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Arsenic Tracking in Iranian Rice: Analysis of Agricultural Soil and Water, Unpolished Rice and White Rice

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ABSTRACT: Since rice is a staple food most consumed world-wide, there have been increasing health concerns regarding exposure to arsenic through rice consumption. Several studies have reported the accumulation of arsenic in rice grains cultivated in regions with elevated levels of arsenic in groundwater or contaminated soil. Therefore the principal aim of this study was determining the amount of arsenic in Iranian rice cultivated in the same farms in two consecutive years and arsenic tracking through agricultural water and soil. Therefore different provinces (Mazandaran, Gilan, Fars, Ghazvin, Lorestan and Khozestan) with high harvested area and various weather conditions were selected. Since the arsenic levels in water and soil that were used for rice cultivation are effective on adsorbed arsenic by the rice, therefore to specify the origin of the contamination, sampling from soil, water and unpolished rice of each selected farms were carried. Total arsenic content was determined from the digests by hydride generation atomic absorption spectrophotometer. The results indicated that accumulation and the increase in arsenic content of agricultural soil causes an increase in the arsenic content of white rice. The results have also shown that arsenic contents in all the investigated white rice samples are less than the maximum limit specified by the national standard (0.15 mg/kg), and no significant difference was observed in the arsenic content in two consecutive years of rice cultivation. Therefore the control of arsenic content in water used for irrigation, limited the usage of chemical fertilizers and pesticides that might affect the concentration of arsenic in the soil and maintain the aerobic conditions during either the vegetative or the reproductive stages of the rice growth that might reduce the concentration of arsenic in the soil and consequently in the rice.

Keywords: *Arsenic, Atomic Absorption Spectrophotometry, Soil, Unpolished Rice, Water, White Rice.*

Introduction

Arsenic is described as a metalloid occurring naturally in the earth’s crust in more than 200 mineral compounds. Arsenic compounds have been widely used in many industries, and as components in pesticides for agricultural applications. Both natural processes and human actions cause arsenic contamination in the air, water, soil, and food (Watson *et al*., 2014).

Arsenic can be easily accumulated in animals and plants, thereby causing excess arsenic contamination in food. Food is the major vector to deliver arsenic; one of the five most toxic heavy metals in the world; to the human body. Arsenic is present in food in many different chemical forms based on their physical, chemical, biological properties, and toxicity levels. Arsenic in food can be generally divided into two

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species: inorganic arsenic and organic arsenic (Chen *et al*., 2016). Arsenic enters the body in a solution form, therefore its toxicity mainly depends on their chemical structure and solubility. The inorganic arsenic species (As^{III} and As^{V}) are the most toxic forms of arsenic, and are carcinogenic in humans (Watson *et al*., 2014).

Inorganic arsenic has been classified by the International Agency for Research on Cancer as Group I carcinogen; while Monomethyl arsonic acid (MMA) and Dimethyl arsinic acid (DMA) are also considered as potential carcinogen.

Arsenic is released to environment through natural phenomena and anthropogenic inputs. Arsenic is also redistributed in the environment by rain, underground water, and human activities. Although arsenic in the environment comes mainly from minerals and geogenic sources, human activities such as mining, burning of fossil fuels, and indiscriminate use of arsenical pesticides during the early to mid1900s resulted in extensive soil contamination. A large number of sites around the globe have been reported to be contaminated by arsenic from natural and anthropogenic sources (Watson *et al*., 2014).

Inorganic arsenic is predominant in soils and ground waters, As (V) in aerobic and As (III) in anoxic conditions. Arsenic methylation can occur in soil systems due to microbial activity. The main route for arsenic bioavailability for humans is the chain food (via dietary intake and drinking water) (Mihucz *et al*., 2007).

Arsenic in food comes from 2 sources: one is from the gaseous, water or solid wastes, which are produced manually from industrial manufacture, mining, arsenic-containing pesticides, and volcanism. These contaminations can directly pollute air, water and soil, and further to be absorbed by animals and plants. The other source is from the machine, pipe, container, packaging materials, and additives which may contaminate food during processing, storage, transportation, and sales stages (Chen *et al*., 2016).

Widespread human exposure to arsenic from drinking water and foods, and associated incidences of human health hazards (non-carcinogenic effects, including dermal symptoms, respiratory problems, neurologic complications, obstetric effects, etc., and carcinogenic effects on different organs, affecting the skin, lung, bladder, liver etc.) have been a growing concern during the past three decades globally. Arsenic in groundwater has been reported in many parts of the world, including North America, Europe, Australia, and several Latin American countries. However, groundwater contamination with elevated levels of arsenic and associated health problems has reached critical levels in recent years in several South (S) and Southeast (SE) Asian countries (Watson *et al*., 2014).

The source of arsenic in the ground water remains undiscovered. However, it is believed that long term geological changes led to the release of arsenic from its core compound called arsenopyrites due to its oxidation by air reaching the underground aquifers through the tube wells conduits. The other theory indicates that reduction of iron and manganese oxy-hydroxide is associated with arsenic release to groundwater. Irrigation with arsenic contaminated groundwater is likely to increase its concentration in top soils of paddy fields and eventually in agricultural crops (Azizur Rahman *et al*., 2006).

Rice is the dominant staple food for over half of the world′s population, especially in developing Asian countries, contributing 70% of the energy provided by their daily food intake (Sommella *et al*., 2013). The world’s annual paddy rice production between 2008 and 2011 was fairly stable, at 686.1, 686.9, 683.4, and 702.2 million tons, respectively. Daily rice consumption varies considerably – from more than 600 g/day in

Myanmar and Laos to less than 50 g/day in some countries in Europe (Watson *et al.*, 2014).

Rice production is heavily concentrated in Asia, with just four countries, China, India, Indonesia, and Bangladesh, accounting for nearly 70% of global production (FAO, 2011). In the European Union, Italy is the leading rice producer with approximately 50% of the total EU harvest (Italian Grain and Feed Report, 2012) (Sommella *et al.*, 2013).

In Iran the total area under rice cultivation is about 600 000 ha and rice is grown in 16 provinces once a year, from April - May to August - September. However, more than 80 percent of rice harvested area is distributed in the two northern provinces of Mazandaran and Gilan coastal areas around the Caspian Sea where the climate is sub-tropical humid and very different to the rest of the country.

Rice is usually grown in anaerobic (flooded) conditions in which arsenic exists mainly as dissolved As (III) form and is readily taken up by the rice plant. Therefore, rice grown under flooded conditions was found to accumulate much more arsenic than that grown under aerobic conditions. As (V) was found to be the main arsenic species in the aerobic soil, and arsenic accumulation in rice grain was observed to be 10- to 15-fold higher in flooded than in aerobically grown rice. Maintaining aerobic conditions during either the vegetative or the reproductive stage of rice growth decreases arsenic accumulation in rice grain significantly as compared with rice grown under flooded conditions (Watson *et al.,* 2014).

In Iran, the production of rice is mainly based on river water irrigation. Additionally, the general cultivation practice of rice based on continuous flooding of the irrigation land leads to the development of anaerobic condition in the rice field and consequently increases the concentration of arsenic. Therefore the accumulation of arsenic in rice can become up to 10 fold higher than in other cereals (Halder *et al.*, 2014).

It has been reported that rice grains can accumulate arsenic from the soil. Rice accumulates arsenic in approximately 13- and 20-fold greater amounts as compared to wheat and barley, respectively.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a Provisional Tolerable Weekly Intake (PTWI) for inorganic arsenic of 0.015 mg/kg of bodyweight/week in 1988. However, the European Food Safety Agency (EFSA) has recently concluded that this PTWI is no longer appropriate as inorganic arsenic produces cancer of the lung and urinary bladder in addition to skin cancer, and a range of adverse health effects have been reported at exposures lower than those reviewed by the JECFA.

Recently, the JECFA proposed a maximum level of 0.2 mg/kg of inorganic arsenic in polished rice (Signes-Pastor *et al.*, 2016)

According to the Codex Standard No. 198-1995, rice shall be free from heavy metals in amounts which may represent a hazard to human health (Anon, 1995).

European Union in April 2016 proposed a maximum level of inorganic arsenic (0.25 mg/kg) in husked rice.

According to the Institute of Standards and Industrial Research of Iran (No. 12968) the maximum amount of arsenic in white rice is 0.15 mg/kg.

The main sources of arsenic in rice grain are arsenic rich paddy soils and irrigation water. Arsenic chemistry in paddy soils is extremely complex because of frequent redox cycles in the soil, and arsenic uptake in rice from soil is influenced by a range of factors, including bioavailability, rhizosphere processes, and metabolism in rice plants. Therefore, agronomic measures such as water management and fertilization practices would be effective methods to reduce arsenic uptake in rice grain (Watson *et al*., 2014).

Several studies have reported the accumulation of arsenic in rice grains cultivated in regions with elevated levels of arsenic in groundwater (Halder *et al*., 2014).

Because of high levels of arsenic in paddy soils, from contaminated underground irrigation water, rice contains relatively higher amounts of arsenic as compared to other agricultural crops.

Meharg and Rahman (2003) reported 10-fold elevation of arsenic levels in rice grain gown in arsenic contaminated soil. They also reported variations in arsenic content for different rice varieties. Thus, though drinking water is the main source of arsenic in the environment, it is not the only source for human being. For the population living on subsistence rice diets, arsenic contaminated rice grain contributes greatly in its dietary intake (Azizur Rahman *et al*., 2006).

Previously, 1.83 mg of As/kg have been reported in rice grain collected from the arsenic affected areas of Bangladesh (Meharg and Rahman, 2003). Thus, arsenic concentration in rice grain is a newly uncovered disaster on a massive scale for the population of subsistence rice diet. Onken and Hossner (1995) reported that plants grown in soils treated with arsenic had higher rates of its uptake for similar rates of growth as compared to that of untreated soils. However, use of arsenic contaminated groundwater for rice cultivation could be another major route for arsenic intake in human body (Azizur Rahman *et al*., 2006).

It has been shown that plants, including rice, can accumulate arsenic at different concentrations in various edible parts (Watson *et al*., 2014).

Therefore the aim of this research was to determine the amount of arsenic in Iranian rice cultivated in the same farms in two consecutive years and arsenic tracking through agricultural soil and water.

Materials and Methods

- *Sampling in 2016*

The principal aim of this study was to determine the amount of arsenic in Iranian rice. Therefore five different provinces (Mazandaran, Gilan, Fars, Ghazvin and Lorestan) with the high harvested area and various weather conditions were selected. In all the areas river water has been used for the rice cultivation.

Since the arsenic levels in water and soil that were used for rice cultivation are effective on adsorbed arsenic by the rice, therefore to specify the origin of the contamination, sampling from soil, water and unpolished rice of each selected farms were carried from 15th of September to 15th of October 2016.

Soil samples (1 kg) were also collected from three points of 2 m^2 areas and 10–15 cm depth of the selected plots.

Water samples (1 L) were collected from the river nearby the rice field where the irrigation has been performed. Water was sampled in polyethylene bottles and then filtered through 0.45 Millipore filter paper and preserved in the refrigerator at 4 °C temperature.

Unpolished rice samples were collected during harvest and sun dried immediately after collection, tagged properly, air tight in the polyethylene bags and kept in room temperature for further laboratory analysis.

The dehusking and milling process were carried out to remove the outer part of unpolished rice (husk and bran) to make it edible. The samples of the white rice were packed in polyethylene bags and kept at room temperature.

- *Sampling in 2017*

Sampling from the white rice was carried out exactly from the same selected farms in Mazandaran, Gilan, Ghazvin and Lorestan from the 7th of September to the 7th of October 2017. White rice samples cultivated in three farms in Khozestan were also collected from the 20th of June to the 20th of July 2017.

- *Methods*

Unpolished and white rice samples were milled to particles of less than 0.5 mm using a food mill (Molinex, France). The pretreatment and measurements were carried out according to the Iranian National Standardization Organization, No. 16722.

5 ml of the concentrated HNO_3 was added to 250 mg of dry sample in each of the 250ml quartz glass digestion tubes and allowed to evaporate slowly to dryness. The tubes with dried samples were placed in a muffle furnace with an initial and final temperatures of 150 and 425±25°C respectively programed at 50°C per hour. Furnace was kept at the final temperature of 425±25°C for 12 hours. Distilled water (1 ml) and hydrochloric acid (5 ml) were added to the samples and then diluted to 25 ml with diluted hydrochloric acid (6 mol/l).

Total arsenic content was determined from the digests by hydride generation atomic absorption spectrophotometer (Varian 20plus AA). The absorptions of the samples were read at the wavelength of 193.7 nm.

All the instruments were calibrated using matrix-matched standards. In each analytical batch at least two reagent blanks and three duplicate samples were included in the acid digests to assess the accuracy of the chemical analysis.

- *Statistical Analysis*

All the experiments and/or measurements were carried out in triplicate order. The data were statistically analyzed using the Statistical Analysis System software package on replicated test data. Analyses of variance were performed by application of ANOVA procedure. Significant differences between the mean values were determined using Duncan multiple range test. Microsoft Excel 2016 were used for data analysis.

Results and Discussion

- *Arsenic in water samples*

According to the Environmental Protection Agency (EPA) and World Health Organization (WHO) the maximum contaminant levels of arsenic in drinking water is 10 µg/L (or 10 ppb). The maximum arsenic levels in drinking water according to the Iranian National Standards Organization (Standard No. 1053) has been set at 0.01mg/L.

According to the European Food Safety Authority (2009) the average arsenic content of seawater is about 1.5-1.7 µg/L but increased concentrations have been reported in some coastal areas. The concentrations of arsenic measured in european rivers vary between 0.1 and 1.7 µg/L (Anon, 2009). The mean arsenic concentrations in groundwater has been reported between 0.1-2 µg/L, but in areas with volcanic rock or sulfide mineral deposits the concentrations might be increased up to 3,400 µg/L. In some mining areas the arsenic concentrations can occur up to 48,000 µg/L (Anon, 2009).

Several studies have been reported concerning the accumulation of arsenic in rice grains cultivated in regions with elevated levels of arsenic in groundwater (Halder *et al.*, 2014). Countries in south and southeast asia have been affected by arsenic due to the groundwater containing high arsenic content namely Bangladesh, several states of India, Nepal, Myanmar, Pakistan, Vietnam, Lao People’s Democratic Republic, Cambodia, Taiwan, and several provinces of China, and, more recently, the lowlands of Sumatra in Indonesia (Watson *et al.*, 2014).

In this research river water was used to irrigate rice paddies and the amounts of arsenic in all the samples were less than the detection limit of the instrument (<4 µg/L). Although the low amount of arsenic in used water does not have significant effect on the absorption of arsenic in rice, it should be noted that rice is cultivated in anaerobic (flooded) conditions that even the low concentrations of arsenic in water is accumulated in the soil and this leads to increased concentration of arsenic in soil and consequently in the rice.

- Arsenic in soil samples

The arsenic concentrations in soil samples obtained from different regions have been indicated in Table 1. The amount of arsenic in eleven soil samples belonged to five different regions ranged from 1.46±0.04 mg/kg to 8.20±0.10 mg/kg (Figure 1).

The mean value of arsenic in soil samples obtained from different regions of Gilan and Lorestan were the lowest (2.115 mg/kg) and the highest (8.10 mg/kg), respectively.

The results indicated that there are significant differences between the amount of arsenic in soil of Gilan with the other samples ($p<0.05$). Whereas there are not significant differences between the amounts of arsenic in soils of Ghazvin, Mazandaran, Fars and Lorestan.

The irrigation of rice paddies in the studied areas was through river water and arsenic was determined in trace quantities in used water samples therefore the difference between the amount of arsenic in soils of different regions might be due to the nature of the soil of each region and in particular the use of chemical fertilizers and pesticides.

Arsenic in soil could be derived from natural and anthropogenic sources. Background arsenic levels in surface soils range from 0.1 to 55 mg/kg (Anon, 2009).

Levels of arsenic in soil has been reported 0.1-40 mg/kg (Nicholson *et al.*, 2003). Upper limits of “normal” arsenic concentration in sand, sandy loam, loam and loam/clay non-polluted soils have been determined 10, 15, 20 and 30 μg/g, respectively (Pais & Jones, 2000).

The maximum acceptable concentrations of arsenic considered as phytotoxic in agricultural soils in Japan, United Kingdom, Canada, Poland, Germany and Austria has been proposed 15, 20, 25, 30, 40 and 50 mg/kg, respectively (Pais & Jones, 2000) while the maximum amount of arsenic in the soil of China has been determined ≤30 mg/kg (Dong, 2001)

Atmospheric pollution and application of chemical fertilizers and pesticides appear to be the major contributors to the anthropogenic arsenic deposition in agricultural soils. The arsenic content of fertilisers depends on its concentration in raw material used for the production. Rock phosphate used for the manufacturing of fertilizers and detergents, can contain up to 200 mg arsenic/kg (Anon, 2009).

Table 1. Arsenic content of soil, unpolished rice and white rice samples obtained from different regions (Mean ± SD)

Region		**Soil (ppm)**	**Unpolished Rice (ppb)**	**White Rice (ppb)**
Gilan	1	2.77 ± 0.05	149.29 ± 3.72	75.43 ±1.44
	2	1.46 ± 0.04	104.70 ± 3.35	49.20 ± 1.17
Mazandaran	1	6.00 ± 0.10	159.17 ± 8.69	94.90 ± 0.38
	2	6.10 ± 0.15	156.99 ± 6.48	111.04 ± 1.59
	3	6.80 ± 0.20	201.22 ± 1.00	75.16 ± 3.93
Lorestan	1	8.20 ± 0.10	193.14 ± 5.14	123.03 ± 1.23
	2	8.00 ± 0.20	136.12 ± 2.32	92.49 ± 3.95
Fars	1	6.70 ± 0.10	201.67 ± 2.10	118.19 ± 4.17
	2	6.25 ± 0.05	219.03 ± 2.70	113.10 ± 4.91
Ghazvin	1	6.80 ± 0.25	132.83 ± 4.61	109.20 ± 3.29
	2	7.50 ± 0.30	183.00 ± 1.88	113.03 ± 0.20

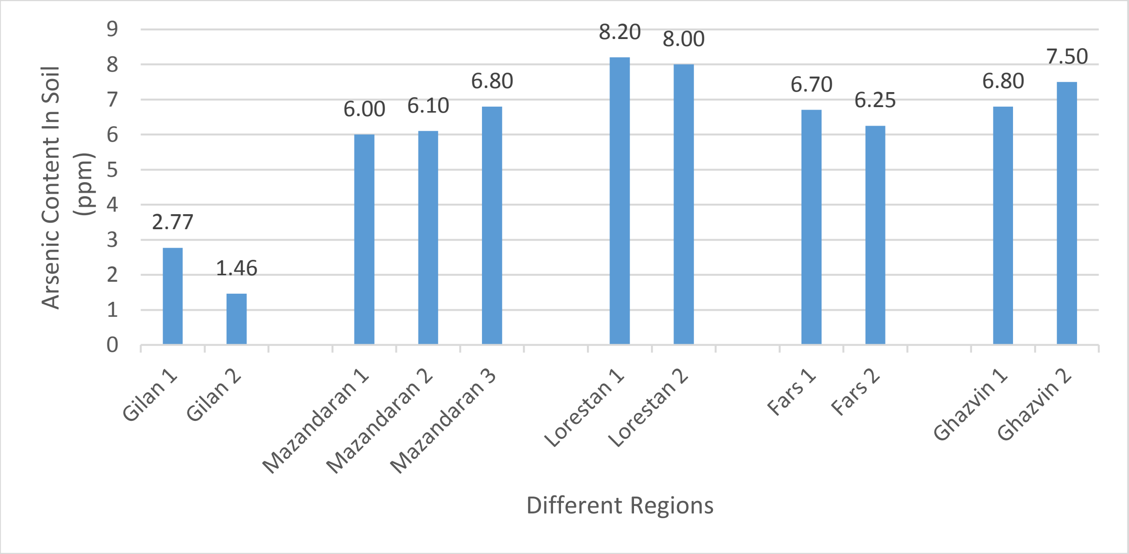

Fig. 1. Arsenic content of soil samples obtained from different regions

Jayasumana *et al*. (2015) indicated that almost all the agrochemicals available to the farmers in Sri Lanka especially phosphate fertilizers are contaminated with arsenic. The highest amount was in triple super phosphate with a mean value of 31 mg/kg. Also arsenic contamination in pesticides used in the studied area varied from 0.18 mg/kg to 2.53 mg/kg.

Therefore it seems that regular consumption of chemical fertilizers and pesticides can cause accumulation and increase the arsenic content of soil in long term.

Williams *et al*. (2006) stated the background levels of arsenic in rice paddy soils ranging from 4 to 8 µg/g that can reach up to 83 µg/g in areas where the crop land has been irrigated with arsenic-contaminated groundwater (Azizur Rahman & Hasegawa, 2011).

Due to the amount of arsenic in the investigated soils that is less than 10 mg/kg; lower than the standard concentration, problems concerned with arsenic contamination in the soils of studied regions were not observed.

- *Arsenic in unpolished samples*

Table 1 presents the amount of arsenic in 11 unpolished rice samples related to 5 different regions. The concentration of arsenic ranged between 0.105 to 0.219 mg/kg (Figure 2).

According to the mean values of arsenic in unpolished rice samples from different areas of study the highest concentration of arsenic was identified in Fars province (0.210 ppm) and the least was detected in Gilan samples (0.127 ppm).

There are significant differences between the amount of arsenic in unpolished rice of Gilan and Fars regions ($p<0.05$). There are not significant differences between the results of arsenic in unpolished rice of these two areas with other studied regions.

Although the regression analysis reveals that the extent of arsenic in unpolished rice is positively correlated with the arsenic concentration in the soil. In other word it might be suggested that the arsenic concentration in unpolished rice to some extent is related to the arsenic concentration in the soil. The correlation factor between the amount of arsenic in the soil and unpolished rice samples ($r=0.53$) is moderate that indicates the increased arsenic in the soil to some extent has some effects on the concentration of arsenic content in the unpolished rice.

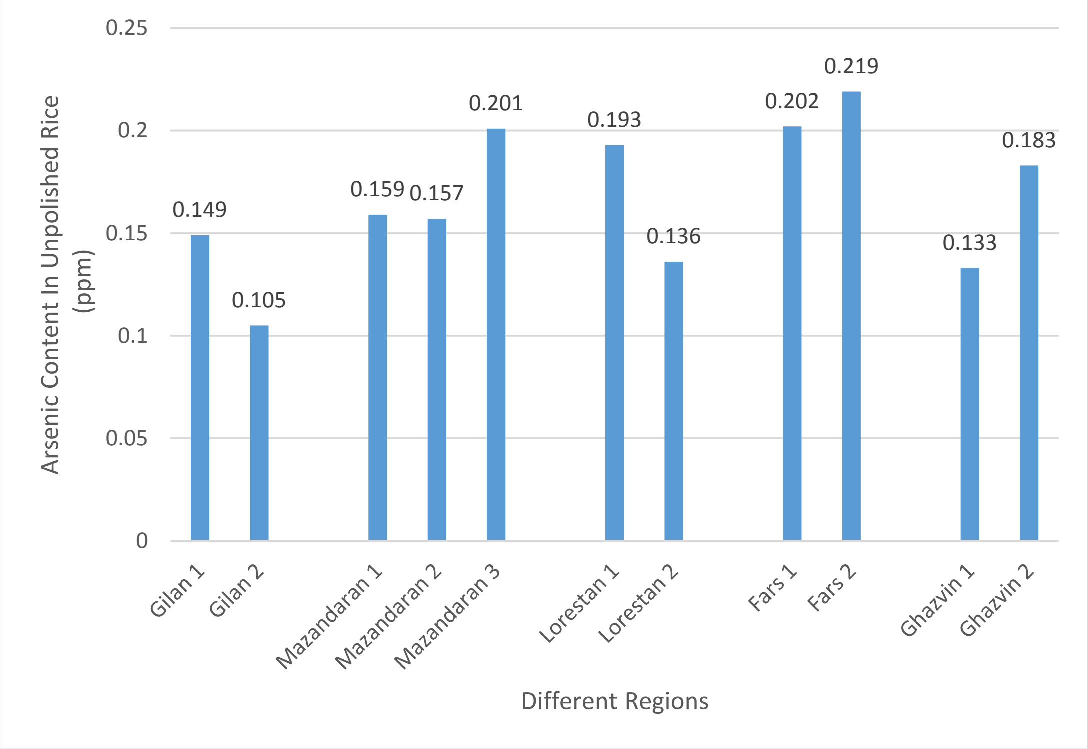

Fig. 2. Arsenic content in unpolished rice samples obtained from different regions

- Arsenic in white rice samples cultivated in 2016

The arsenic content in rice samples obtained from different regions has been indicated and presented in Figure 3. The concentrations ranged from 0.049 mg/kg to 0.123 mg/kg. According to Figure 4 that indicates the mean value of arsenic in rice samples obtained, the highest amount of arsenic was detected in Fars (0.115 mg/kg) and Ghazvin (0.111 mg/kg) samples while Gilan area had the lowest concentration (0.062 mg/kg). There are significant differences between the arsenic contents of rice cultivated in Gilan with other studied regions ($p<0.05$).

The Codex Alimentarius Commission (2014), an international body coordinated by WHO and the Food and Agriculture Organization of the United Nations, has proposed a maximum level of 0.2 mg/kg (or 200 μg/kg) for inorganic arsenic in white (or polished) rice (Newbigging *et al*., 2015).

European Union in April 2016 proposed a maximum level of inorganic arsenic (0.25 mg/kg) in husked rice also this commission has proposed a limit of 0.2 mg/kg for white rice, 0.3 mg/kg for puffed rice and 0.1 mg/kg for rice products intended to be used for children (Erickson, 2015).

According to The Institute of Standards and Industrial Research of Iran (No. 12968) the maximum amount of arsenic in white rice is 0.15 mg/kg.

Therefore the results indicated that arsenic content in all the investigated samples is less than the maximum limit specified by standards (Figure 3).

The arsenic levels in the rice grain are influenced by environmental conditions (arsenic in soil and water), agricultural conditions (materials, cultivars, and control of irrigation water), genetic variation, rice-processing and preparation methods and soil biogeochemistry (Sommella *et al.*, 2013; Halder *et al*., 2014).

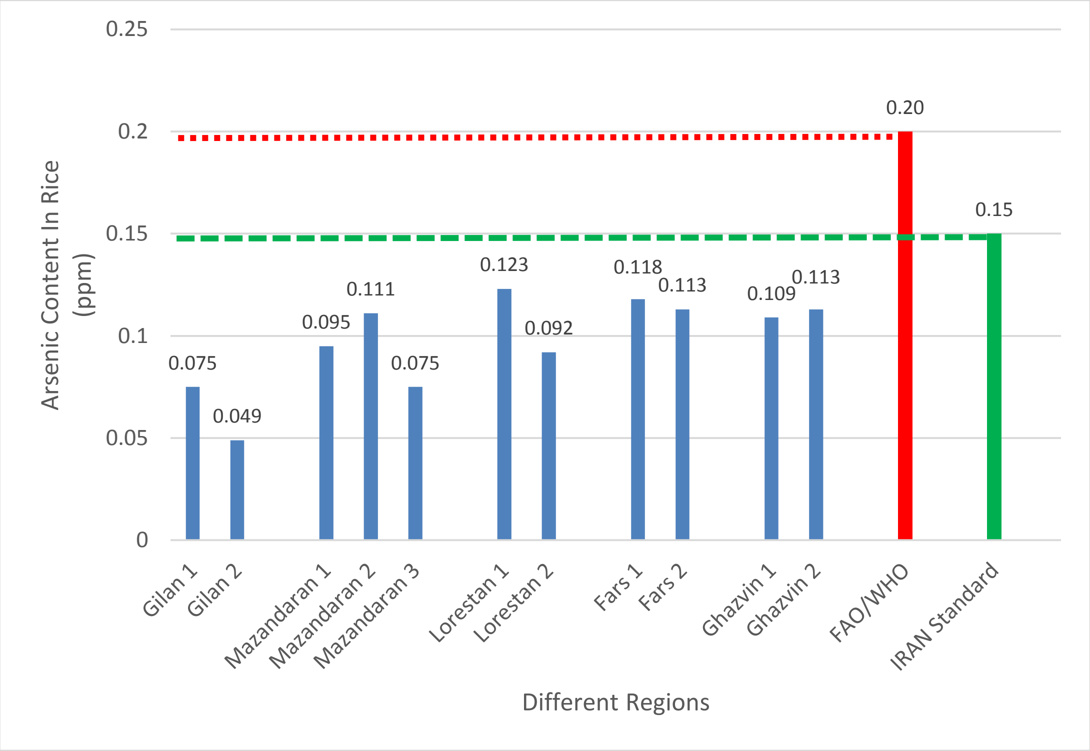

Fig. 3. Arsenic contents in rice samples obtained from different regions in 2016 as compared to the standards

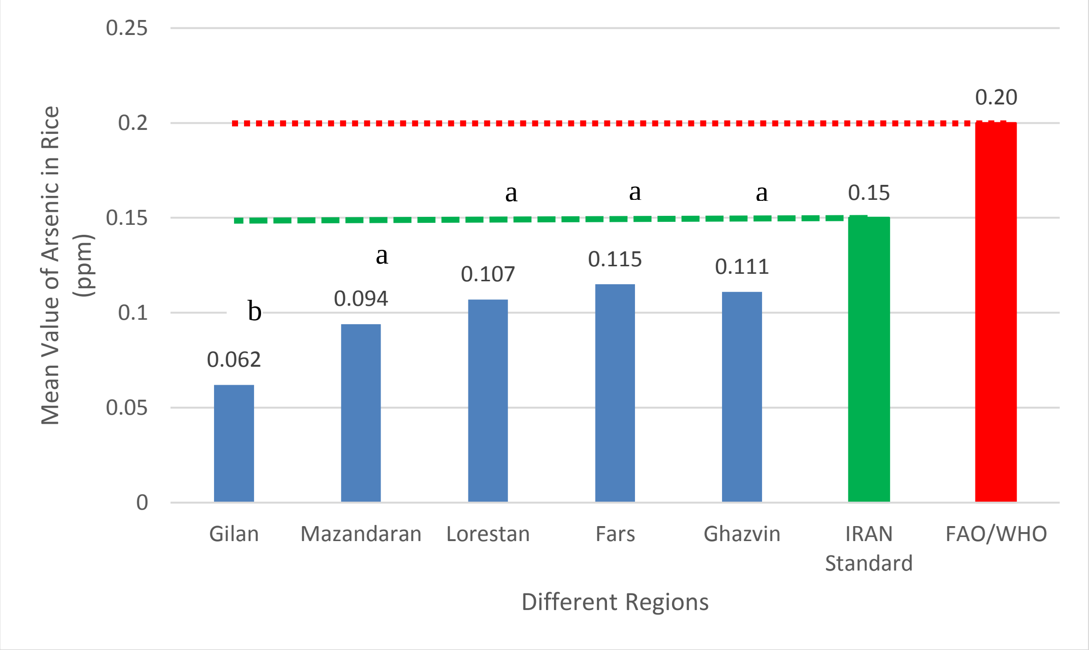

Fig. 4. The mean values of arsenic in rice samples obtained from different regions in 2016 as compared to the standards

The main sources of arsenic in rice grain are arsenic rich paddy soils and irrigation water. Arsenic chemistry in paddy soils is extremely complex because of frequent redox cycles in the soil, and arsenic uptake in rice from soil is influenced by a number of factors, including bioavailability, rhizosphere processes, and metabolism in rice plants. Therefore, agronomic measures such as water management and fertilization practices would be effective methods to reduce arsenic uptake in rice grain (Watson *et al*., 2014).

Accumulation of arsenic in rice is at higher levels as compared to other crops, such as wheat (Schoof *et al*., 1993; Williams *et al*., 2007). There have been many studies focusing on rice physiology and arsenic uptake (Meharg and Hartley-Whitaker, 2002; Ma *et al*., 2008; Zhu *et al*., 2008; Rahman and Hasegawa, 2011; Zhang *et al*., 2011; Zhao *et al*., 2009 and 2013), but there is still no common consensus on the underlying mechanism for the higher arsenic uptake by rice (Newbigging *et al*., 2015).

Under anaerobic conditions arsenic in soil is converted readily to arsenite which is mobile, leading to arsenic in rice grain. This occurs in soils which have no or limited anthropogenic contamination. Rice grain arsenic level is elevated further when grown in soils that are subjected to anthropogenic contamination namely arsenical pesticide use, base and precious mining and smelting impacted soils and contaminated water irrigated soils (Raab *et al*., 2006).

The regression analysis reveals that the extent of arsenic in white rice is positively correlated with the arsenic content in the soil. Therefore accumulation and increase in the arsenic content of agricultural soil causes an increase in the arsenic content of white rice.

The correlation factor between the concentration of arsenic in the soil and white rice samples (r=0.77) shows a relatively high dependency that indicates the increased arsenic in the soil has significant effects on the increase of arsenic content in the white rice (Figure 5).

According to the Figure 6 the arsenic content in all the rice samples decreased after dehusking and milling. The percent reduction of arsenic content in the samples after milling is shown in Figure 7. The highest reduction of arsenic in rice samples was related to Gilan samples with 51% reduction and the lowest was related to Ghazvin sample with 28% as presented in Figure 8.

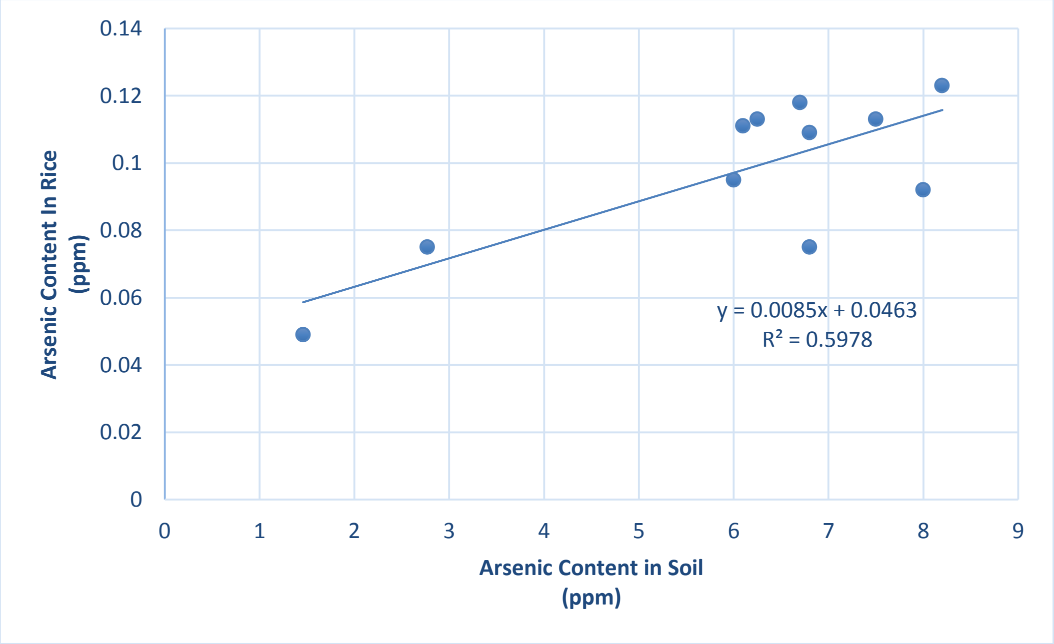

Fig. 5. Correlation of the extent of arsenic between soil and white rice samples

Therefore the results indicated that the maximum amount of arsenic is accumulated in the outer layers and during milling process, in order to produce white rice, the arsenic content is reduced up to 50% depending on the milling condition.

Meharg *et al.* (2012) utilized synchrotron-based X-ray fluorescence (S-XRF) to investigate the location of arsenic in polished (white) and unpolished (brown) rice grains. The study showed that most arsenic is localized on the surface, consisting of the pericarp and aleurone layer of brown rice. The polishing process removes all of the pericarp and most of the aleurone layer, thus producing rice bran that contains higher levels of total and inorganic arsenic as compared to the amount found in the endosperm (white rice) (Watson *et al.*, 2014).

Naito *et al*. (2015) reported that the total arsenic content in brown rice stored in grain form at 15°C or 25°C was stable for one year.

It should be noted that the assessment of arsenic exposure by only quantifying arsenic content in the raw rice could provide misleading information on the actual health risk (Halder *et al*., 2014).

The preparation of rice for cooking, and the cooking methods, have a substantial influence on arsenic retention in cooked rice.

- *Arsenic in white rice samples cultivated in 2017*

The arsenic content in rice samples obtained from different regions in 2017 has been indicated in Figure 9. According to this figure the highest amount of arsenic was detected in one of the Lorestan samples (0.174 ppm) while Khozestan area had the lowest concentrations (0.035-0.055 ppm) that might be due to the aerobic condition of cultivation. In anaerobic (flooded) conditions arsenic exists mainly as dissolve As (III) form and is readily taken up by the rice plant. Therefore, rice grown under flooded conditions was found to accumulate much more arsenic than that grown under aerobic conditions. Maintaining aerobic conditions during either the vegetative or the reproductive stage of rice growth decreases arsenic accumulation in rice grain significantly as compared to the rice grown under flooded conditions.

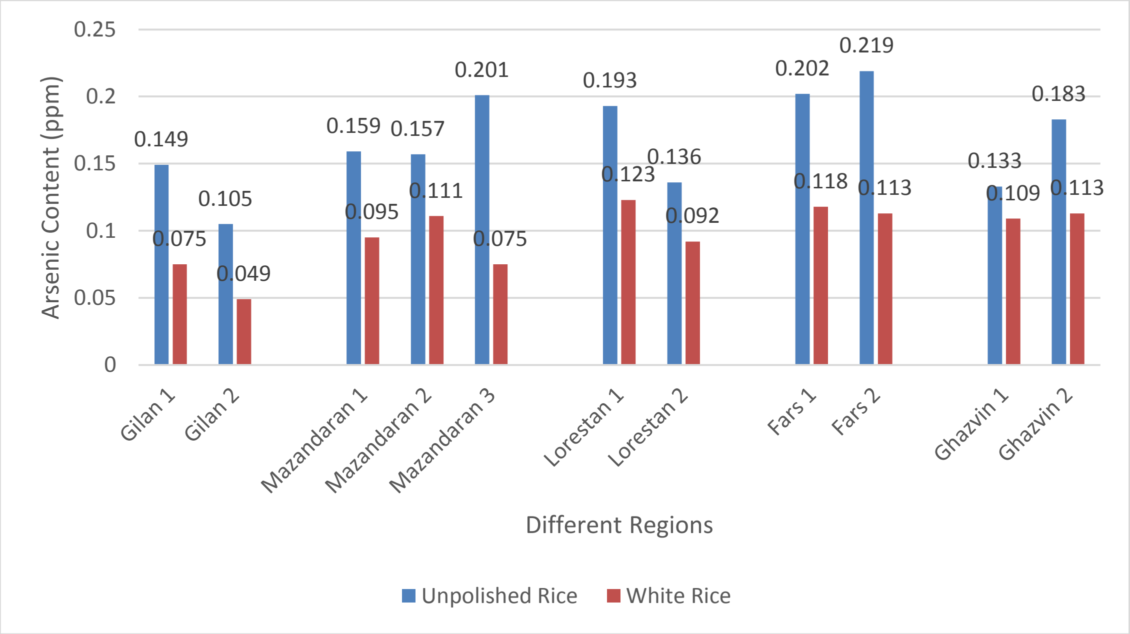

Fig. 6. Arsenic contents in unpolished and white rice samples obtained from different regions

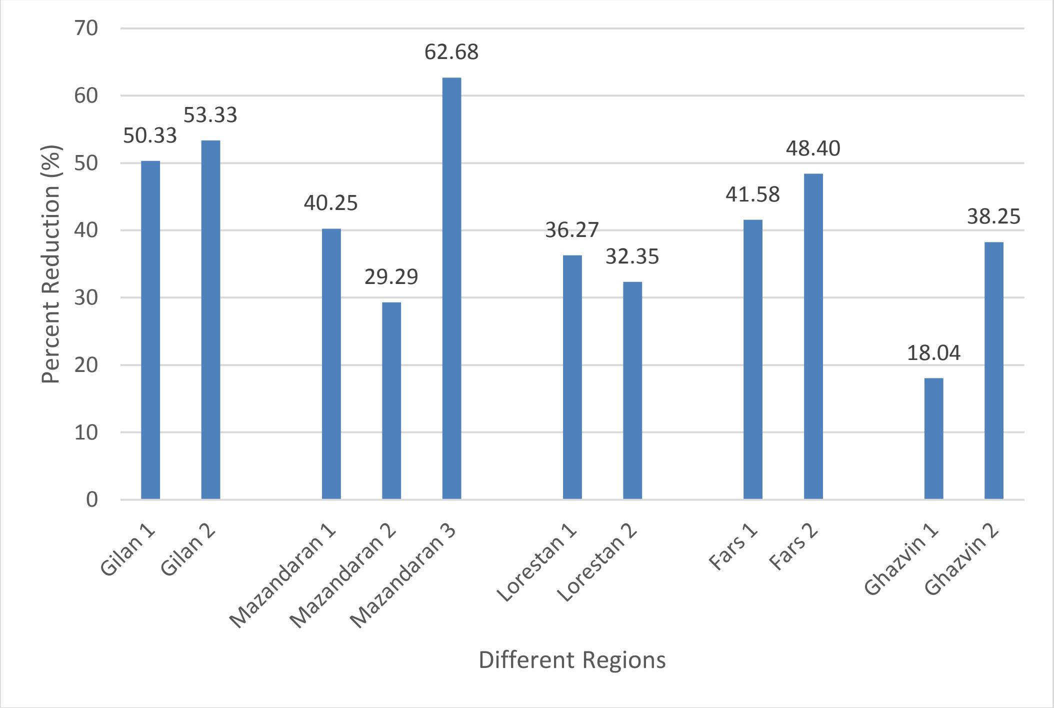

Fig.7. Percent reduction of arsenic contents in samples after milling

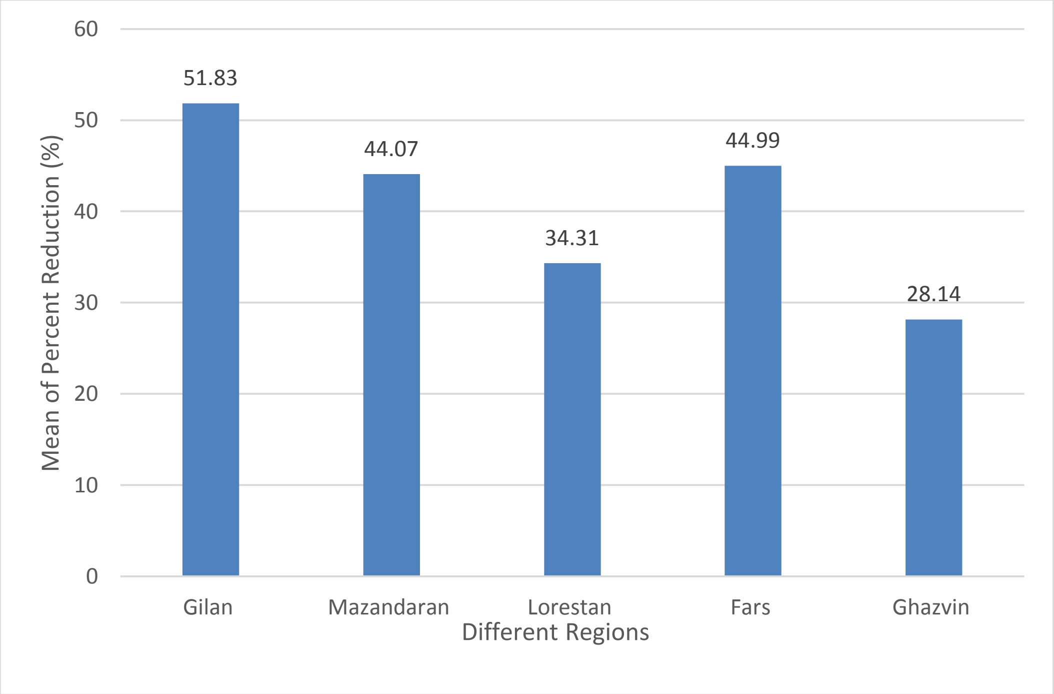

Fig. 8. The mean values of percent reduction of arsenic contents in samples after milling

There are significant differences between the arsenic contents of all rice samples except of Lorestan 2 and Gilan 1 samples ($p<0.05$).

The results indicated that arsenic contents in all the investigated samples except Lorestan 1 sample is less than the maximum limit specified in standards.

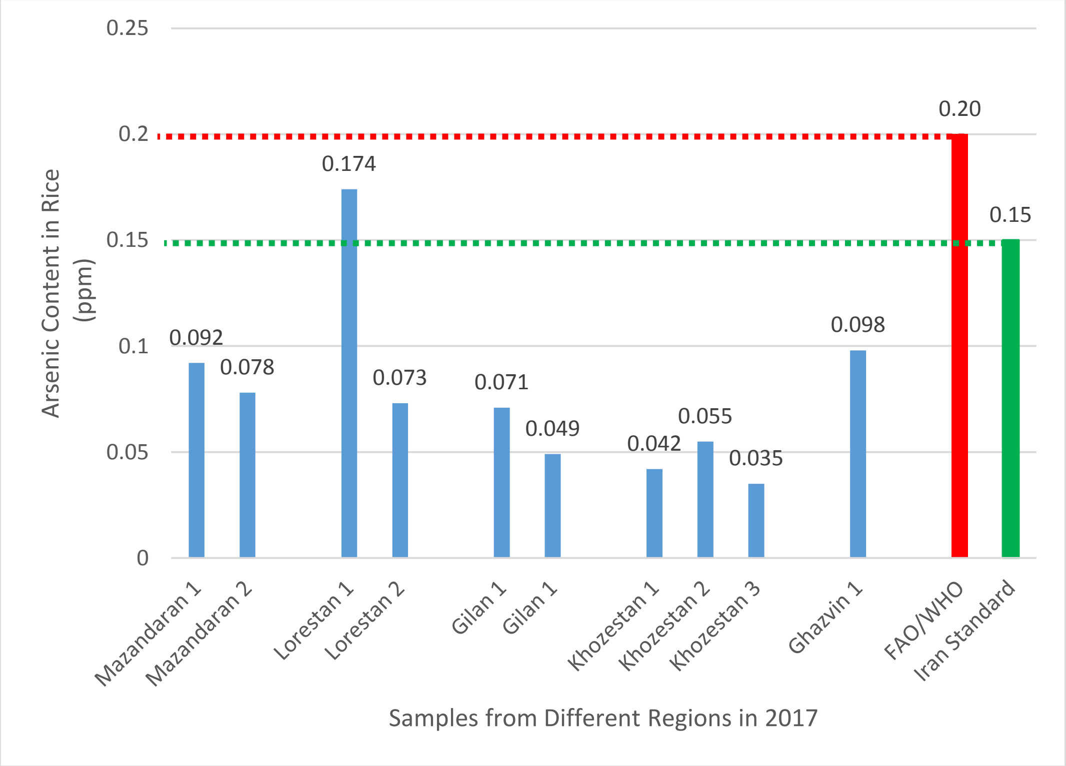

Fig. 9. Arsenic contents in rice samples from different regions in 2017

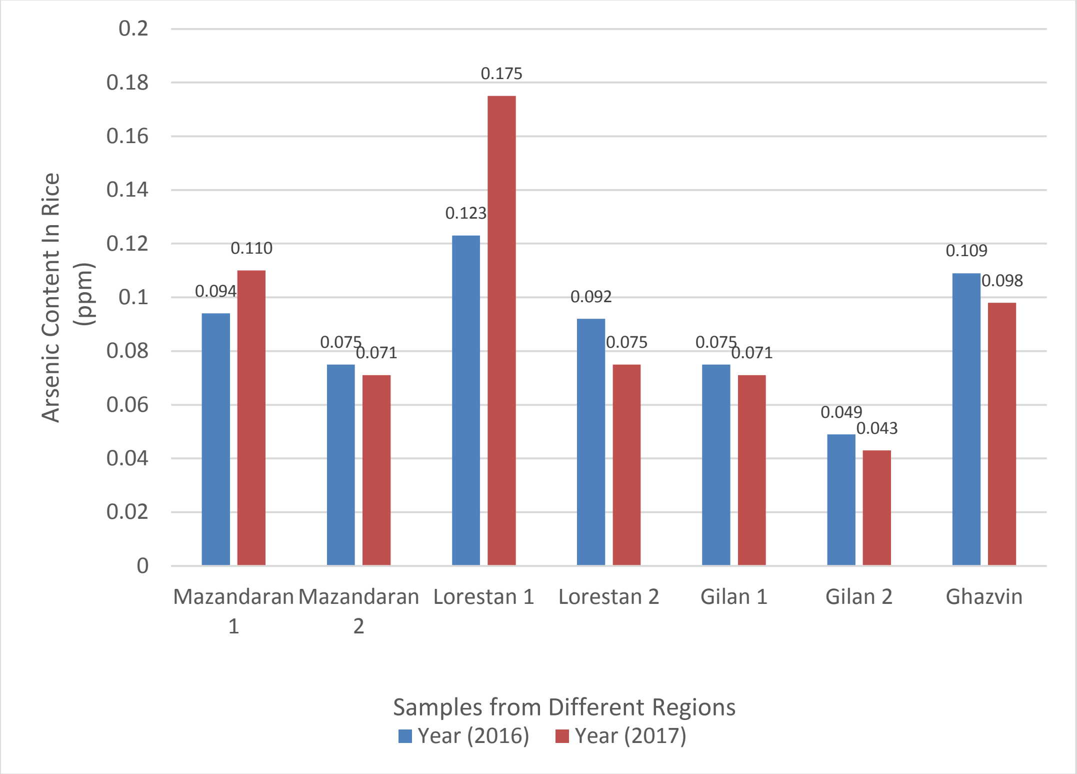

Fig. 10. Arsenic contents of the rice samples obtained from the same farms in the years 2016 and 2017

- Comparison of arsenic content of the rice samples obtained from the same farms cultivated in 2016 and 2017 years

The arsenic contents in rice samples obtained from the same farms in 2016 and 2017 years are presented in Figure 10. According to the results that are shown the highest amount of arsenic was detected in Lorestan samples.

The results indicated that the arsenic contents in all the investigated samples except Lorestan 2017 sample is less than the maximum limit specified by the standards and except Lorestan samples there is no significant difference between the arsenic contents of rice cultivated in same farm in two consecutive years ($p<0.05$).

Conclusion

The results have indicated that the amount of arsenic in the investigated soils was less than 10 mg/kg; lower than the standard concentration; therefore problems concerned with arsenic contamination in the soils of studied regions were not observed.

The regression analysis reveals that the extent of arsenic in white rice is positively correlated with the arsenic content in the soil. Therefore accumulation and the increase in arsenic content of agricultural soil causes an increase in the arsenic content of white rice.

The results of this research study indicated that the maximum amount of arsenic is accumulated in the outer layers of unpolished rice and during milling process in order to produce white rice the arsenic content is reduced by approximately 50% depending on the milling condition.

The results have also shown that arsenic contents in all the investigated white rice samples in 2016 are less than the maximum limit specified by the national standard (0.15 mg/kg).

Among the six studied provinces the lowest amount of arsenic was observed in Khozestan and Gilan provinces rice samples.

No significant difference was observed in the arsenic content in two consecutive years of rice cultivation.

Therefore the control of arsenic content in water used for irrigation, limited usage of chemical fertilizers and pesticides that might affect the concentration of arsenic in the soil and maintaining aerobic conditions during either the vegetative or the reproductive stage of rice growth might reduce the concentration of arsenic in the soil and consequently in the rice.

It should be noted that the assessment of arsenic exposure by only quantifying arsenic content in the raw rice could provide misleading information on the actual health risk because the preparation of rice for cooking, and the cooking methods, have a substantial influence on arsenic retention in cooked rice that is recommended to be investigated.

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