Journal of Nuts 12(3) (2021) 215-225

Journal of Nuts

Journal homepage: ijnrs.damghaniau.ac.ir

Risk Assessment of Contamination of Soil, Water and Plants to Arsenic in Pistachio Orchards of Kerman Province, Iran

Simin Yazdanpanah–Ravari[1], Hossein Heidari Sharifabad[*2], Hossein Abbaspour[3], Alireza Iranbakhsh[1]

[1] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2] Department of Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran

[3] Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran

A R T I C L E I N F O

Keywords:

Arsenic contamination;
Food chain;
Heavy metal;
Pistachio plant

A B S T R A C T

Arsenic (As) is a natural occurring element which poses a potential risk to groundwater quality, as well as food safety through As transportation in soil-water-plant systems. The present study investigated the level of As contamination in water, soil, and pistachio plants (leaves and seeds) in 7 regions of Kerman province, as one of the most important pistachio cultivation regions in Iran. The results revealed that the concentration in the soil of the sampled areas varied from 15 to 1200 mg/kg The highest As concentrations was observed in the Shahr-e Babak, Bayaz, and Sirjan regions. The As concentration in the water of the sampled areas was between 0.62 and 483 µg/L, and the regions of Sirjan and Shahr-e Babak had the highest levels of arsenic contamination. The highest accumulation of As in pistachio leaves and seeds was observed in the Shahr-e Babak, Bayaz, and Sirjan areas, which had a positive correlation with the As concentration in the soil and water of the area. Thus, the level of As in fresh pistachios in Shahr-e Babak, Sirjan, and Bayaz regions can be considered a threat to the safety of the Iranian population.

Introduction

Arsenic (As) is found as a toxic element in water, soil, plant, and air resources. The toxicity of As depends on the oxidation state, whose concentration in the environment increases naturally or in response to industrial activity (Mahzuz *et al.,* 2009; Villa-Lojo *et al.,* 2002). Arsenic is found in organic and inorganic forms in the soil, and since the inorganic form is easily transferred from the soil, water, and plants to humans and is more toxic than the organic form, the inorganic form is more important (Babel and Opiso, 2007). Arsenic has been shown to induce skin, intestinal, bladder, kidney, and hepatic cancers in humans (Smith *et al.,* 1992). Drinking polluted water is one of the main ways for As to enter the human body, which has been reported in many parts of the world, including China, Bangladesh, Taiwan, Vietnam, India, Chile, Argentina, Iran, and the United States. On the other hand, the use of groundwater and running water contaminated with As to irrigate crops increases the risk of As transfer from contaminated water and soil to the plant, as well as its entry into the human food chain (Jack *et al.,* 2003; Ghorbani *et al.,* 2011). For example, in some parts of Bangladesh, India, and Pakistan, water contaminated with As is used to irrigate rice plants, which has contaminated rice in these areas (Abedin *et al.*, 2002; Williams *et al.*, 2005; Ghorbani *et al.*, 2009). It has been shown that the soil in some parts of Iran, including the provinces of Khorasan, Kurdistan, and Kerman, is contaminated with As, which has negatively affected the ecosystem and living organisms in these areas (Ghassemzadeh *et al.,* 2006; Zandsalimi *et al.,* 2011).

Pistachio (*Pistacia vera* L.) is one of the most important plants of the Anacardiaceae family that is commonly planted in arid and semi-arid regions of Iran (Norozi *et al.*, 2019), Greece, Italy, Syria, Turkey

[*]**Corresponding author**: Email address: h.heidari1871@hotmail.com
Received: 13 December 2020; Received in revised form: 7 June 2021; Accepted: 14 August 2021
DOI: 10.22034/jon.2021.1917726.1102

and USA. In Iran, pistachios are known as nuts containing vitamins, minerals, fatty acids, sterols and phenolic compounds (Roozban *et al.*, 2005; Alipour 2018; Eslami *et al.*, 2019; Behzadi Rad *et al.*, 2021), which are consumed as a salted, roasted and fresh nut snacks (Ardakani, 2005). One of the major concerns in food safety is that they are contaminated with heavy metals. Toxic heavy metals not only negatively affect the quality of crops, but also cause biological, chemical and physical problems, as well as mutagenic and cytotoxic effects in animals and humans (Al-Othman *et al*., 2012). Accordingly, to protect public health against the dangers of heavy metals, it is important to monitor the presence of heavy metals in strategic horticultural products, especially pistachios (Taghizadeh *et al*., 2017). In recent years, the joint FAO/WHO Expert Committee on Food Additive (JECFA) recommended permissible tolerable weekly intake (PTWIs) (Joint, 2003). Kerman province is the most important region for pistachio cultivation in Iran, which has the highest cultivated area of pistachios. Therefore, in current study, in addition to the study of the soil and water of the contaminated areas in terms of As concentration and related variables, As accumulation in different parts of pistachio and its relationship with As concentration of the soil and water in different regions was investigated to study the As transfer cycle between the soil, water and plant.

Material and Methods

Study areas and plant materials

Kerman province, with an area of 181,714 km^2, is the second largest province in Iran, located between 54º21´E and 59º34´E, also between 26º29´N and 31º58´N. Sampling of soil, water and pistachio plants was done in 7 different areas of Kerman (Sirjan, Shahr-e Babak, Bayaz, Anar, Kabootarkhan, Kazem Abad, Zarand and Ravar) in August and September 2019 (Fig. 1). In the current study, sampling of leaves and fruits of one of the most important commercial cultivars, ‘Akbari’ cultivar was performed. After separating the soil and hard shells of the fruit, the kernels were dried at room temperature for 72 hours and stored at -20 ºC for chemical analysis.

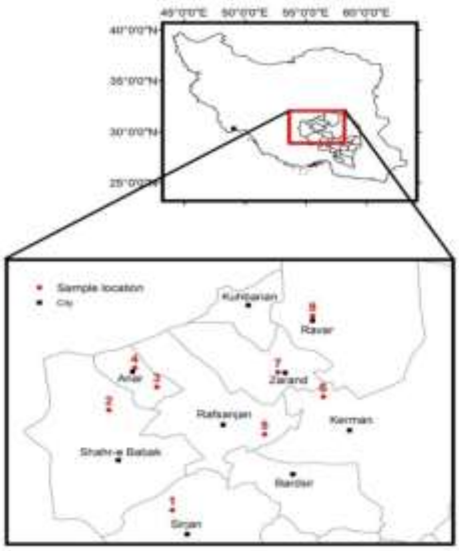

Fig. 1. Location of the studied areas in Kerman province, Iran. (1= Sirjan, 2= Shahr-e Babak, 3= Bayaz, 4= Anar, 5= Kabootarkhan, 6= Kazem Abad, 7= Zarand and 8= Ravar)

Water and soil sampling

Irrigation water was collected from the study areas in sterile polypropylene bottles. To prevent microbial contamination, HNO_3 (1 M) was added to the bottles and stored in the refrigerator. Soil samples were also collected from selected cultivation areas from 0 to 50 cm depth. Then, after crushing and drying in air, they were screened with a 2 mm mesh sieve and kept at room temperature for further analysis. Water and soil sampling were performed with 4 replications, each of which consisted of 3 independent samples.

Water and soil analysis

After water filtration using a 0.45 µm membrane filter, qualitative parameters of water such as pH, electrical conductivity (EC) and total dissolved solids (TDS) were measured using a portable combine meter (Milwaukee, model SM802). The total hardness (TH) of the water was determined by the following equation: TH = $(2.5 \times Ca^{2+}) + (4.1 \times Mg^{2+})$ (Todd, 1980). The major anions and cations of water such as nitrate, bicarbonate, chloride, magnesium, calcium, potassium and sodium were measured according to the standard specifications of APHA *et al*., (1992). After acidifying the water with HNO_3 (0.2%, v/v), the As content of the water was measured using an atomic absorption spectrometry (Shimadzu, 6200).

pH and Organic matter of soil were measured potentiometrically in water-saturated soil paste and dichromate oxidation by Tiurin method (Zandsalimi *et al*., 2011), respectively. Total phosphorus was measured according to the method previously described by Ghorbani *et al*., (2019). The hydrometer method was used to determine soil texture (clay, silt and sand). To measure As, the soil was extracted using a HCl/HNO_3 (3:1) mixture in a Kjeldahl digestion tube. Then, after incubating at room temperature for 24 h and filtering with A moistened Whatman No. 40, the As content was determined using an atomic absorption spectrometry (Shimadzu, 6200).

Plant analysis

The plant samples (leaves and kernels) were digested in HNO_3:H_2O_2 (1:4) mixture after rinsing with deionized water and drying at 68 ºC for 72 h (Ghorbani *et al*., 2020). The As concentration of plant tissues was determined using an atomic absorption spectrometry (Shimadzu, 6200).

Statistical analysis

All measurements were performed with 4 replications, each of which was calculated with three independent samples (Ghorbani *et al*., 2011; Ghasemi-Omran *et al*., 2021). The data were analyzed using SAS 9.1.3 software and the mean comparison was carried out with a least significant difference (LSD) test (at the 5% level).

Results

Water characteristics

The results of water analysis in eight regions showed that there was no significant difference between the pH of water in the study areas, but there was a significant difference between the EC, TDS and TH characteristics of water between different regions (Table 1). The results showed that EC varied from 442 to 831 ds m^{-1} in the water of the studied areas so that the highest EC was observed in Shahr-e Babak (831 ds m^{-1}) and Sirjan (803 ds m^{-1}) regions and the lowest EC was observed in Ravar (442 ds m^{-1}) region (Table 1). The highest water TDS and TH were related to Shahr-e Babak area. Also, the lowest levels of water TDS and TH were observed in Ravar and Zarand regions, respectively (Table 1).

Table 1. Characteristics of water analysis in the studied areas

	pH	EC (ds/m)	TDS (mg/L)	TH (mg/L $CaCO_3$)
Sirjan	7.14 ± 0.02a	803.4 ± 19.5a	452.1 ± 30.0b	20.47 ± 3.78f
Shahr-e Babak	7.16 ± 0.03a	831.1 ± 30.0a	563.2 ± 20.1a	54.28 ± 2.18a
Bayaz	7.17 ± 0.03a	548.0 ± 20.3c	312.4 ± 19.5d	53.00 ± 2.20ab
Anar	7.15 ± 0.02a	651.1 ± 30.2b	367.2 ± 20.0c	47.56 ± 3.72bc
Kabootarkhan	7.16 ± 0.02a	613.0 ± 30.0b	359.2 ± 19.7c	42.35 ± 4.29cd
Kazem Abad	7.15 ± 0.04a	549.4 ± 29.8c	345.4 ± 38.1cd	40.93 ± 2.65d
Zarand	7.15 ± 0.03a	499.1 ± 30.1d	304.5 ± 29.5d	29.90 ± 0.44e
Ravar	7.16 ± 0.02a	442.1 ± 30.2e	253.6 ± 19.6e	33.74 ± 4.56e

Values marked with same alphabets are not significantly different (LSD, $p < 0.05$). All the values are means of four replicates ± SD.

The results of the present study showed that all the studied areas except Zarand and Ravar had higher As concentration than the World Health Organization standard level. The highest concentration of As was obtained in the water of Sirjan region with 483 µg L^{-1} (Table 2). The highest concentrations of Ca and Mg were observed in Shahr-e Babak water, while the lowest concentrations of Ca and Mg were recorded in the water of Kazem Abad and Sirjan regions, respectively (Table 2). The concentration of K in water ranged from 0.045 to 0.581 meq L^{-1}, with the highest and lowest concentrations of K in Bayaz and Sirjan, respectively (Table 2). Among the sampled areas, the highest concentrations of Cl and NO_3 were 3.62 and 0.98 meq L^{-1}, respectively, which were observed in Shahr-e Babak region, however, the lowest levels of Cl and NO_3 were recorded in the water of Sirjan and Ravar regions, respectively (Table 2). The level of water HCO_3 in the studied areas varied between 2.1 to 18.34 meq L^{-1}, with the highest levels of HCO_3 in the Ravar region and the lowest levels of HCO_3 in the Zarand region (Table 2).

Table 2. The average comparison of arsenic (As), calcium (Ca), magnesium (Mg), potassium (K), chloride (Cl), nitrate (NO_3) and bicarbonate (HCO_3) concentrations in the water of the studied areas

	As (µg L^{-1})	Ca (meq L^{-1})	Mg (meq L^{-1})	K (meq L^{-1})	Cl (meq L^{-1})	NO_3 (meq L^{-1})	HCO_3 (meq L^{-1})
Sirjan	483 ± 11.9a	5.21 ± 0.97b	1.82 ± 0.33e	0.045 ± 0.005f	1.78 ± 0.13d	0.176 ± 0.016e	6.03 ± 0.49b
Shahr-e Babak	357.1 ± 20.5b	6.59 ± 0.75a	9.22 ± 0.99a	0.269 ± 0.010d	3.62 ± 0.30a	0.982 ± 0.097a	6.52 ± 0.50b
Bayaz	177.8 ± 12.4c	6.32 ± 0.49a	9.08 ± 0.82ab	0.581 ± 0.051a	3.31 ± 0.27ab	0.937 ± 0.081a	5.61 ± 0.50bc
Anar	162.4 ± 15.2c	5.86 ± 0.48ab	8.02 ± 0.62bc	0.476 ± 0.026b	3.10 ± 0.28b	0.676 ± 0.081b	4.94 ± 0.51cd
Kabootarkhan	65.7 ± 10.1d	4.90 ± 0.50bc	7.34 ± 0.74c	0.371 ± 0.027c	2.21 ± 0.22c	0.566 ± 0.049c	4.25 ± 0.49d
Kazem Abad	28.3 ± 4.2e	3.40 ± 0.25d	7.91 ± 0.49bc	0.346 ± 0.027c	2.26 ± 0.14c	0.354 ± 0.032d	3.26 ± 0.33e
Zarand	4.66 ± 0.2f	3.59 ± 0.50d	5.10 ± 0.41d	0.333 ± 0.029c	2.13 ± 0.15c	0.247 ± 0.027e	2.10 ± 0.16f
Ravar	0.62 ± 0.1f	3.91 ± 0.47cd	5.84 ± 0.82d	0.192 ± 0.013e	2.05 ± 0.14cd	0.174 ± 0.013e	18.34 ± 0.98a

Values marked with same alphabets are not significantly different (LSD, $p < 0.05$). All the values are means of four replicates ± SD.

Soil characteristics

The soil texture, organic matter and pH of each region along with the concentration of As and P are shown in Table 3. The analysis of soil texture showed that there was a significant difference in the soil of the sampled areas. The highest amount of clay and silt were observed in Shahr-e-babak region with aa and 39.1 % while the sites of Zarand and Ravar had the lowest amount of clay and silt, respectively (Table 3). The clay amount in the studied areas was between 17 to 62%, with the highest and lowest clay levels recorded in Zarand and Shahr-e-babak regions, respectively (Table 3). Between different regions, there was a significant difference in the amount of organic matter.The highest and lowest levels of organic matter were observed in the regions of Anar and Shahr-e-babak by 0.97 and 0.56 %, respectively

(Table 3). Soil analysis showed that soil pH was alkaline in all areas (pH > 8). The EC in the studied areas varied from 0.52 to 1.72 ds m^{-1}, and the highest EC was recorded in the Ravar region. While the Anar and Shahr-e-babak regions had the lowest EC (Table 3). The highest concentrations of As were observed in the soil of Shahr-e-babak and Biaz regions with 1200 and 1055 mg kg^{-1}, respectively. In general, the Shahr-e-babak, Biaz, Sirjan, Anar, Kabotarkhan and Kazemabad regions had much higher As concentrations than the average threshold of toxicity (40 mg kg^{-1}) for crops (Table 3). The results also showed that the soil P concentration ranged from 9.63 to 21.23 mg kg^{-1}, with the highest P concentrations observed in the Shahr-e-babak region (Table 3).

Arsenic analysis in plant and the relationship between arsenic concentration in plants with soil and water

The results of the present study showed that the As concentration in pistachio leaves in the sampled areas varied from 1.17 to 8.42 mg kg^{-1} DW, so that the highest and lowest concentrations of As were observed in Shahr-e-babak and Ravar regions, respectively (Fig. 2). The results of As concentration in kernels showed that in only two regions, Ravar and Zarand, the concentration of As was in the normal range (from 0.1 to 0.5 mg kg^{-1} DW according to Lombi and Nolan (2005)), While in other regions, the As concentration was more than 0.5 mg kg^{-1} DW. The highest As accumulation in kernels was observed in Sirjan (2.07 mg kg^{-1} DW) and Shahr-e-babak (2.01 mg kg^{-1} DW) regions (Fig. 3).

Table 4 shows the correlation between As concentration in pistachio seeds and leaves with soil and water characteristics of the studied areas. The correlation results showed that there was a significant positive correlation between the As concentration in pistachio leaves and kernels with the As concentration, EC, TDS, TH, Ca, Cl and NO_3 in the water of the studied areas (Table 4). The As concentration in kernels and leaves had a significant positive correlation with pH, EC and total As of soil, and a significant negative correlation with the clay and organic matter of soil (Table 4).

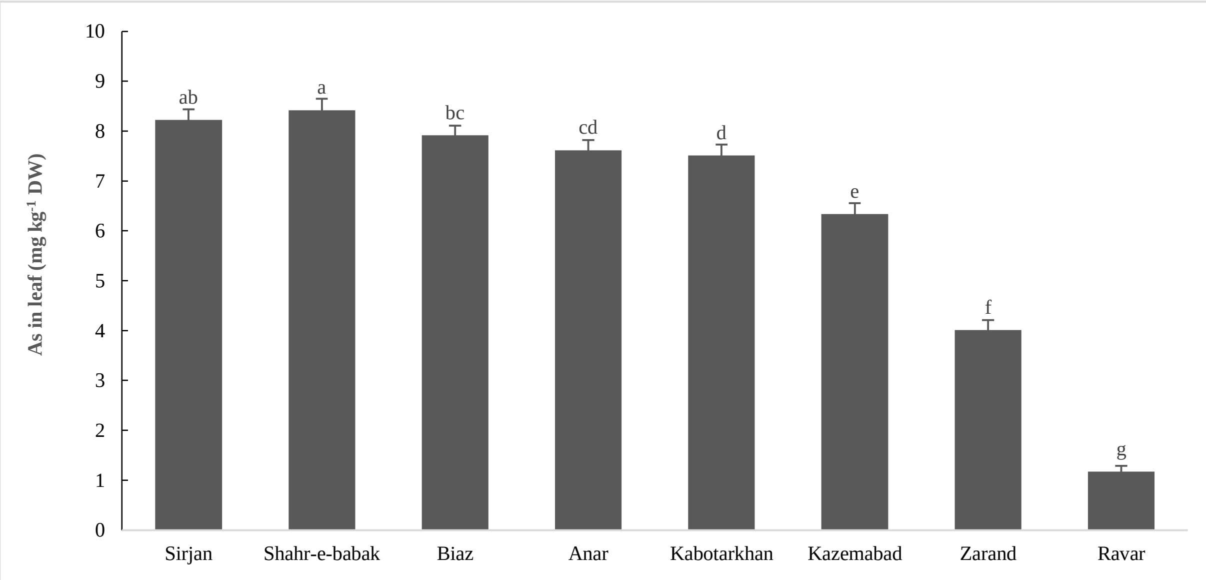

Fig. 2. Arsenic (As) concentration in pistachio leaves collected from 8 regions. Values marked with same alphabets are not significantly different (LSD, $p < 0.05$). All the values are means of four replicates ± SD.

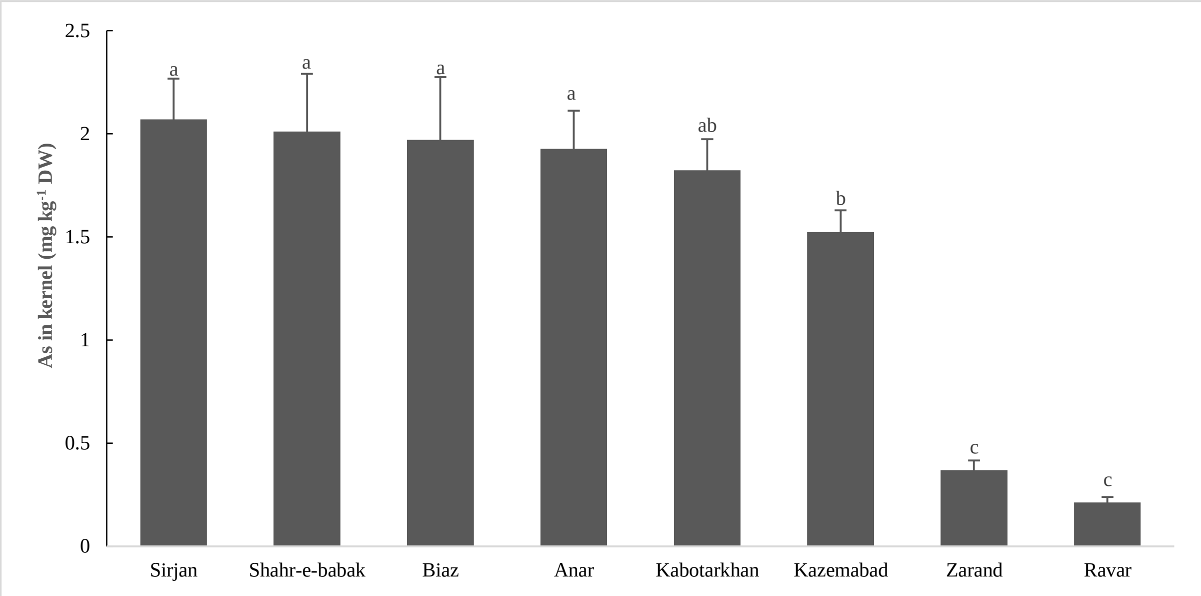

Fig 3. Arsenic (As) concentration in pistachio kernels collected from 8 regions. Values marked with same alphabets are not significantly different (LSD, $p < 0.05$). All the values are means of four replicates ± SD.

Table 3. The average comparison of soil characteristics of the studied areas

	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	pH	EC (ds m^{-1})	Total As (mg kg^{-1})	P (mg kg^{-1})
Sirjan	26.03 ± 2.95b	32.07 ± 2.10bc	39.07 ± 3.00c	0.74 ± 0.03cd	8.23 ± 0.15a	1.02 ± 0.07c	1023 ± 42bc	12.83 ± 0.75e
Shahr-e Babak	44.00 ± 3.50a	39.10 ± 3.85a	17.00 ± 1.70d	0.56 ± 0.02f	8.50 ± 0.21a	0.58 ± 0.04e	1200 ± 62a	21.23 ± 0.70a
Bayaz	28.00 ± 3.00b	36.00 ± 1.51ab	36.00 ± 3.00c	0.78 ± 0.04bc	8.47 ± 0.15a	0.71 ± 0.04d	1055 ± 44b	18.43 ± 0.45b
Anar	26.03 ± 2.45b	37.00 ± 2.03a	36.00 ± 2.60c	0.97 ± 0.05a	8.53 ± 0.25a	0.52 ± 0.03e	998 ± 36bc	19.27 ± 0.65b
Kabootarkhan	24.07 ± 1.90b	29.07 ± 2.91c	46.03 ± 2.93b	0.71 ± 0.04de	8.20 ± 0.21a	0.69 ± 0.04d	989 ± 27c	15.37 ± 0.43d
Kazem Abad	27.03 ± 1.95b	39.03 ± 2.92a	35.07 ± 2.91c	0.81 ± 0.03b	8.27 ± 0.14a	0.77 ± 0.03d	551 ± 27d	17.23 ± 0.57c
Zarand	14.07 ± 1.41c	22.07 ± 1.89d	62.07 ± 2.93a	0.68 ± 0.04e	8.33 ± 0.23a	1.33 ± 0.05b	34 ± 2e	12.27 ± 0.66e
Ravar	16.03 ± 1.93c	20.03 ± 1.94d	61.00 ± 3.00a	0.74 ± 0.05cd	8.30 ± 0.20a	1.72 ± 0.07a	15 ± 2e	9.63 ± 0.46f

Values marked with same alphabets are not significantly different (LSD, $p < 0.05$). All the values are means of four replicates ± SD.

Table 4. Correlation coefficient between the arsenic (As) concentration in pistachio leaves and kernels with water and soil characteristics of the studied areas

	Water										
	As in water	pH	EC	TDS	TH	Ca	Mg	K	Cl	NO_3	HCO_3
As in leaf	0.67**	-0.04ns	0.75**	0.69**	0.44*	0.66**	0.22^{ns}	0.22^{ns}	0.46*	0.62**	-0.63**
As in seed	0.67**	0.03ns	0.72**	0.62**	0.47*	0.71**	0.22^{ns}	0.19^{ns}	0.45*	0.58**	-0.45*
	Soil										
	Clay	Silt	Sand	Organic matter	pH	EC	Total As	P			
As in leaf	-0.89**	-0.06^{ns}	0.59**	-0.67**	0.87**	0.73**	0.65*	0.60^{ns}			
As in seed	-0.98**	0.27^{ns}	0.47^{ns}	-0.90**	0.91**	0.88**	0.86**	0.85**			

*, ** and ^{ns}: Significant difference values $P \leq 0.05$, $P \leq 0.01$ and non-significant.

Discussion

Increasing the concentration of As in drinking water as one of the most toxic heavy metals is known as one of the major public health problems in recent years in various parts of the world (Urík *et al*., 2009). The five studied areas (62.5%) had the As concentration of more than 50 µg/L, which is much higher than the maximum allowable As concentration of drinking water (50 µg L^{-1}) (WHO 1993 and 1996). As contamination in water and soil was reported in some parts of Kerman province (Dehghani and Abbasnejad 2011). Increased accumulation of heavy toxic metals such as As in environment (water, soil, etc.) can be a serious risk to human health due to uptake by plants and entry into food chains or entry into drinking water (Davis *et al*., 2001). The results of the present study showed that, not only As, but also high concentrations of NO_3^- can be a threat to the health of the local people, and their negative effects on public health could become apparent in the coming years. The findings of the current study could be useful for assessing the risk posed to the environment by natural processes in the contaminated areas (Ghorbani *et al.*, 2019).

The results of soil analysis showed that 6 regions of Sirjan, Shahr-e Babak, Bayaz, Anar, Kazem Abad and Kabootarkhan had As concentration higher than the average threshold of As toxicity for crops according to Sheppard (1992). In a report, Ghadiri Soufi *et al*., (2017) indicated that As contamination in the regions of Kerman province could be due to mining activities. The toxicity and bioavailability of heavy metals, including As in the soil, depends on various soil characteristics. In addition to soil characteristics, the amount of As accumulation in the plant varies from species to species (Davis *et al*., 2001). The use of groundwater contaminated with As in the studied areas can contaminate soil and crop products and thus lead to the entry of toxic metal As into the food chain. The results of the present study showed that increasing the concentration of As in soil and water increased the accumulation of As in pistachio leaves and seeds, which is contrary to the results reported by Zandsalimi *et al*. (2011).

It is well accepted that arsenates are absorbed by phosphate carriers by plant roots, although these carriers have a higher affinity to phosphate than arsenate. Competitive inhibitor of phosphate uptake by As has been reported by Meharg and Macnair (1990). The results showed that the soil of the studied areas had a concentration of normal phosphorus, which is assumed to have no significant effect on As uptake and accumulation in the plant. In general, many factors, including age and part of plant, plant species, pedological factors (drainage and minerals conditions, soil texture, colloid contents, organic matter and pH) and chemical speciation are involved in the uptake of As by plants (Singh *et al*., 2007; Casado *et al*., 2007). In general, the results showed that the main cause of the As accumulation in pistachio leaves and seeds was the As concentration in soil and water, so that areas with high concentrations of As (Sirjan and Shahr-e Babak), more As accumulation in pistachio leaves and seeds were observed.

Conclusions

In the present study, the As accumulation in pistachio seeds and leaves along with soil and water characteristics of 7 regions of Kerman province was investigated. According to the results of water, soil and plant analysis in 7 regions, the highest levels of As contamination were observed in Shahr-e Babak, Sirjan and Bayaz regions. The results of the present study showed that the difference in the As concentration of pistachio leaves and seeds in different regions is related to different concentrations of As in soil and water in the sampled areas. Therefore, the level of As in fresh pistachios in Shahr-e Babak, Sirjan and Bayaz regions can be considered a threat to the safety of the Iranian population, which needs further investigation.

Conflict of interest

The authors declare no conflict of interests

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