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Health risk assessment of heavy metals via dietary intake of five pistachio (*Pistacia vera* L.) cultivars collected from different geographical sites of Iran

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ABSTRACT

Pistachio is an important horticultural product and Iran is considered as a main pistachio producing country. Assessment of heavy metals in this export fruit is crucial for protecting public health against toxic heavy metals. The concentration of selected heavy metals in soil, water and five pistachio cultivars from four geographical regions of Iran were measured. Although none of the elements were detected in water irrigation, infield metal content in the soil had good correlation with that of pistachio. The highest amounts of Al, As, Co, Ni and Se were reported in samples collected from Sarakhs, Iran. Considering both cultivar and region effects on selected heavy metals concentration, Kaleghoochi cultivar from Sarakhs site showed the highest amount of Al, As, Ni and Se. The maximum concentration of Hg was found in Akbari cultivar collected from Damghan. In the Akbari and the Ahmad aghaei cultivars collected from Sarakhs and Damghan cultivation zones, respectively, the highest amount of Co were observed. Based on our results, the HI value for the consumers of Iranian pistachio was 0.066. It seems that the levels of heavy metals in these pistachio samples pose no risk to consumers.

1. Introduction

Pistachio (*Pistacia vera* L.) is a member of Anacardiaceae family, which is often cultivated in arid and semi-arid regions. Iran, USA, Turkey, Syria, Italy and Greece have the highest production and exportation rates of pistachio. Iranian people consume the pistachios as fresh, roasted and salted nut snacks. They use also non-split ones as the processed products such as pistachio halva, pistachio milk and pistachio butter (Ardakani, 2005). Pistachio is recognized as a nut with nutritional properties and contains fatty acids, minerals, vitamins, sterols and phenolic compounds (Brufau et al., 2006). Several clinical researches have shown a positive correlation between intake of nuts and prevention of coronary heart diseases, cancer, cholesterol levels and diabetes (Moayedi et al., 2011). The presence of heavy metal in foods is one of the most important concerns about food safety (Khan et al., 2008). Accordingly, monitoring of toxic heavy metals in pistachio kernel as a strategic horticultural production, is important for protecting public health against the hazards of heavy metals. It should be noted that the existence of heavy metals can affect the quality of agricultural crops, it can cause physical, chemical and biological problems, and it can have cytotoxic and mutagenic consequences for humans and animals (Al-Othman et al., 2012). Heavy metals are classified as potentially toxic (arsenic, cadmium, lead, etc.), probably essential (vanadium and cobalt), and essential (copper, zinc, iron, manganese, etc.). Moreover, long-term ingestion of toxic heavy metals may have very detrimental effects (Yurt Lambrecht,

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Durkan and Unak, 2007). For example, the presence of the toxic metalloid arsenic in the environment can seriously affect human health (e.g. cardiotoxicity) (Alamolhodaei et al., 2015). Prolonged consumption of unsafe concentrations of lead, may result in accumulation of Pb in the kidney (Järup, 2003). Additionally, Pb and Hg may cause various abnormalities in kids (Hartwig, 1998) such as mental retardation, cerebellar ataxia, primitive reflexes and dysarthria (Mergler et al., 2007). The mechanism considered is the induction of oxidative DNA damage, either owning to generation of reactive oxygen species by Fenton-type reactions, or by inactivating enzymes involved in the cellular defense against reactive oxygen systems (Sugiyama, 1994). Also long term intake of Cd causes prostate and ovarian cancers in rats (Şaplakoğlu et al., 1997). There are several sources of heavy metals contamination of food such as industrial pollution, factory ejaculation, agrochemical fertilizers (which may contain cadmium and organic mercury), arsenic-based pesticides (Adare et al., 2008), water containing heavy metal residues and air polluted by coal mining (Pip, 1991). Several studies suggested that plants which were grown in soil contaminated by heavy metals may be considerably toxic (Bhagure and Mirgane, 2011). Furthermore, it was shown that crops which were grown in soil polluted by Cd and Pb, have higher amounts of hazardous elements than those collected from non-polluted soils (Dowdy and Larson, 1975). Accordingly, accumulation of toxic elements in the nut or related food is a food safety concern and may pose potential health risk. So, monitoring of heavy metal levels and estimation of the intake levels of these contaminants via pistachio consumption are essential for a clear risk assessment of its consumption. In recent years, the joint FAO/WHO Expert Committee on Food Additive (JECFA) recommended permissible tolerable weekly intake (PTWIs) (Joint, 2003). So far, effort has been made to investigate heavy metals in Iranian pistachio, with a focus on the plant genetic and cultivation geographical zone effects. The present study aimed (1) to determine the concentration of Al, As, Cd, Co, Cr, Hg, Ni, Pb and Se as non-essential and toxic elements in five pistachio cultivars from different regions of Iran using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) as a fast and sensitive technique, (2) to introduce the best cultivar which was near the standards index, (3) to evaluate the effect of geographical, soil and water properties on the elements levels, (4) to calculate hazard index value of collected pistachio cultivars for Iranian consumers.

2. Materials and methods

2.1. Plant materials and climate factors

Rafsanjan, Damghan, Sarakhs and Feizabad are among the main areas of pistachio cultivation in Iran (Esfandiyari et al., 2012). The ripe pistachios (*Pistacia vera* L.) were collected from arid and semiarid sites of Iran namely, Damghan, Feizabad, Rafsanjan and Sarakhs in August and September 2015. These pistachio cultivars are the most important commercial ones followed by Akbari, Ahmad aghaei, Badami-e-sefid, Kalehghoochi and Owhadi. Soft hulls and hard shells of fruits were easily separated and the kernels were air-dried at room temperature for 48 h. Then kernels were stored at −18 °C until chemical analysis. Climate and topographic factors of collected sites are shown in Fig. 1.

2.2. Soil and water sampling

Soil and water samples from the four cultivation sites were collected from September 2015 until September 2016. Soil samples were collected from 0 to 30 and 30–60 cm depth in triplicate, air dried, crushed, passed through 2 mm mesh size sieve, and stored in ambient temperature before analysis. Also, 100 ml of irrigation water from the selected geographical sites were collected in triplicate in washed polypropylene bottles and 1 ml HNO_3 was added in water samples to avoid microbial contamination. Before digestion the water samples were kept in refrigerator.

2.3. Reagents

All of the chemicals used for digestion procedures and the multi element standard solutions for ICP-OES were purchased from Merck (Darmstadt, Germany).

2.4. Digestion procedures

2.4.1. Dry ashing

This process had two phases; in the first phase 10 g of each homogenized sample (soil and pistachio) were incubated for circa 2 h at 120 °C for stabilization. Then, samples were weighted and compared with their pre-incubation weights in case of weight loss; heating was repeated until the weight was fixed. Samples were heated on a hotplate until no smoke was coming out of the ash. The second phase was conducted for 10 h at 550 °C in an oven with the initial temperature set at 200 °C increased with a rate of 50 °C/h until the temperature reached 550 °C. Ash samples were moisturized with distilled water and evaporated to dryness over a water bath. Then, the residue was dissolved in 10 ml HNO_3 and made up to a final volume of 100 ml with deionized water (Mirlohi et al., 2013).

2.4.2. Wet ashing

For measurement of As and Hg escape 30 ml of concentrated nitric acid was added to each sample (soil and pistachio). The acidified samples were kept at room temperature for 24 h. After the digestion, samples were heated using a hotplate. The temperature was increased until the solution boiled and kept simmered until turned transparent. Next, 3 ml of each sample was filtered through a Whatman filter paper No. 42. Then, deionized distilled water was added, until the final volume was 100 ml. (Mirlohi et al., 2013).

2.5. Instrumentation

After dry and wet ashing, liquid samples were analyzed by using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (SPECTRO ARCOS, Germany) for detection of the levels heavy metals (Al, As, Cd, Co, Cr, Hg, Ni, Pb and Se). SPECTRO ARCOS is different from conventional ICP spectrometers as it has a unique optical system which exhibits unique resolution, validity and stability. In this method, the optical system chamber is filled with argon. Records represented with 32 linear CCD detectors to cover the entire wavelength range of 130–770 nm. This CCD benefits from special minus temperature cooling. This system is self-stabilized on 15 °C, and it is also independent from environmental temperature changes.

2.6. Human risk assessment of heavy metals in selected pistachio cultivar consumption

2.6.1. Estimated daily intake (EDI)

The two determinative factors in estimated daily intake (EDI) are the heavy metal concentration in food and the daily food consumption, however, the body weight may change the tolerance. The EDI was calculated for the adult population according to the following equation:

Fig. 1. Geographical location, topographic and climate characteristics of the cultivation zones.

$$\mathrm{EDI} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{FIR} \times \mathrm{C}}{\mathrm{WAB} \times \mathrm{TA}}$$

Where EF is exposure frequency (365 days/year), ED is the exposure duration (70 years, equivalent to the average lifetime), FIR is the food ingestion rate (0.82 g/person/day), C is the heavy metal concentration in pistachio (μg/g), W_{AB} is the average body weight (70 kg) and T_A is the average exposure time for non-carcinogens (365 days/year × ED) (Qian et al., 2010). (Shirani et al., 2017; Balarastaghi et al., 2017; Nejabat et al., 2017).

2.6.2. Target hazard quotient (THQ)

For the determination of the potential health risks of some of the pollutants the non-cancer risk characterization methodology of THQ (Target Hazard Quotient) was, are used (Storelli, 2008). The THQ for the consumers of potentially contaminated pistachio was measured by comparing the provisional tolerable weekly intake (PTWI) of each element with those measured in kernel samples. THQ was determined according to the following equation (Qian et al., 2010).

$$\mathrm{THQ} = \frac{EDI \times 7}{PTWI}$$

PTWIs for Al, As, Cd, Co, Cr, Hg, Ni, Pb and Se presented by Joint FAO/WHO Expert Committee on Food Additives (JECFA) are 1000 μg Al/kg bw/week, 15 μg As/kg bw (body weight)/week, 7 μg Cd/kg bw/week, 700 μg Co/kg bw/week, 35 μg Cr/kg bw/week, 4 μg Hg/kg bw/week, 35 μg Ni/kg bw/week, 25 μg Pb/kg bw/week, and 55 μg Se/kg bw/week (Bilandžić et al., 2011) (Commission, 2011).

2.6.3. Hazard index (HI)

To assess the total potential risk from non-carcinogenic and carcinogenic effects posed by two or more pollutants the calculation of Hazard index (HI) was used (Tsakiris et al., 2015). The HI represents the potential risk of adverse health effects caused by a mixture of chemical constituents. The HI values through daily consumption of pistachio for human beings were evaluated using the following equation (Qian et al., 2010).

$$\mathrm{HI} = \sum_{n=0}^{i} \mathrm{THQn}$$

2.7. Statistical analysis

Statistical analysis was performed as a factorial complete randomized design with three replications by JMP 8 (SAS Campus Drive, Cary, NC 27513) and Excel software. All the results were presented as mean ± standard deviation. Significant differences among mean values were determined by using LSD at the level of 0.05.

3. Results and discussion

3.1. Operating condition for ICP-OES SPECTRO ARCOS

ICP-OES SPECTRO ARCOS has been widely used for elemental analyses in industry and research as it allows to analyze more samples within less time. This system can complete a single analysis in 30 s; so, it has the highest speed in its class. Also, it is equipped with Multi View mechanism that lets the operator turn a radial-view instrument into an axial-view device or vice-versa, in 90 s or less.

3.2. Heavy metals concentrations in pistachio samples

3.2.1. Aluminum

Aluminum is the third most abundant metal in the earths. It seems that it is a non-essential element and does not have any biological functions in plant organisms. The toxic effects of aluminum compounds on male reproductive system include necrosis of spermatocytes and spermatids and reduction of fertility (Guo et al., 2005). Based on WHO statistics (Organization, 1987) the permissible aluminum dose for an adult is quite high (60 mg/day). In simple effects among selected pistachio cultivars we showed statistically significant differences between them at $P < 0.05$. The highest Al level (4.8 ± 0.02 mg/kg) was found in Kaleghoochi cultivar (Table 1). Moreover, we focused on the zone of cultivation and the results showed that the pistachio collected from Sarakhs had the highest amount of Al followed by Rafsanjan, Damghan and Feizabad (Table 2). The Al content of plants depends on several factors. The average levels of Al in our samples were lower than those found in Beneh, Sefid, Garmeh cultivars (3.22–9.59 mg/kg) as reported by Davarynejad et al (DAVARYNEJAD, ZAREI, & NAGY, 2013). which seems to be due to the type of cultivar.

3.2.2. Arsenic

The major sources of arsenic contamination are industrial activities waste, and chemical and pesticide application which pollute soils, surface and ground water that finally enter the food chain (Gonzaga et al., 2006). Arsenic exists in two forms, namely organic and inorganic, that could be found in the environment and human body (Jomova and Valko, 2011). Organic form is a combination of arsenic, hydrogen and carbon, while the inorganic forms contain elements like oxygen and sulfur (Jomova and Valko, 2011). Inorganic arsenic is more toxic than the organic one (Manna et al., 2008). Arsenic seriously affects human health. For instance, arsenic poisoning may lead to cardiovascular diseases by induction of arrhythmias and ischemia (Alissa and Ferns, 2011). When arsenic is consumed via contaminated water it can be hazardous for humans and animals. The amount of arsenic uptake by plants depends on changing in speciation (methylated or complexes like phytochelatins) which affects the level of toxicity (Smith et al., 1998). Among the collected pistachio cultivar and sites, Kaleghoochi cultivar (with means 1.963 ± 0.005 mg/kg) and sarakhs region showed the highest level of arsenic (Tables 1 and 2, respectively). In an evaluation of different types of nuts in Turkey, As was found at very low concentrations and samples were safe for human (Divrikli et al., 2006).

3.2.3. Cadmium

Environmental pollution is the main source of cadmium content in the food chain. Cadmium is a trace element that is found at high levels in industrial sites. Cd contamination usually has an anthropogenic source (Akbari et al., 2012). High amount of Cd can cause kidney dysfunction, hypertension and hepatic diseases (Cabrera et al., 1998). In our study Cd was not detected in any cultivars collected from different cultivation zones (Tables 1 and 2). Our results are in agreement with a previous study, in which Cd was not detected in pistachio samples as evaluated by ICP technique. In that study, authors suggested that their pistachio samples were safe for humans (Tošić et al., 2015).

3.2.4. Cobalt

Cobalt, a silvery grey solid, not only can be released into the atmosphere in particulate form but it could be also released into water and soil. Though it is an essential element for physiological functions of plants its concentration in the environment should be

Table 1
The influence of cultivar type on heavy metals content in samples collected from the same region.

Heavy metals	LOD	LOQ	Ahmadaghaei	Akbari	Kaleghoochi	Owhadi	Badami-e-sefid
Al	0.056	0.182	4.60 ± 0.01^{b}	4.61 ± 0.01^{b}	4.80 ± 0.02^{a}	4.60 ± 0.01^{b}	4.37 ± 0.02^{c}
As	0.0828	0.275	1.655 ± 0.005^{c}	1.576 ± 0.005^{e}	1.963 ± 0.005^{a}	1.9 ± 0.005^{b}	1.626 ± 0.005^{d}
Cd	0.0828	0.2759	n.d.	n.d.	n.d.	n.d.	n.d.
Co	0.01	0.03	n.d.	n.d.	n.d.	n.d.	n.d.
Cr	0.0588	0.196	0.777 ± 0.005^{c}	0.9 ± 0.005^{a}	0.661 ± 0.005^{d}	0.839 ± 0.005^{b}	0.299 ± 0.005^{e}
Hg	0.01	0.03	Trace	Trace	Trace	Trace	Trace
Ni	0.182	0.302	0.598 ± 0.001^{b}	0.597 ± 0.001^{b}	0.66 ± 0.001^{a}	0.426 ± 0.001^{c}	0.355 ± 0.001^{d}
Pb	0.129	0.730	Trace	Trace	Trace	Trace	Trace
Se	0.02	0.06	0.133 ± 0.001^{b}	0.190 ± 0.001^{a}	0.194 ± 0.001^{a}	0.115 ± 0.002^{c}	0.088 ± 0.002^{d}

Data expressed as mean of heavy metals content (mg/kg) of samples ±SD (standard deviation) in three replicates.
The lowercase superscripts (a, b, c and d) express statistically significant differences among different cultivars collected from the same region. In each row, similar superscripts show that there were no significant differences in the specific heavy metal.
Means ± SD within rows with the same superscripts letters (a, b …) are significantly different at $P < 0.05$.
“n.d.: not detected, shows a level below LOD”.
Trace is defined as a level between LOD and LOQ.

Table 2
The influence of zone of cultivation on heavy metals content of pistachio cultivars.

Heavy metals	LOD	LOQ	Damghan	Feizabad	Rafsanjan	Sarakhs
Al	0.056	0.182	4.027 ± 0.005^{c}	3.071 ± 0.005^{d}	4.22 ± 0.004^{b}	7.064 ± 0.005^{a}
As	0.0828	0.275	1.632 ± 0.001^{c}	1.31 ± 0.001^{d}	1.815 ± 0.001^{b}	2.22 ± 0.001^{a}
Cd	0.0828	0.2759	n.d.	n.d.	n.d.	n.d.
Co	0.01	0.03	n.d.	n.d.	n.d.	n.d.
Cr	0.0588	0.196	0.197 ± 0.005^{c}	1.416 ± 0.003^{a}	Trace	0.974 ± 0.002^{b}
Hg	0.01	0.03	Trace	n.d.	n.d.	Trace
Ni	0.182	0.302	Trace	0.32 ± 0.001^{c}	0.734 ± 0.001^{b}	0.836 ± 0.001^{a}
Pb	0.129	0.730	Trace	Trace	Trace	Trace
Se	0.02	0.06	0.078 ± 0.001^{c}	Trace	0.183 ± 0.002^{b}	0.280 ± 0.002^{a}

Data expressed as mean of heavy metals content (mg/kg) of samples ±SD (standard deviation) in three replicates.
The lowercase superscripts (a, b, c and d) express statistically significant differences among different cultivars collected from the same region. In each row, similar superscripts show that there were no significant differences in the specific heavy metal.
Means ± SD within rows with the same superscripts letters (a, b …) are significantly different at $P < 0.05$.
“n.d.: not detected, shows a level below LOD”.
Trace is defined as a level between LOD and LOQ.

controlled in order to reduce its presence in the food chain. Several lethal cardiomyopathies were reported in people who consumed high levels of Co. These types of cardiomyopathy were similar to alcoholic cardiomyopathy and beriberi. Liver injury with hepatic necrosis and increased levels of serum bilirubin and serum enzymes was also reported (Kim et al., 2006). In our study Co was not detected in any cultivars collected from different cultivation zones (Tables 1 and 2). Co concentrations in our study were lower than pistachio in local market of Serbia (0.0182–0.0207 mg/kg) (Tošić et al., 2015).

3.2.5. Chromium

One of the toxic metals that could be released through industrial pollution is chromium. Plants absorb chromium leading to human and environmental toxicity. The concentration of Cr in wastewater must be less than 0.05 mg/L, but much higher levels have been seen in the wastewater of leather tanneries, petroleum refineries and wood preservation industries. Cr concentration in soil is not affected by short-term use of chemical fertilizers but 40-year application increased its concentration by 17.1% compared with control soil (Saviozzi et al., 1999). Based on our results the highest level of chromium pollution among the cultivation zone was found for Feizabad (Table 2). Akbari pistachio cultivar represented as the most chromium-polluted ones (with means 0.9 ± 0.005 mg/kg) among five selected cultivars (Table 1). Our results were similar to the study which evaluated eleven pistachio cultivars in which the chromium level ranged from 0.60 to 1.86 (mg/kg ((DAVARYNEJAD et al., 2013). A range of chromium intake between 50 and 200 μg/day is tentatively recommended for adults (Saracoglu et al., 2009).

3.2.6. Mercury

The unique characteristic of mercury is that it could be found in various forms as HgS, $Hg^{+2,}$ Hg° and methyl-Hg, but in agriculture soil is mostly detected in the ionic form (Hg^{+2}) (Han et al., 2006). Some heavy metal-based pesticides are the main cause for the mercury pollution of the orchard soil (Ross, 1994). Toxic levels of Hg cause physiological problems in plants, as stomata closing via binding to water channel protein and affected water absorption (Zhang and Tyerman, 1999). We found that Hg was not detected or trace in any cultivars collected from different cultivation zones (Tables 1 and 2).

3.2.7. Nickel

Human activities such as sewage, sewage sludge, phosphate fertilizers, and pesticides, significantly increase Ni concentration in soil (Fan et al., 2013). High concentrations of Ni cause toxicity in soil and various physiological disorders, such as chlorosis and necrosis, in plant species (Rahman et al., 2005). Nickel toxicity can affect the skin and it can cause respiratory allergies and cancers (Teissedre et al., 1998). According to WHO (Kanis, 1994) the daily intake of nickel is recommend to be between 100 and 300 μg. In our results the highest level of nickel was found in Sarakhs followed by Rafsanjan and feizabad (Table 2). Additionally, among different types of pistachios Kaleghoochi had the highest level of Ni

(0.66 ± 0.001 mg/kg) (Table 1). Ni concentrations in our samples were lower than some commercial and wild pistachios (1.50 and 0.94 mg/kg, respectively) measured by Davarynejad et al. (DAVARYNEJAD et al., 2013). The levels of Ni in pistachio as reported by a Spanish and a Turkish report were 0.34 and 13.8 μg/kg, respectively (Cabrera et al., 2003; Divrikli et al., 2006).

3.2.8. Lead

Lead is the most abundant toxic element found in the soil. Oxidative stress, inhibition of seed germination, root growth, enzyme activity and metabolic disorders are some of the effects of Pb on plants. Moreover the toxic effects of Pb in induction of brain damage in children are well known (Ahmedna et al., 2004). Pb concentrations in teeth, as indicator of lead presence in the body, revealed a relationship between environmental exposure factors and body accumulation of Pb (Amirabadi et al., 2016). Prolonged intake even at low concentrations causes toxic problems and for this reason determining Pb in dietary supplements is of great interest. In this study, we found trace levels of lead in different cultivars collected from different cultivation zones (Tables 1 and 2). High lead concentration showed that it is one of the most abundant and widespread contaminants (Tošić et al., 2015).

3.2.9. Selenium

Selenium serves as a key factor in many physical and metabolic functions in plants. For example Brazil nuts that have high concentrations of Se (up to 0.5 g/kg) are important sources of this essential nutrient. Se is a microelement which plays an important role in maintenance of human health due to its antioxidant characteristics. This element has been determined in different nuts (Moayedi et al., 2011). In some plants, selenium accumulates without causing any toxicity symptoms. As shown in Table 2 similar pattern was seen for Se; as, Sarakhs represented as the cultivation zone with the highest Se pollution. Kaleghoochi and Akbari pistachio cultivars had the highest level of Se (with means 0.194 ± 0.001 mg/kg and 0.190 ± 0.001 mg/kg, respectively) (Table 1). In another study Se content was 0.658 and 0.752 mg/kg in pistachio samples which is about 3.5–4 times higher than that found in our samples. Se concentrations in samples are affected by soil composition (Tošić et al., 2015). Selenium content is dependent on the amount of this element in the soil in which plant species grow. For instance Se content showed variations in Brazil nuts collected from different countries of the South America (Brazil, Bolivia and Peru) (Parekh et al., 2008).

According to our results, the zone of cultivation and type of cultivars have exerted significant influence on the levels of heavy metals in pistachio samples (Table 3). These results clearly showed that the collection zone and the type of cultivar influenced the heavy metal concentrations. Considering the effect of cultivation zone and cultivar type showed that the maximum concentrations of Al, As, Ni and Se were found in Kaleghoochi cultivar from Sarakhs cultivation zone and the minimum concentrations of these metals the results were found in Badami-e-sefid cultivar from Feizabad (for Al and As). Regarding Cr content, the maximum and minimum concentration were found in Owhadi Feizabad and Ahmad aghaei Damghan, respectively. The variations in the concentrations of heavy metals in the pistachio cultivars may be ascribed to the physical and chemical nature of the cultivation site soils, the capacity of the pistachio root stock for absorption of heavy metals and their accumulation in the scion, and atmospheric deposition of heavy metals which can be influenced by innumerable factors such as temperature, moisture and wind velocity (Zurera-Cosano et al., 1989). The variation in the heavy metals concentrations may also be due to the variation in anthropogenic activities such as brick kiln activities, addition of phosphate fertilizers or use of metals-based pesticides and also the distance from the industrial activities (Sharma et al., 2009).

3.3. Levels of heavy metals in soil samples

Table 4 summarizes the heavy metal concentrations in soils of four cultivation zone (namely, Damghan, Feizabad, Rafsanjan and Sarakhs), Iran. Statistically significant variations that were found ($P < 0.05$) in our study indicate that samples collected from these

Table 3
Concentrations of heavy metals in five different cultivar types collected from four cultivation zones.

Station	Cultivar	Al	As	Cd	Co	Hg	Ni	Pb	Se
Damghan	Ahmad aghaei	4.03 ± 0.006^{j}	$1.2 \pm 0.004^{\circ}$	n.d.	n.d.	n.d.	Trace	Trace	0.08 ± 0.005^{ij}
	Akbari	4.01 ± 0.005^{j}	1.5 ± 0.005^{l}	n.d.	n.d.	n.d.	Trace	Trace	0.13 ± 0.005^{h}
	Kalehghoochi	4.32 ± 0.003^{g}	1.8 ± 0.005^{g}	n.d.	Trace	n.d.	Trace	Trace	0.091 ± 0.004^{i}
	Owhadi (Fandoghi)	4.116 ± 0.003^{i}	1.86 ± 0.005^{f}	n.d.	Trace	n.d.	Trace	Trace	0.07 ± 0.005^{j}
	Badami-e-sefid	3.66 ± 0.002^{k}	1.8 ± 0.002^{g}	n.d.	n.d.	n.d.	n.d.	Trace	Trace
Feizabad	Ahmad aghaei	3.109 ± 0.004^{l}	1.544 ± 0.001^{k}	n.d.	n.d.	n.d.	0.342 ± 0.001^{h}	Trace	Trace
	Akbari	3.06 ± 0.005^{m}	1.334 ± 0.001^{m}	n.d.	n.d.	n.d.	0.34 ± 0.001^{h}	Trace	Trace
	Kalehghoochi	3.115 ± 0.005^{l}	1.351 ± 0.001^{m}	n.d.	n.d.	n.d.	0.341 ± 0.002^{h}	Trace	Trace
	Owhadi (Fandoghi)	3.088 ± 0.005^{m}	1.228 ± 0.001^{n}	n.d.	n.d.	n.d.	Trace	Trace	n.d.
	Badami-e-sefid	2.983 ± 0.005^{n}	1.093 ± 0.001^{p}	n.d.	n.d.	n.d.	Trace	Trace	Trace
Rafsanjan	Ahmad aghaei	4.23 ± 0.003^{h}	1.77 ± 0.005^{hi}	n.d.	n.d.	n.d.	0.866 ± 0.001^{d}	Trace	0.18 ± 0.003^{fg}
	Akbari	4.15 ± 0.005^{i}	1.693 ± 0.005^{j}	n.d.	n.d.	n.d.	0.860 ± 0.003^{d}	Trace	0.192 ± 0.001^{f}
	Kalehghoochi	4.398 ± 0.002^{f}	1.973 ± 0.005^{d}	n.d.	n.d.	n.d.	0.970 ± 0.001^{b}	Trace	0.243 ± 0.004^{c}
	Owhadi (Fandoghi)	4.218 ± 0.002^{h}	1.89 ± 0.005^{e}	n.d.	n.d.	n.d.	0.558 ± 0.001^{f}	Trace	0.167 ± 0.001^{g}
	Badami-e-sefid	4.116 ± 0.005^{i}	1.75 ± 0.005^{i}	n.d.	n.d.	n.d.	0.416 ± 0.002^{g}	Trace	0.133 ± 0.003^{h}
Sarakhs	Ahmad aghaei	7.054 ± 0.005^{c}	2.106 ± 0.002^{c}	n.d.	n.d.	Trace	0.97 ± 0.003^{b}	Trace	0.221 ± 0.002^{d}
	Akbari	7.226 ± 0.3^{b}	1.78 ± 0.001^{gh}	n.d.	n.d.	Trace	0.90 ± 0.005^{c}	Trace	0.39 ± 0.002^{b}
	Kalehghoochi	7.363 ± 0.1^{a}	2.731 ± 0.001^{a}	n.d.	n.d.	Trace	1.08 ± 0.004^{a}	Trace	0.411 ± 0.003^{a}
	Owhadi (Fandoghi)	6.966 ± 0.2^{d}	2.623 ± 0.001^{b}	n.d.	n.d.	Trace	0.653 ± 0.003^{e}	Trace	0.208 ± 0.001^{e}
	Badami-e-sefid	6.713 ± 0.4^{e}	1.862 ± 0.001^{ef}	n.d.	n.d.	Trace	0.576 ± 0.002^{f}	Trace	0.17 ± 0.002^{fg}

Data expressed as mean of heavy metals content (mg/kg) of samples ±SD (standard deviation) in three replicates.
The lowercase superscripts (a, b, c, d, e f, g, h, i, j, k, l, m, n, o, p) express statistically significant differences among different cultivars collected from the same region. In each row, similar superscripts show that there were no significant differences in the specific heavy metal.
Means ± SD within rows with the same superscripts letters (a, b …) are significantly different at $P < 0.05$.
"n.d.: not detected, shows a level below LOD".
Trace is defined as a level between LOD and LOQ.

Table 4
Mean concentration of heavy metals in the soil of four cultivation zones.

Heavy metals	LOD	LOQ	Damghan	Feizabad	Rafsanjan	Sarakhs
Al	0.056	0.182	4.18 ± 0.05[c]	3.14 ± 0.03[d]	4.68 ± 0.03[b]	7.00 ± 0.02[a]
As	0.0828	0.275	1.7 ± 0.001[c]	1.41 ± 0.001[d]	1.9 ± 0.001[b]	2.30 ± 0.001[a]
Cd	0.0828	0.2759	n.d.	n.d.	n.d.	n.d.
Co	0.01	0.03	Trace	Trace	n.d.	Trace
Cr	0.0588	0.196	0.213 ± 0.005[c]	1.69 ± 0.005[a]	0.2 ± 0.005[c]	1.1 ± 0.005[b]
Hg	0.01	0.03	Trace	Trace	Trace	Trace
Ni	0.182	0.302	0.3 ± 0.002[d]	0.40 ± 0.005[c]	0.80 ± 0.005[b]	0.89 ± 0.005[a]
Pb	0.129	0.730	Trace	Trace	Trace	Trace
Se	0.02	0.06	0.09 ± 0.005[c]	0.049 ± 0.005[d]	0.194 ± 0.005[b]	0.33 ± 0.005[a]

Data expressed as mean of heavy metals content (mg/kg) of samples ±SD (standard deviation) in three replicates.
The lowercase superscripts (a, b, c and d) express statistically significant differences among different cultivars collected from the same region. In each row, similar superscripts show that there were no significant differences in the specific heavy metal.
Means ± SD within rows with the same superscripts letters (a, b …) are significantly different at $P < 0.05$.
“n.d.: not detected, shows a level below LOD”.
Trace is defined as a level between LOD and LOQ.

four locations vary in terms of mean metal concentrations. It seems that the lower concentrations of heavy metals are due to various reason: (1) the continuous removal of heavy metals by pistachio trees grown in these areas, (2) the leaching of heavy metals into the deeper levels of soils, and (3) the site soil physicochemical characteristics which respond to all agrochemical materials and types of water irrigation used for pistachio trees.

Also the regression equations and correlation coefficients (r^2) between heavy metal concentrations in the pistachio samples and metal concentration in the soil for Al was $y = 0.948\ X + 0.383$ ($r^2 = 0.983$), for As was $y = 0.983\ X + 0.111$ ($r^2 = 0.999$), for Co was $y = 2.661\ X + 0.004$ ($r^2 = 0.997$), for Cr was $y = 1.2\ X - 0.03$ ($r^2 = 0.998$), for Hg $y = 1.164\ X + 0.003$ ($r^2 = 0.965$), for Ni $y = 0.935\ X + 0.111$ ($r^2 = 09997$), for Pb $y = 1.55\ X - 0.086$ ($r^2 = 0.98$) and for Se $y = 1.135\ X + 0.002$ ($r^2 = 0.991$).

3.4. Levels of heavy metals in water samples

The heavy metal concentration in irrigation water were very low or below the level of detection.

3.5. Human risk assessment of intake of heavy metals through consumption of selected pistachio cultivars

3.5.1. Estimated daily intake (EDI)

The EDIs of heavy metals for Iranian people via consumption of selected pistachio cultivar are presented in Table 5. The EDIs for heavy metals in selected pistachio cultivar are in the following order: Al > As > Cr > Ni > Se.

3.5.2. Target hazard quotient (THQ)

The THQ is a valuable parameter for assessing the risks associated with consumption of selected product contaminated with heavy metals. THQ resulted lower than 1 indicates no risk for human health derived from consumption (Adel et al., 2016). The THQs for assessments of heavy metals risk for Iranian population posed by pistachio consumption, are shown in Table 6. For all selected pistachio cultivars the THQs for heavy metals were in the following

Table 5
Estimated daily intake (EDI) of heavy metals for Iranian people via consumption of selected pistachio cultivars.

Heavy metals	Ahmadaghaei	Akbari	Kaleghoochi	Owhadi	Badami-e-sefid	Mean
Al	0.26	0.264	0.27	0.25	0.25	0.25
As	0.094	0.09	0.112	0.109	0.092	0.099
Cd	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Co	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Cr	0.044	0.051	0.037	0.047	0.017	0.0392
Hg	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Ni	0.034	0.034	0.037	0.024	0.02	0.029
Pb	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Se	0.0076	0.01	0.011	0.0066	0.005	0.008

N.E.: In cases that the level of a specific heavy metal could not be detected or was trace, it excluded from the calculation.

Table 6
Target hazard quotient (THQ) for assessment assessments of heavy metals risk for Iranian population posed by pistachio consumption.

Heavy metals	Ahmadaghaei	Akbari	Kaleghoochi	Owhadi	Badami-e-sefid	Mean
Al	0.001	0.001	0.001	0.001	0.001	0.001
As	0.043	0.042	0.052	0.05	0.042	0.045
Cd	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Co	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Cr	0.0088	0.01	0.0074	0.0094	0.0034	0.0078
Hg	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Ni	0.0068	0.0068	0.0074	0.0048	0.004	0.0059
Pb	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Se	0.0009	0.001	0.0014	0.0008	0.0006	0.00094

N.E.: In cases that the level of a specific heavy metal could not be detected or was trace, it excluded from the calculation.

order: As > Cr > Ni > Al > Se. Results showed that As intake has the highest potential health risk of interaction effects and Co ingestion has the minimum risk. However, all values a well below 1 which indicates no risk.

3.5.3. Hazard index (HI)

The accepted value for HI should be equal or below 1 indicating no significant health risk. The probability of experiencing long-term health effects increment with the increasing HI value and according to Lemly (1996) is for HI = 1.1–10 moderate risk while HI > 10 reflects to highly risk (Ogunkunle et al., 2013). HI value for collected pistachio cultivars consumed by the Iranian population, is 0.066. This means that pistachio intake pose no risk for the health of the Iranian population.

4. Conclusion

In the present study heavy metals concentrations were evaluated in five commercial pistachio cultivars collected from four cultivation zones in Iran. In addition, the possible role of type of cultivar and region in heavy metals concentration in pistachio samples and associated risk for consumers were investigated. We showed that the differences in content of detected heavy metals in the samples were related to the type of the cultivar, their ability to absorb the heavy metals, the geographical origin and the characteristics of the soil and water of the cultivation zone. According to our measurements among the tested cultivars Kaleghoochi had the highest amount of the majority of trace elements (Al, As, Ni and Se). In respect of geographical sites Sarakhs was the most polluted region. Overall, the level of heavy metals in fresh pistachio samples could be considered as of no health risk to Iranian population safety but it necessitates a continuous monitoring.

Conflict of interest

Authors declare that there is no conflict of interest. Also, it should be noted that the present article may not be considered as an EFSA scientific output and it is published under the sole responsibility of the co-author Marina Goumenou. The positions and opinions presented in this article are those of the authors alone and do not represent the views/any official position or scientific works of EFSA. To know about the views or scientific outputs of EFSA, please consult its website under http://www.efsa.europa.eu."

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