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Research article

COMPARING SOME PHYSICAL AND CHEMICAL PROPERTIES OF GREEN OLIVE (*OLEA EUROPEA* L.) IN IRAN ASSOCIATION WITH ECOLOGICAL CONDITIONS

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ABSTRACT: Enhancing the under cultivation area of olive is one of the most important programs of the project of Iran. Per capita consumption of olive stands at 160 grams annually. Due to importance of olive quality and considering some environmental factors, the aim of this research was to determine the physical and chemical properties, fatty acid, mineral and heavy metal contents of green olive fruit cultured in top olive growing regions of the Iran country: Association with ecological conditions. Green olive fruits were collected from 10 different farmlands in Rudbar-Aliabad from Gilan Province And Tarom From Zanjan Province which are considered the most important regions of olive cultivation in Iran . In each location, the chosen cultivars were those predominating in the respective area. The mineral elements and heavy metals: K, Na, Ca, Zn, Cu, Fe, Mn, Pb and Cd contents in olive fruits were measured using an atomic absorption spectrometer. Fatty acids were determined by gas chromatography. According to variance analyses of data, oil rate and most of the fatty acids in olive oil samples were significantly affected by growth location. There was a positive correlation between the locations of growing with the weight of olive fruits ($p < 0.005$). The results showed high levels of Pb and Cd in all olive samples studied. Correlation matrix for lead content shows a highly significant positive correlation among locations and its content. The mean content of lead and Cadmium levels in the 1100 samples analyzed exceeded both limits set for these heavy metals.

Key Words: Green Olive, Heavy Metals, Mineral Contents, Fatty acid, Zanjan, Gilan

INTRODUCTION

Olive, whose scientific name is *Olea europea* L. is a plant from Oleaceae family and *Olea genus* [1]. The olive tree (Olea europea L.) grows in a subtropical climate as a traditional main crop, familiar in Mediterranean countries [2] and this region alone produces 99% and consumes 87% of the world's olive oils [3] . It may have originated in Syria, Asia Minor, Ethiopia, Egypt, or India. Likewise the olive is found widely in Cyprus, the coasts of Turkey, Syria, Lebanon, Israel, the south of Spain, France, Italy, and the coast of North Africa. Spanish migrants spread the olive to Mexico, Argentina, and Uruguay in Latin America, and Italians took it to Australia [2]. Due to the popularity of this fruit and the oil extracted from it, the areas under cultivation has been increased areas under cultivation has been increased other part of the world, particularly middle other part of the world, especially Middle East and to some extent, Iran [4]. According to the statistics provided by Food and Agriculture Organization of United Nations (FAO) in 2009 olive cultivation in Iran has been 31,114 hectares and the rate of the product 40,025 ton[1]. A high degree of variability has been observed empirically in each of these cultivars but thorough study of the extent and attributes of this variability is still lacking. According to the National Research Centre for Genetic Engineering & Biotechnology, the following are the agronomic and commercial characteristics of the most important varieties: Mari, Zard, Rowghani**,** Gelooleh, Dakal, Fishomi [5]. Most of Iran's varietal heritage is located in the valley of Sefi-Rud, Tarom and Manjil about 60–70 km from the coast of the Caspian Sea.

Olive cultivars are distributed across the provinces of Gilan, Zanjan, and Golestan in the North and Khozestan and Fars in the South. Some parts of Gilan (Loshan, Manjil, Rodbar, Aliabad, Jodaky, Vakhman, Bahramabad, Kalashtar, Koshk, Rostamabad, and Ganjeh) are the most important olive growing areas in the country [6].

The fruit contains water (up to 70%) which is called "vegetable" water. The average chemical composition of the olive fruit is: water, 50%; protein, 1.6%; oil 22%; carbohydrates, 19%; cellulose, 5.8%; minerals (ash) 1.5% [7] . Olive fruit is classified as a drupe because it has a woody stone containing the embryo, or seed, surrounded by flesh (the pericarp) enclosed by a protective skin. The general physical features of olive fruit are: Length 1–3 cm, diameter 1–2 cm, Weight from 0.5–15 grams or more, Flesh makes up 60–90% of total fruit weight, Stone makes up 10–40% of fruit weight, Embryo (seed) inside stone makes up 1–2% of fruit weight. Olive shapes include pear, egg and heart; other features include asymmetry, nipples and points. The water content of olive flesh is around 60–70% w/w. It follows, therefore, that the lower the water contents of olive flesh, the higher its nutritive and energy value. Olive flesh has a low sugar content (2–6%w/w), high oil content (10–30% w/w) and contains the unique extremely bitter glycoside oleuropein([8] . Researchers have investigated the relationships between mineral elements [9, 10] and flower bud formation in different fruit types as well as the olive tree [11]. Olive is a rich source of valuable nutrients and bioactive of medicinal and therapeutic interest. The mineral element contents in fruit trees is acquiring interest for their effect on characteristics of the fruits quality in general, and olive oil quality in particular [12]. This fruit contains appreciable concentration of hydrophilic (phenolic acids, phenolic alcohols, flavonoids and secoiridoids) and lipophilic (cresols) phenolic compounds that are known to possess multiple biological activities such as antioxidant, anti-carcinogenic, anti-inflammatory, anti-microbial, antihypertensive, anti-dyslipidemia, cardio tonic, laxative, and antiplatelet. Other important compounds present in olive fruit are pectin, organic acids, and pigments. Virgin olive oil (VOO), extracted mechanically from the fruit, is also very popular for its nutritive and health-promoting potential, especially against cardiovascular disorders due to the presence of high levels of monounsaturated and other valuable minor components such as phenolics, phytosterols, tocopherols, carotenoids, chlorophyll and squalen [13]. However, heavy metals are natural components of the Earth's crust that main rout enter to the human body is through Food and intake of heavy metal-contaminated fruit or vegetables may pose a risk to the human health [14, 15, 16, 17, 18, 19]. Some of these elements are essential for human health such as Cu, Fe, Mn, Zn while others, if present even at low levels and tendency to accumulate in human organs can be toxic (e.g., Cd, Pb, Hg, As) [20,21,22]. Elements As, Cr, Cd and Pb are very important on account of their toxicity and metabolic role because they catalyze the decomposition of hydro-peroxides, aldehydes, ketones, acids and epoxides. These compounds may develop pathological effects on the digestive system and increase carcinogenic effect by reacting with other food components such as proteins and pigments. Some of these metals may be harmful if present in the final product, even at low concentrations [23]. High concentrations of lead in the body can cause death or permanent damage to the central nervous system, the brain, and kidneys. This damage commonly results in behavior and learning problems (such as hyperactivity), memory and concentration problems, high blood pressure, hearing problems, headaches, slowed growth, reproductive problems in men and women, digestive problems, muscle and joint pain. Cd appears to accumulate with age, especially in the kidney and it is considered also as a cancer and cardiovascular diseases [24].

In recent years, however, cancer has become the third major cause of mortality in Iran after cardiovascular disease and accidents. Breast cancer is the major cause of cancer morbidity and mortality between women in the world especially in Iran. Metals involved in environmental toxicology are closely related to tumor growth and cancer. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide. In Iran, the incidence of breast cancer is rising and ranked first among malignancies in women [25]. Lung cancer remains the leading cause of cancer death in the world to date, the rate of lung cancer has been relatively low in Iran. Most lung cancer can be explained by external environmental or behavioral factors. Of these, cigarette smoking is the main risk factor but heavy metals are significantly associated with lung cancer [26]. Cancer of the pancreas is an important cause of cancer mortality in developed countries. This cancer is still the disease of elderly people in Iran. Iran where there is rapid industrial development, the amount of toxic metals that accumulate in the human body from a polluted environment is expected to increase. It has become evident that there is an intimate relationship between trace elements and cancer. The high levels of Cadmium (Cd) and lead (Pb) may be linked with a number of physiological disorders in humans [27, 28]. Due to the importance role of heavy metals play in health status of the human body with regard to the incidence of cancer in Iran, the present study was initiated to investigate the levels of contamination with them and then determine and emphasis on their toxicological implications in olive cultivated in the farmlands in Rudbar-Aliabad from Gilan Province and Tarom From Zanjan Province which are considered the most important regions of olive cultivation in Iran.

It is necessary to know the amount of these metals in green olives in Olives and it has been reported that the old commercial olive orchards are located mainly in the north of Iran and more than 85% of olive production belongs to these regions [25]. The aim of this investigation was to:

Determine the physical and chemical properties, fatty acid, mineral and heavy metal contents of olive fruit cultured in top olive growing regions of Iran (Rudbar-Aliabad from Gilan Province and Tarom from Zanjan Province): Association with ecological conditions.

MATERIAL AND METHODS

Study Area

The provinces of Fars, Kerman, Hormuzgan, Sistan-Baluchistan, Qazvin, Gilan, Golestan and Zanjan are the top olive growing regions of the Iran. In Iran, the olive tree is usually grown under poor soil conditions not suitable for field crops and three-quarters of the areas in Iran under olive cultivation are located in hillsides. Soil fertility is often considered invariable at the small scale, such as the olive orchard level[29] . In this research two locations of the most important regions of olive cultivation was studied:

1- Tarom and Gilvan farms in Zanjan province have the highest acreage under olive cultivation. The experimental field was located in a 320 hectare irrigated olive orchard located in Tarom region, of Zanjan Province in Iran (between 48° 56′ and 50°5′ E; and 36° 47′ and 37° 36′ N). The orchard is covered with 18 year-old olive trees cv ‘Zard. The trees have been planted at a density of 204 trees/ha(7×7 m) and the soil is sandy. 2- IRIB in 08 September 2013 reported that Roudbar ranks third nationwide in terms of production of olive [30]. Rudbar is 268 kilometres (167 mi) from Tehran and with a variable climate. It is located on the fringes of a valley through which the Sefid River (Rud-e Safid) flows. Rudbar can be considered as a gateway to the Gīlān Province from central Iran. Its name (meaning "by the river" in Persian) is a reference to the river named Sepid Rood (Sepid Roud) that passes by the town. Rudbar is also called "Roodbar Zeitoun (olive)" for the olive gardens in the area. Rudbar's local economy notably includes olives and olive-based products. Groves of olive trees surround the city and olive oil is produced locally. Loshan and Roudbar have been converted into olive farms.

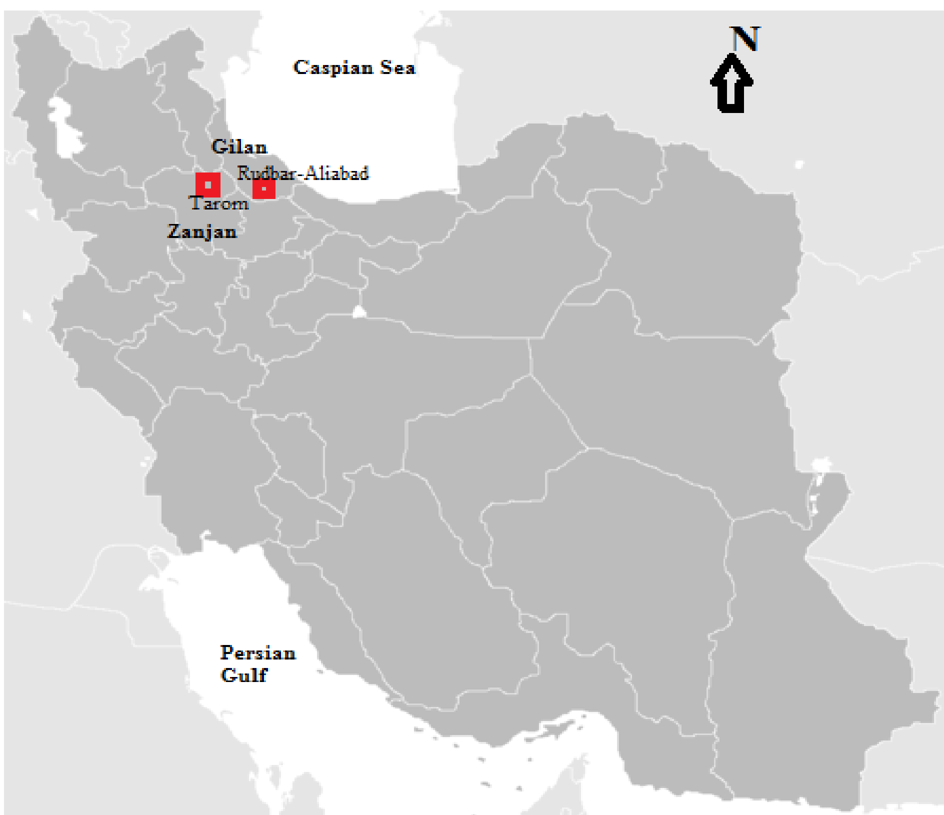

Figure-1. The map of sampling localities, including two studied provinces

Sampling Method

1100 samples from green olive fruits were collected from 10 different farmlands in Rudbar-Aliabad from Gilan Province And Tarom From Zanjan Province(figure-1), which are considered the most important regions of olive cultivation in Iran were purchased in summer, autumn and winter 2013 and 2014. In each location, the chosen cultivars were those predominating in the respective area. The samples were collected during the period when olives are usually harvested for oil production. Each sample (about 500-1000 g) of olive fruits were manually collected from the same three olive trees, and kept at +4°C. Due to this descriptive study the effect of processing method, samples were studied in 2 different conditions: crude and processed forms .Samples were randomly purchased for analysis and analyzed according to standardized international protocols by wet digestion method [31, 32]. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines.

Quantitative determination of mineral contents

The mineral elements: K, Na, Ca, Zn, Cu, Fe and Mn in olive fruits were measured using an atomic absorption spectrometer with flame atomization (PerkinElmer PinAAcle 900T atomic absorption (AA) spectrophotometer) in research Laboratory pharmaceutical Sciences Branch. The measurements were made in hold mode with air acetylene flame, where the air (as oxidant) was maintained at a flow of 50 mL/ min and the acetylene (as fuel) was maintained at a flow of 20 mL/ min, to reach a flame temperature of 2,600°C. The hollow-cathode lamps were specific for each element analyzed. Previously, to achieve maximum sensitivity and precision, the equipment was equilibrated by alignment of the lamp and lighter and adjustment of the selected wavelength [29, 30].

Quantitative determination of Heavy Metals

For heavy metal analyses approximately 20.0 g of each sample (fresh green olive seeds and packaged olive seeds) accurately weighed and digested in accordance with U.S. Analar grade nitric acid, hydrogen peroxide (about 30%) , Sulfuric Acid (about 98%) and concentrated per chloric acid (37%) [3:1:2:3] were used for the digestion. Application of concentrated HNO_3 along with thirty percent hydrogen peroxide H_2O_2 (Merck) for mineralization of samples to the complete digestion of samples [16,33] following Environmental Protection Agency (EPA) Method 3052 was done.

All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% volume/volume nitric acid at least overnight, and rinsed abundantly in deionized water and dried in such a manner to ensure that any contamination does not occur. Five-point calibration curves (five standards and one blank) were constructed for each analyte. The calibration curve correlation coefficient was examined to ensure an $r^2 \geq 0.998$ before the start of the sample analysis. The digested samples were diluted with 10% HNO3 and brought up to 50 mL and analyzed by a graphite furnace atomic absorption spectrophotometry, (GFAAS). The measurements were performed using a PerkinElmer PinAAcle 900T atomic absorption (AA) spectrophotometer and using at least five standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines [15, 16, 31, 33] certified standard reference material (Alpha – Line, Chem Tech Analytical, England) was used to ensure accuracy, and the analytical values were within the range of certified values. All recoveries of the metals studied were greater than 95%.

Determination of fatty acids

Fatty acids were derived using the boron trifluoride method [34]. The working conditions of gas chromatography was determined using methyl ester method as described by Hisil [34,35] .The oil was extracted three times for 2 g air-dried seed sample by homogenization with ether do petrol. The oil samples (50-100 mg) were converted into fatty acid methyl esters (FAME). The software was run by an Optiplex GX150 Dell computer with Windows 98. The GC column was a Chrompack Capillary Column, CP-Sil 8 CB LOW BLEED/MS, measuring 30m, ID 0.25mm, coated with 0.25mm particles. The MS used an Ion Trap technique with both an internal diffusion pump and an external Varian DS 102 rotary vane pump. 99.999% grade He was used for the inert gas with a Chrompack Gas-Clean GC-MS Filter for oxygen and moisture. Stockroom chemicals of various grades were used for the runs.

FAME (5 μl) was analyzed using a Varian 2100 chromatograph, under the following temperature programme: 90°C (7 min), 5 °C /min to 240 °C (15 min). Temperature of both injector and flame ionization detector was 225 °C. The fatty acids were converted to their methyl esters by heating them in 10% BF3-methanol. Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times [36, 37, 38, 39, 40]. Results are given as mean values of two replicates.

RESULTS

Results of Fatty acids

The fatty acid compositions of olive oils (crude fruit olive samples) were determined by gas chromatography. Oleic acid (63.7- 85.2 %) was present in the highest concentration followed by palmitic (8.6 - 17.9 %), linoleic (3.3-17.2 %), stearic (2.3-6.6 %) and linolenic (0.2 -2.8 %). Differences between fatty acids of oils were significant (p □ 0.01) . The highest concentration of palmitic acid were found in olive samples from Rudbar while linoleic and stearic acid in Zanjan samples were significantly higher (p □ 0.01). As the fatty acid composition in oils is affected by species, genetics, variety, growing conditions, locality, climatic conditions and postharvest treatment the results were variable in depend on the locations.

According to variance analyses of data, oil rate and most of the fatty acids in olive oil samples were significantly affected by growth location. There was a positive correlation between the location of growing with the weight of olive fruits too (p □ 0.005). Differences between the weight of fruits were significant (p □ 0.01) for all farmland locations, although even in the same farm there was a variety number of weight. The highest fruit weight 9.15 g belongs to Rudbar (Gilan province). The mean of Rudbar samples weight was higher than Tarom samples significantly.

Table 1- The mean content (mg/kg DW) of mineral contents in crude fresh green olive samples from two different regionals.

Mineral Element	Mean Content (mg/kg DW ± SD) Tarom (Zanjan)	Mean Content (mg/kg DW ± SD) Rudbar-Aliabad (Gilan)	Mean content (mg/kg DW ±SD) (n= 600 Crude olive fruit samples)
K	9053 ± 229	11193 ± 317	10123 ± 273
Ca	1965 ± 87	1833 ± 111	1899 ± 99
Na	923 ± 46	1026 ± 53	9745 ±49
Zn	24.9 ± 0.7	9.3 ± 0.6	17.10 ± 0.65
Cu	17.61 ± 0.53	2.3 ± 0.8	9.96 ± 0.66
Mn	6.8 ± 0.3	6.4 ± 0.9	6.6 ± 0.6
Fe	236.3 ± 15.4	222.3 ± 9.4	229.3 ± 24.8
Pb	83.211 ± 9.245	57.447 ± 11.011	70.324 ± 10.128
Cd	4.4611 ± 0.9667	5.0222 ± 0.8719	4.7416 ± 0.9193
SD = Standard Deviation			

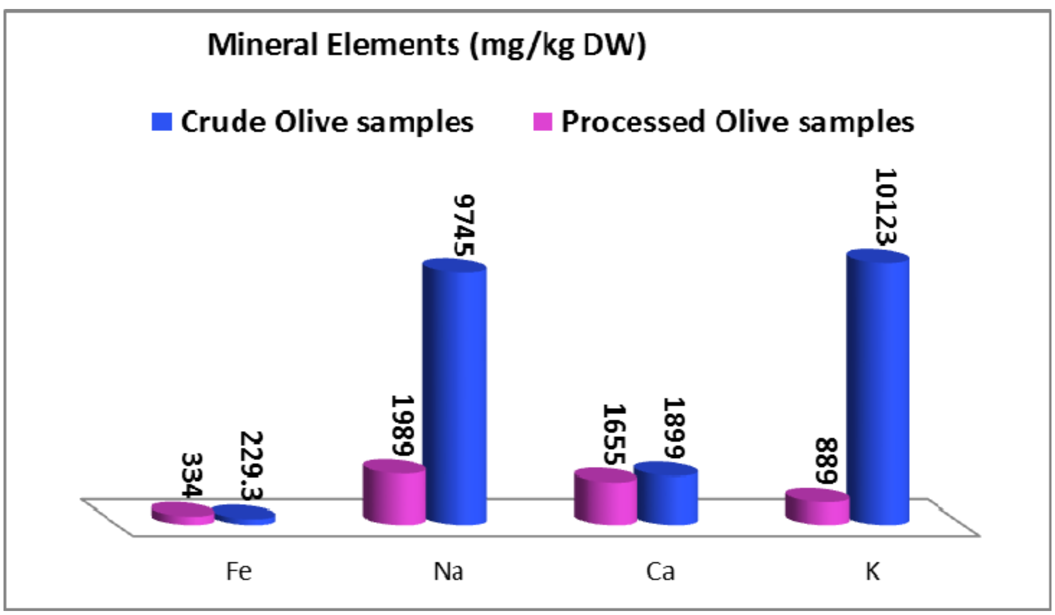

Figure 2- The mean contents of Iron, Sodium, Calcium and Potassium (mg/kg DW) in crude olive fruit and traditionally processed samples

Results of mineral Contents

The mean of mineral contents of Rudbar and Tarom samples were summarized in table 1. The mean content of potassium and sodium in Rudbar samples were higher while Iron, Zinc, Calcium and copper in Zanjan samples were much higher than Rudbar olive samples. The mean contents of mineral elements in crude fresh olive samples compared with processed and locally treated green olive samples are compared in figures 2 and 3. Results showed that except Fe and Mn all other elements in crude samples were higher than treated olive studied samples.

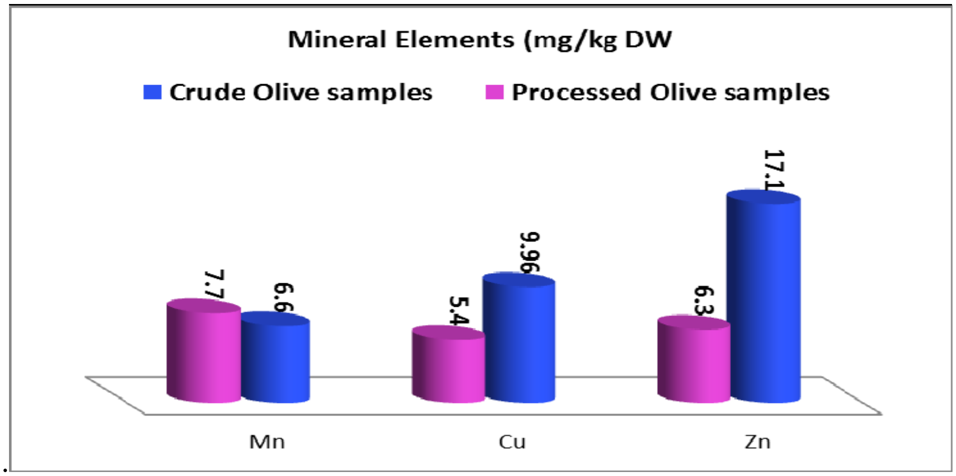

Figure 3- The mean contents of Copper, Zinc and Manganese (mg/kg DW) in crude olive fruit samples

Results of Heavy metals

Acceptable oral limits of Cadmium include 0.09 ug/kg bw/day [41], 0.05ppm in food (FAO/WHO) [42,43] and 0.005ppm for water [58]. The WHO has established a Provisional Tolerable Daily intake for Cadmium of 1 ug/kg bw/day. The allowable set standard limits of the Environmental Protection Agency (EPA) for lead in foods is at 0.5ppm and 0.015 ppm for water [44]. The results show high levels of Pb and Cd in all olive samples studied (figure 4). Correlation matrix for lead content shows a highly significant positive correlation among locations and its content. No significant correlation has been found for Cadmium content in two studied provinces. Based on the standard limits set by the US EPA for lead and FDA for Cadmium for food at 0.5ppm and 0.05ppm, respectively, all of the olive samples exceeded the specifications. It was observed that lead content in Zanjan crude samples and Cadmium in Gilan samples are higher than the other samples.

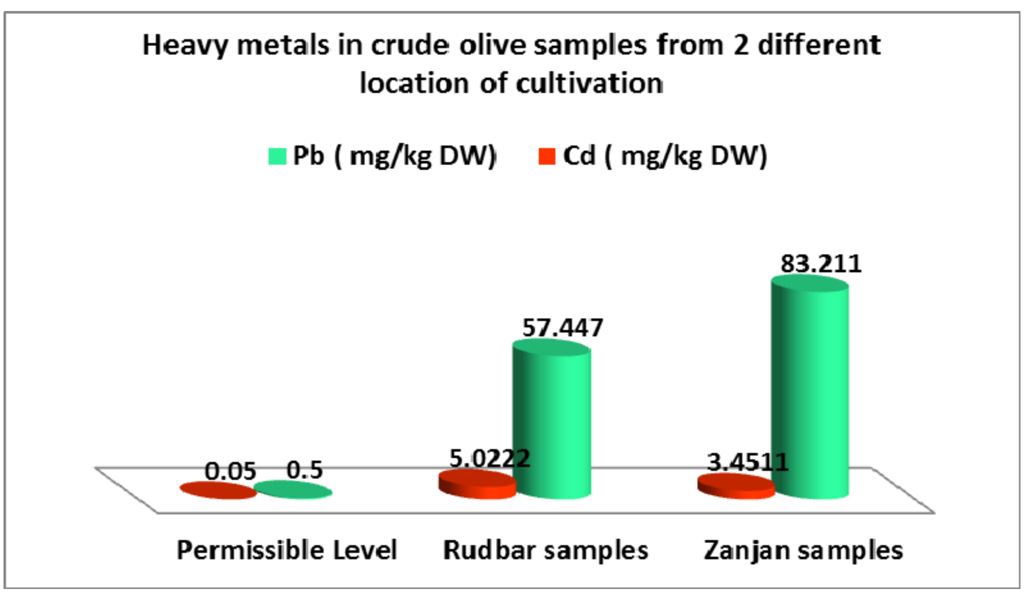

Figure 4- The mean contents of Cadmium and Lead (mg/kg DW) in crude olive fruit, comparing by allowable standard limit.

DISCUSSION

Our results are similar in fatty acid composition, when compared to the values in the other literatures [38, 39, 40, 45,46] . The fatty acid composition has been studied as oil content and fatty acid composition was different based on region [45, 46] . Comparison of olive fatty acid has a great importance due to olive oil quality depends on cultivar, environmental condition and influents [46, 47, 48].

The weight of olive fruits showed that Rudbar samples had high quality as in other studies from Turkey, the weight of edible oil olives fruit reported as 3.5 g-3.9 g [45] , 2.5-4.1 g[46], and 3.3-4.2 g [47], 1.7-3.6 g [48,49]. The results obtained from some experiment showed that the oil profile of olive from the northern region, with upper altitude has a superior quality proportion than the south region [50,51,52]. Many factors can affect the chemical and physical properties of olive oils such as the type of soil, temperature. Even night temperature in the high altitudes is generally lower; it affects the fatty acid composition [53]. One of the other major factors is soil structure. Soil organic matter enhances both olive tree productivity and soil structure, and helps the soil maintain several nutrients in forms available for the roots. Soil water retention capacity is enhanced by the presence of humus and, thus, the tree can better resist water shortage during the dry season [54,55,56,57]. As a result, one can assure the sustainability and the autonomy of olive farming by preserving soil richness in organic matter. Aims of this study were evaluation of nutritional status of Zard Olive cultivar in different orchards and assessment of the methods for improving chemical fertilizers efficiency. Results indicated that almost all of the olive orchards of Tarom suffer from imbalances in nutritional status that limit commercial production in the area. The main nutrient deficiencies are in Nitrogen, Potassium, Zinc and Boron, therefore, these nutrients must be used in orchards and over-application of Phosphorus must be avoided [58].

CONCLUSION

The mean content of lead and Cadmium levels in the 1100 green olive samples analyzed exceeded both limits set for them. The public must be made aware of the findings of heavy metals. Researches in Iran should on the simplest, most cost effective remediation of the environment and moves to revolutionize processes that affect integrity of commodities must be initiated. As a conclusion, this study revealed that different ecological and topographic conditions especially altitude and temperature and soil structure resulted in significant changes in fatty acid composition, mineral and Heavy metal contents in olive fruits. Apart from growing condition, one should also consider some other factors such as type of soil fertility, cultivar oil type, planting time and irrigation in order to obtain a desire yield and quality. It should be consider that better understand the combined reasons in olive tree nutrition and production could make possible to greater development and achievements in optimum productions. Much more work to be done is recommended to integrate all significant factors affected the physical and chemical properties of vegetables and crops.

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