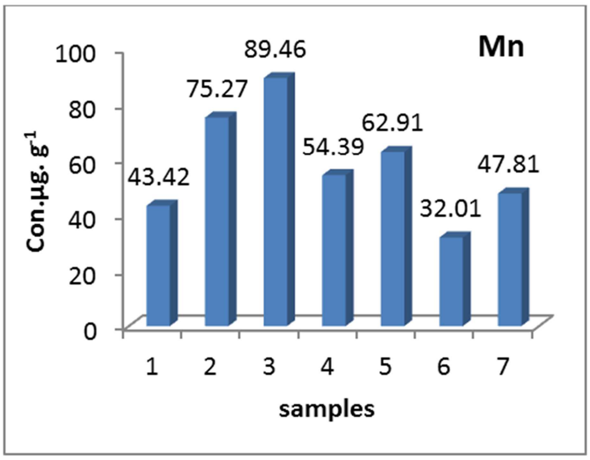


***Figure 6.*** *Distribution of Manganese in Tea Leaves Sample.*

Our study on the herbal tea leaves Ni levels revealed that they ranged between 0.0833 and 2.349 µg. $g^{-1}$ with a mean value of 1.044 µg. $g^{-1}$ (Figure 7). The lowest Ni content was found in brand 6 teas and the highest in brand 1 tea. Nickel in tea samples ranging between 2.89 and 22.6 µg. $g^{-1}$ was previously reported by other investigator [39] and the nickel content in black tea was slightly higher than in green tea. It is believed that nickel contamination mainly occurs through foliar absorption and through the application of low quality fertilizers and micro nutrients to the soil [40]. Since Ni is a toxic element, not having any tolerance limit in tea, the agricultural inputs used in tea plantations should be monitored for heavy metal impurity.

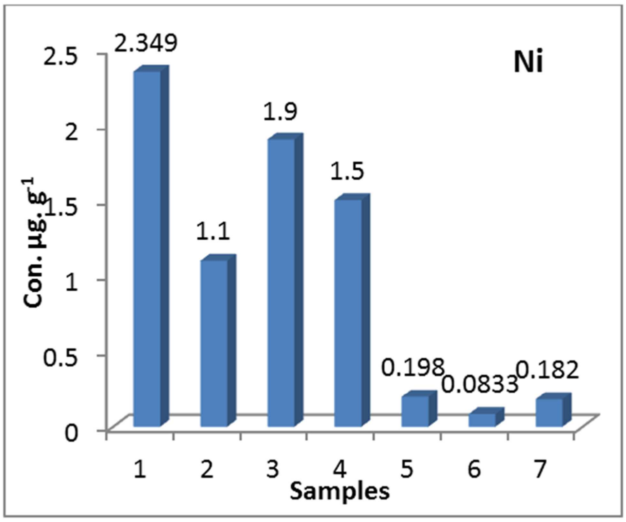


***Figure 7.*** *Distribution of Nickel in Tea Leaves Sample.*

For cadmium, the concentration range found in this study was from 0.035 to 0.38 µg. $g^{-1}$, with an average of 0.142 µg. $g^{-1}$ (Figure 8). Seenivasan et al. [41] reported a lower mean Cd concentration in black tea samples from South India as 0.14 ± 0.06 µg $g^{-1}$. Waqar and Mian [42], and Narin et al. [43] reported a higher mean Cd concentration as 1.1 ± 0.5 and 2.0 ± 0.8 µg $g^{-1}$ in black tea leaves from Saudi Arabia and Turkey, respectively. Shen and Chen [44], in their study of metal concentration in green and black tea in Taiwan reported a mean Cd concentration of 0.07 µg $g^{-1}$ in black tea, which is lower than the present report. The Cd concentration of tea leaves from several tea estates in different regions varied from small amounts to a large amount depending on the soil structures.

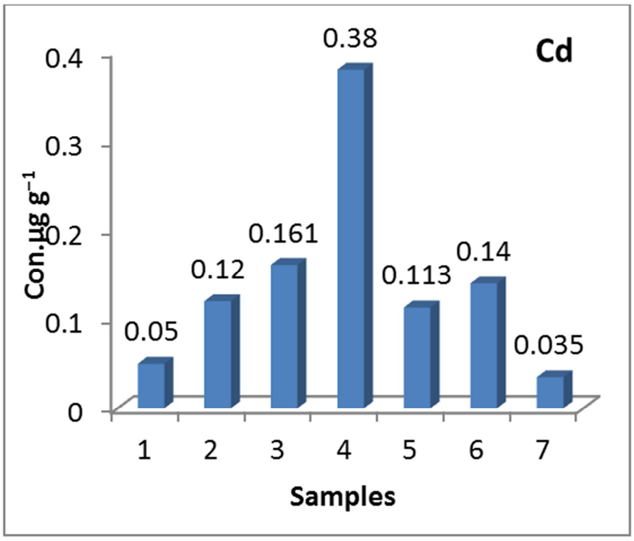


***Figure 8.*** *Distribution of Cadmium in Tea Leaves Sample.*

In order to estimate possible correlation between elements, the results obtained are analyzed using statistical software. The results demonstrated significant Pearson correlations (at the 0.01 level) between Zn-Cu-Mn, Fe-Cd, Fe-Mg, Pb-Cd, Mn-Mg and Ni-Cr: 0.79; 0.71; 0.69; 0.68; 0.80 and 0.77, respectively. Our results did not show any significant correlation between other elements.

Table 4 shows the survey of metal contents for various herbal teas from different regions. The conclusion can be drawn that there are significant differences in the heavy metal contents in herbal teas, which can be ascribed to the different soil quality on which the plants had been grown. It is a rather complex deduction which must bear in mind the geographical distances between the different regions on one hand, and on the other hand the ability of the different tea plants to accumulate the individual heavy metals. It is well known that some plants have an extraordinary ability to accumulate heavy metals and are used for bioremediation of the soil [51].

***Table 4.*** *Comparison of metal contents (µg. $g^{-1}$) of herbal teas marketed in Misurata-Libya with others from different areas.*

| Country | Zn | Mn | Fe | Pb | Ni | Cu | Cd | Reference |
|---|---|---|---|---|---|---|---|---|
| Egypt | 8–68.8 | 9.8–289 | 26.96–1046 | 0.5–14.4 | 0.61-2.85 | 1.8–11.4 | 1.06–2.44 | 45 |
| Turkey | 21.9–48.4 | 23-244 | 224.8-810 | 0.26-4.80 | 0.90-5.4 | 3.92–35.8 | 0.004–0.44 | 46 |
| Iran | - | - | - | 2.08-2.59 | - | 17.59-32.8 | - | 47 |
| India | - | - | - | 0.48–1.03 | 1.1–5.3 | 15.9–32.2 | 0.05–0.38 | 48 |
| Pakistan | 55.3–70 | 24.6–28.9 | 125.2-151.1 | - | - | 12.2–14.3 | - | 49 |
| Serbia | 15.0–43.0 | 25.0-111 | 75.0–546 | - | - | 5.92–14.79 | - | 50 |
| Libya (Misurata) | - | 32.01-89.46 | 79.01-167.1 | 0.463-0.901 | 0.0833-2.349 | 5.141-17.12 | 0.05-0.38 | Present study |

## 4. Conclusion

As tea is an indispensable part of everyday life for many people in Misurata, Libya, as elsewhere, these studies can be considered as a preliminary experiment which should be expanded and continued to ensure that public health is properly monitored and screened. Many scientific studies have concluded that tea drinking is beneficial and helps prevent many diseases, including skin cancer, Parkinson's disease, myocardial infarction, and coronary artery disease.

The samples studied here contain metals in trace levels, with concentrations varying from sample to sample; the most dangerous metals for human health (lead and cadmium) are present in only low concentrations, which is perhaps a rather surprising result initially in view of the previous military activity which has seemingly not affected the uptake of these metals from soil. The determination of the metal content of herbal teas allows the assessment of environmental pollution and the quality and quantity of metal ions made available through their uptake by human beings. Due to their hazard, the content of heavy metals in such products has to be one of the main criteria for the use of plants as raw materials in the production of traditional medicines and herbal infusions. Therefore, it is essential to have a good quality control of plant raw materials used for preparing herbal teas to ensure the safety and efficacy of herbal products.

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