JAEHR
Journal of Advances in Environmental Health Research
J Adv Environ Health Res, 2023; 11(3): 168-173. doi: 10.34172/jaehr.1300
http://jaehr.muk.ac.ir

Original Article

Analysis and Evaluation of Trace Elements in Iranian Olive Samples Using ICP-OES

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Article history:
Received: June 21, 2022
Accepted: January 2, 2023
ePublished: September 29, 2023

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Abstract

Background: One of the concerns faced by many countries, especially developing countries, is the presence of heavy metals in water, air and food, which directly and indirectly cause adverse effects on human health through inhalation and ingestion. Rudbar city is suitable for growing olives due to its climatic conditions, and most of the people living in the area use this product in their meals. The purpose of this study was to determine the amount of heavy metals manganese, iron, copper, zinc (Zn), lead and cadmium in the olives of this area.

Methods: For this purpose, 13 olive samples were selected and their heavy metal concentration was measured by ICP-OES device.

Results: The results indicated that the concentrations of cadmium, lead, manganese, iron, Zn and copper in the samples of Guilan olives were 0.056±0.019, 0.564±0.170, 2.017±0.211, 7.577±1.312, 1.846±0.387 and 4.147±0.901, respectively. Also, in the imported samples it was 0.065±0.014, 0.494±0.153, 1.295±0.353, 3.465±1.066, 1.357. 0.412 and 4.103±1.335, respectively.

Conclusion: Except for one, the amount of lead in all Iranian samples was less than the allowable level. It was also observed that except for lead, the concentration of all metals in foreign samples was lower than Iranian samples. Since olives are one of the most popular food items in the area and considered by travelers, continuous monitoring of the heavy metals in this food is essential.

Keywords: Olive, Heavy metals, Guilan, ICP-OES

Please cite this article as follows: Aboutaleb E, Kazemi Z, Kazemi Z, Azizi Khereshki N. Analysis and evaluation of trace elements in Iranian olive samples using ICP-OES. J Adv Environ Health Res. 2023; 11(3):168-173. doi:10.34172/jaehr.1300

Introduction

Measuring the concentration of heavy metals in food products is a crucial factor for assessing their quality and safety.[1] So, due to the toxicological and nutritional significance of these metals, there is an increasing interest in the measurement of the metal contents of food around the world in the recent decades. The heavy metals in foods in permissible concentrations are useful and essential for human metabolism (e.g., enzymatic activities) and health[2-4] whereas they are harmful and toxic in higher concentrations and can cause health problems to humans and plants.[5-7] Among the heavy metals, cadmium and lead are of special concern due to their potential toxicity at low concentrations. There is more established evidence for their role in the increased risk of various diseases such as Alzheimer[8] and pancreatic cancer.[9] It is found that the industrial, domestic and agricultural activities are the main sources of heavy metals pollution in food.[10, 11]

Olive is considered as one of the important food products around the world which has an important place in the Mediterranean diet as a dietary pattern. It is confirmed that the active polyphenolic compounds in the olive and its oil have the major role in preventing cardiovascular and neurodegenerative diseases, diabetes mellitus and cancer.[12,13]

Spain, Italy, Greece and Turkey with 79% of world production are the largest olive producers. Also, Iran with 29 598 ha area harvested and 37 954 t of olive production was placed in the twelfth rank in 2011.[14] As shown in Figure 1, Guilan Province, located in the north of Iran and on the south of Caspian Sea, is known as the oldest and largest olive producer of this country with about 40% of the annual production.[15] Nowadays, the consumption and demand for olive and its oil is rapidly increasing not only in Iran but throughout the world due to it is recognized dietetic and nutritional value.[16] Therefore, it is

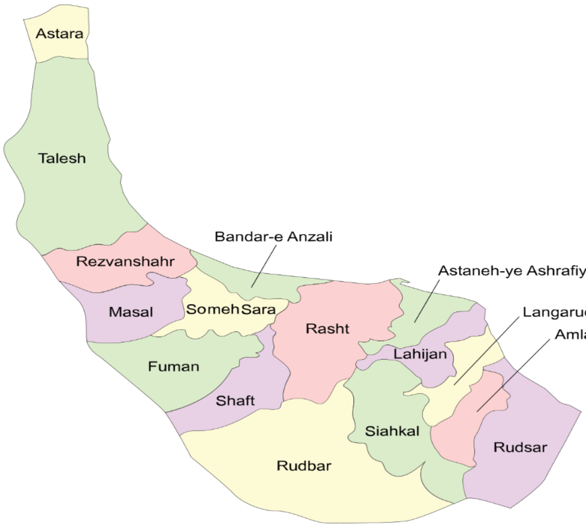

Figure 1. Location of Guilan Province in Iran (as the study area)

necessary to determine their metal content as a significant indicator of toxicological and nutritional assessment. At present, the common techniques for determination of the heavy metals in olive and its oil include electrothermal, flame and graphite furnace atomic absorption spectrometry (ETAAS, F-AAS and GF-AAS)[17] induced coupled plasma atomic emission spectrometry (ICP-AES),[18] induced coupled plasma mass spectrometry (ICP-MS)[19] and coupled plasma optical emission spectrometry (ICP-OES).[20]

The main aim of this study was to investigate and determinate the quantity of the heavy metals including Mn, Fe, Cu and zinc (Zn) especially toxic metals Pb and Cd in consumed olive samples in Guilan province. Accordingly, 13 olive samples including 10 local samples collected from different locations of Guilan province and 3 foreign samples were randomly selected. The samples were digested by wet digestion method for the elimination of organic content of the samples. Then, inductively ICP-OES was used to monitor the concentration of the heavy metals.

Material and Methods

Study Area

Rudbar city is located in a desert area with an area of 2.5 hectares in the south of Rasht city (latitude 36˚ 43′ 43″north and longitude 49˚ 24′ 36″ east) and an altitude of 138.3 meters above sea level. This city is located between the mountains and the adjacent dry cities (Manjil and Lushan) and has a hot and dry climate that is suitable for growing olives. The average annual rainfall is 300.4 mm, the temperature is 20.4 °C and the relative humidity is 61%. The geographical location of the study area is shown in Figure 1.

Digestion of Olive Samples

Each olive sample weighing 0.5 g was dried in the oven and subsequently crushed. Then, 1 mL of deionized water was added and the solution was supplied with an appropriate digestion mixture of $HNO_3/H_2SO_4/H_2O_2$, 4 + 1 + 1 (v/v) (wet digestion method 2). After the reaction, the reaction mixture was heated at 125 °C. The heating was gradually continued until the evolution of fumes ceased and it was stopped when carbonization appeared. After the reactor was cooled to room temperature, 1 mL of HNO_3 was added and the reaction mixture was heated again for a complete digestion. The resulting residue was cooled, dissolved in 2.5 mL HCl and then diluted to 50 mL deionized water. All digestions were performed in triplicate. The blank solutions were prepared under the same conditions without olive/olive oil samples to check the possible metal contaminations in the reagents used. Also, standard metal solutions were freshly prepared by dilution of standard stock solutions (1000 mg L^{-1}) in a 2% HNO_3 solution. In this study, to minimize metal contamination of the samples, all vessels were immersed in freshly prepared 10% HNO_3 for 24 hours, then washed thoroughly with doubly deionized water before use. The prepared solutions were analyzed by ICP-OES.

Statistical Analysis

Analysis of variance (ANOVA) followed by Scheffé's test was used to determine the statistical significance of any difference among the defined groups. A value of $P < 0.05$ was considered to indicate a significant difference. The results were presented as mean value (MV) ± standard deviation (SD). All experiments were carried out using a simultaneous atomic emission spectrometer ICP-OES (SPECTRO AMETEX model: ARCOS FHE12, Germany power 5000 VA). The spectral lines utilized for quantification (in nm) were as follows: Cd (214.438), Pb (220.353), Cu (324.754), Zn (213.856), Fe (259.941), and Mn (257.611).

Results and Discussion

Trace Element Determination

In this study, the concentration of six heavy metals namely Pb, Cd, Mn, Fe, Cu and Zn were analyzed in 13 table olive samples (10 Iranian and 3 Foreign samples). The mean value of the concentration of the metals (MV) along their standard deviation (± SD) are shown in Table 1 and Figure 2. First, we discuss the results of Pb and Cd in the studied olive samples. The results showed that the highest and lowest Pb concentrations in Iranian olive samples were 1.350 ± 0.287 mg/kg and 0.072 ± 0.050 mg/kg, respectively (Table 1, Figure 2), with total mean value of 0.564 ± 0.170 mg/kg (Table 2). The content of Pd in nearly all Iranian olive samples, with the exception of D3 at 1.350 ± 0.287 mg/kg (Table 1, entry 5), was found to be below the maximum permissible limit of 1 mg/kg (Figure 2). According to the United Nations (FAO) and the World Health Organization (WHO)' standards (Codex Alimentarius Commission, 193-2009) [21] and the European Community standards EC (European Commission, 1881-2006),[22] the permissible maximum concentration of Cd and Pb in the

Table 1. The Content of Trace Elements in Olive Samples mg/kg

Entry	Brand	Cd	Pb	Mn	Fe	Zn	Cu
1	D_1	0.068 ± 0.021	0.583 ± 0.117	1.836 ± 0.203	5.573 ± 0.240	2.711 ± 0.515	4.786 ± 0.728
2	D_2	0.040 ± 0.028	0.339 ± 0.184	0.747 ± 0.122	1.150 ± 0.272	0.369 ± 0.097	1.679 ± 0.310
3	D_3	0.082 ± 0.004	0.135 ± 0.003	1.407 ± 0.200	4.504 ± 0.627	1.471 ± 0.077	3.457 ± 0.299
4	D_4	0.058 ± 0.011	0.982 ± 0.329	2.003 ± 0.229	25.922 ± 4.342	2.163 ± 0.152	2.927 ± 0.528
5	D_5	0.034 ± 0.026	1.350 ± 0.287	1.878 ± 0.293	5.459 ± 0.696	1.414 ± 0.125	3.653 ± 0.440
6	D_6	0.057 ± 0.017	0.421 ± 0.370	1.941 ± 0.021	4.397 ± 0.464	1.668 ± 0.810	4.471 ± 1.023
7	D_7	0.078 ± 0.006	0.072 ± 0.050	2.841 ± 0.433	12.285 ± 2.992	2.241 ± 0.666	5.247 ± 2.054
8	D_8	0.050 ± 0.036	0.418 ± 0.156	2.390 ± 0.267	6.259 ± 1.707	1.531 ± 0.140	5.138 ± 0.281
9	D_9	0.047 ± 0.013	0.399 ± 0.017	3.162 ± 0.073	4.984 ± 0.778	2.221 ± 0.459	3.666 ± 0.609
10	D_{10}	0.043 ± 0.025	0.947 ± 0.186	1.964 ± 0.265	5.242 ± 1.000	2.667 ± 0.833	6.444 ± 2.733
11	F_1	0.064 ± 0.006	0.554 ± 0.258	0.978 ± 0.411	2.922 ± 0.756	1.217 ± 0.530	2.808 ± 1.735
12	F_2	0.063 ± 0.009	0.768 ± 0.122	1.235 ± 0.459	3.098 ± 1.858	0.749 ± 0.443	2.907 ± 1.554
13	F_3	0.069 ± 0.016	0.160 ± 0.081	1.673 ± 0.189	4.376 ± 0.583	2.104 ± 0.262	6.595 ± 0.717

Mean value (MV) and Standard Deviation(± SD) were reported:

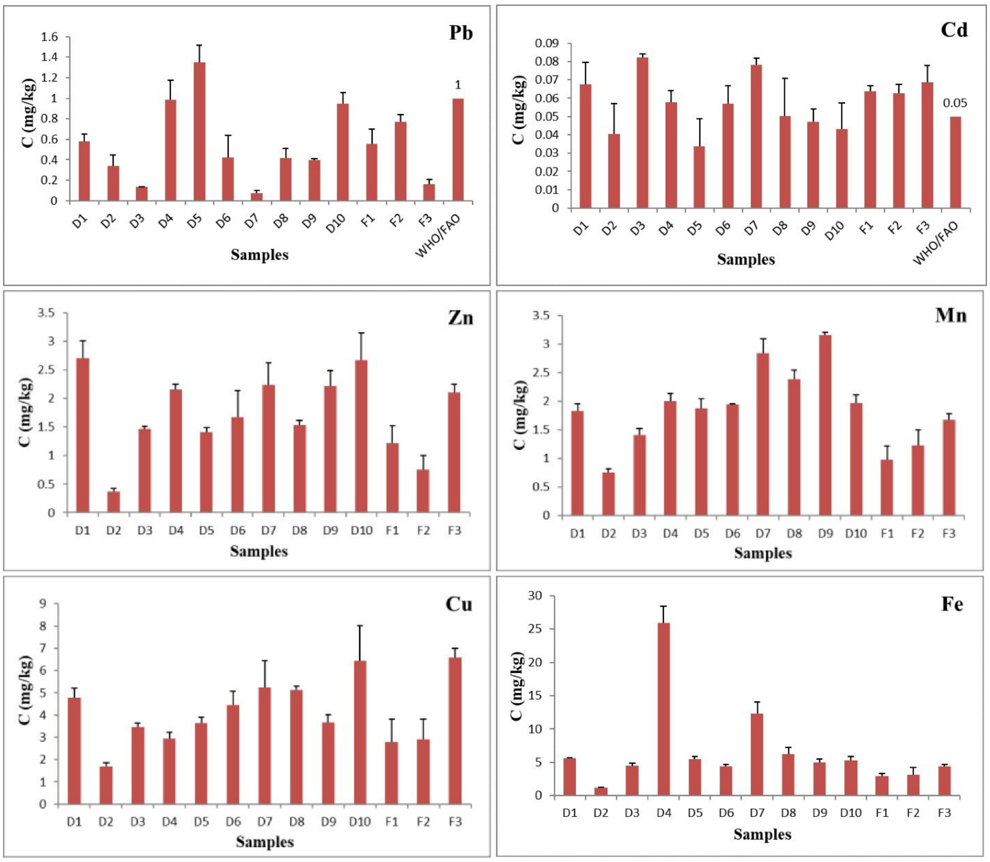

Figure 2. Distribution of the metals in olive samples; The standard limits for Pb and Cd were presented

table olive for human consumption are 0.05 and 1 mg/kg, respectively (Figure 2)[23] which was approved by Institute of Standards and Industrial Research of Iran (ISIRI). On the other hand, the content of Pb in the foreign olive samples ranged from 0.160 ± 0.081 to 0.768 ± 0.122 with total mean value of 0.494 ± 0.153 which are at acceptable levels and a little bit lower than Iranian ones (Table 1, Figure 2). Codex Alimentarius Commission has recently lowered the permissible maximum concentration for Pb in table olive from 1 mg/kg[23] to 0.4 mg/kg.[24] Accordingly, the Pb concentrations in 6 Iranian olive samples were close to the standard and the other 4 samples exceed the permissible limit recommended by Codex Alimentarius Commission.[24] Among the 3 foreign olive samples, only F_3 was less than standard limit and the other 2 samples were higher. The European Community also set the maximum

Table 2. The Total Concentration (Mean ± SD) and ANOVA Table for the Trace Elements in Olive Samples

Entry	Metals	Mean ± SD (mg/kg)		F	P Value[a]
		Iranian	Foreign		
1	Cd	0.056 ± 0.019	0.065 ± 0.014	1.732	0.117
2	Pb	0.564 ± 0.170	0.494 ± 0.153	10.448	0.000*
3	Mn	2.017 ± 0.211	1.295 ± 0.353	18.567	0.000*
4	Fe	7.577 ± 1.312	3.465 ± 1.066	41.343	0.000*
5	Zn	1.846 ± 0.387	1.357 ± 0.412	6.696	0.000*
6	Cu	4.147 ± 0.901	4.103 ± 1.335	4.133	0.001*

[a] Mean are the same $P > 0.05$ and $F < F_{crit}$; Mean are different $P < 0.05$, $F > F_{crit}$.
* Significant.

limit of Pb in olive as 0.1 mg/kg (European Commission, 2015).[25] Applying this standard, the majority of both Iranian and foreign samples did not meet the criteria, with the exception of D7 which aligned well. Additionally, D3 and F3 slightly exceeded the standard limit but remained very close to it.

The levels of Cd concentration in Iranian olive samples were ranged from 0.034 ± 0.026 mg/kg to 0.082 ± 0.004 mg/kg (Table 1 and Figure 2) with total mean value of 0.056 ± 0.019 mg/kg (Table 2). This closely aligns with the standards set by WHO/FAO (Codex Alimentarius Commission, 193-1995, 2011[23] and 2019[24]) as well as EC standards (European Commission, 2015),[25] which specify a Cd concentration limit of 0.05 mg/kg for table olives. Within the set of Iranian olive samples, 7 of them exhibited Cd levels that were either below or nearly equivalent to the maximum permissible limit. Conversely, the remaining 3 samples surpassed this limit. The mean value of Cd concentration in foreign olive samples was 0.065 ± 0.014 mg/kg which is higher than that of Iranian samples and also the standard limits (Table 2).

Lead and cadmium are highly toxic elements. Previous studies have shown that due to the proximity of olive groves and fields to highways and metallurgical industries, these toxic compounds are present in the olive product. In accordance with the guidelines established by the FAO/WHO and international regulations, the acceptable limit for Cu in olives is 0.1 μg/g, while the permissible range for Zn falls between 0.04 μg/g and 0.70 μg/g.[26] Also Cu up to 10 mg is considered safe for adults weighing 60 kg. The permissible level of Zn is recommended for daily consumption of 15 mg for adult men and 12 mg for adult women. Although Zn in the food basket is recommended to increase body growth, according to the study by Zhang et al, exposure to the metal upsets the balance of cholesterol and fertility.[27,28] Zn and copper have also been reported to lead dysfunction of the central nervous, respiratory, and endocrine systems.[29] Research has shown that increasing in the concentration of lead in the body also has detrimental effects on the central nervous system and blood enzymes.[27] In addition, exposure to high concentrations of lead leads to high blood pressure, kidney problems, miscarriage, and increased mortality in children.[30] According to the study by Zukowska and Biziuk, an increase in cadmium in the body is also associated with bone fractures and pulmonary adenocarcinoma.[31] Iron is one of the essential elements of the body, though according to the Toxic Diseases Registry (ATSDR 2012), an increase in its concentration in the body leads to weight loss, joint pain, fatigue and heart and liver problems.[28]

ANOVA Analysis

The significance of metal concentrations was tested by ANOVA, and the results are summarized in Table 2 and Figure 3. For all the investigated metals (Pb, Cu, Mn, Zn and Fe) except for Cd, significant differences ($P < 0.05$) were found among 13 olive samples. The content of Cd in all samples was almost similar and no significant differences was found ($P > 0.05$).

Conclusion

In this study, ICP-OES was used to investigate heavy metals in 13 olive samples (10 Iranian samples and 3 foreign samples) in Guilan province. The results indicated that except for one sample, the amount of lead in all Iranian samples was less than the allowed maximum. The concentration of lead in foreign samples was acceptable and lower than Iranian samples. It was also observed that except for lead, the concentration of all metals in foreign samples was lower than Iranian. ANOVA analysis showed that cadmium metal was present in almost all samples and no significant difference was observed ($P > 0.05$).

Acknowledgments

The authors express their appreciation to the Deputy of Research of Guilan University of Medical Sciences for the financial support.

Authors' Contribution

Conceptualization: Ehsan Aboutaleb, Zahra Kazemi, Nasibeh Azizi Khereshki.
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Funding acquisition: Ehsan Aboutaleb, Nasibeh Azizi Khereshki.
Investigation: Ehsan Aboutaleb, Zahra Kazemi, Zohre Kazemi, Na sibeh Azizi Khereshki.
Methodology: Ehsan Aboutaleb, Zahra Kazemi, Zohre Kazemi.
Project administration: Ehsan Aboutaleb, Zahra Kazemi, Zohre Kazemi.
Resources: Ehsan Aboutaleb, Nasibeh Azizi Khereshki.
Software: Ehsan Aboutaleb, Zohre Kazemi, Nasibeh Azizi Khereshki.
Supervision: Ehsan Aboutaleb.
Validation: Ehsan Aboutaleb, Nasibeh Azizi Khereshki.
Visualization: Ehsan Aboutaleb.
Writing–original draft: Ehsan Aboutaleb, Zahra Kazemi, Zohre Kazemi.
Writing–review & editing: Ehsan Aboutaleb, Zahra Kazemi, Zohre Kazemi, Nasibeh Azizi Khereshki.

Competing Interests

The authors of this article declare that they have no conflict of interests

Ethical Approval

This study was approved by the Ethics Committee of Guilan

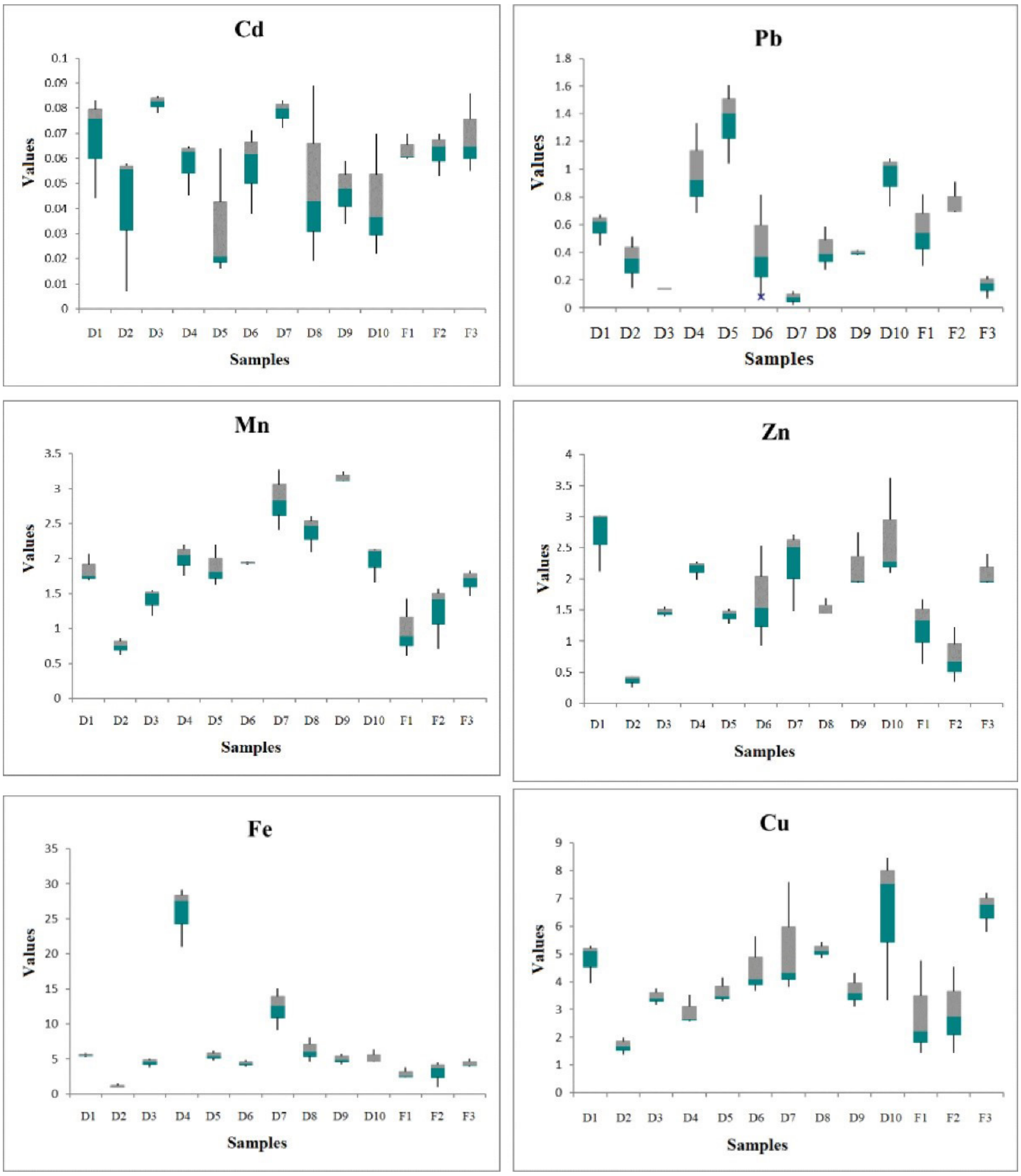

Figure 3. The ANOVA Diagrams Obtained From the Analysis of the Content of Trace Elements

University of Medical Sciences, Rasht, Iran under grant number of 93072209.

Funding

This study was supported by the Deputy of Research at Guilan University of Medical Sciences, Rasht.

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