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Contamination of Rice Milk Samples with Toxic Heavy Metals in Ahvaz, Khuzestan Province, Iran

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ABSTRACT

Background: Rice milk is one of the traditional foods in Khuzestan province. Due to the combination of milk and rice, this product plays a major role as a diet for children under two years old and also as a diet for adults. Heavy metals represent a special group of food contaminants and exposure to heavy metals was shown to be directly related to progression of diseases. This study was conducted to determine the concentrations of some heavy metals in rice milk to evaluate whether the concentrations of these elements correspond to the permissible levels of toxic elements in milk and rice in Ahvaz, Khuzestan Province, Iran.

Methods: Totally, 50 rice milk samples (25 samples from East Ahvaz and 25 samples from West Ahvaz) were collected. The heavy metals in all samples were evaluated using quantified diffuse spectroscopy (OES) or mass spectrometry (MS).

Results: The mean concentration of the arsenic and lead was above the permissible limit. The average nickel and cadmium concentrations in the samples were below the allowable limit values and no mercury was detected in all samples.

Conclusion: The study results showed that consumption of rice milk did not pose a direct and serious threat to the health of consumers. Future studies and continuous monitoring are necessary to assess the content of heavy metals and the risk of their consumption to the consumer of the rice milk.

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Introduction

Milk is considered a complete food and, due to its variety for minerals and vitamins, it plays an important role in the growth and maintenance of human health. Milk and dairy products are the main sources of calcium and their consumption is recommended to maintain the health and strength of bones, teeth, and skin (1). Rice is one of the grains produced all over the world and is consumed daily by billions of people. White and brown rice are the most popular types of rice with similar origins (2). In the United States and many countries, white rice is typically fortified with nutrients, including iron, and B vitamins such as folic acid, niacin, and thiamine (3). Rice milk is one of the traditional foods of Khuzestan province. Due to the combination of milk and rice, this product has a special place in the diet of children less than two years old and also in the diet of adult population. The nutrients in milk are essential for the child's growth, development, and physical and mental health; however, the main way to achieve these benefits are to make sure it is free of heavy metals and other toxic compounds (4).

Heavy metals represent a special group of food contaminants and the entry of heavy metals into the environment has recently increased dramatically due to rapid industrialization and the use of new technologies that require heavy metals (5). The presence of heavy metals in foods that exceed the standard levels can lead to the incidence of various diseases, including neurological and genetic diseases and cancer (6). Cadmium, for example can be accumulated in tissues such as liver and kidneys and causes cancer, anemia, and a high blood pressure. High concentrations of lead were demonstrated to have harmful effects on the nervous system, kidneys, lungs, bone marrow, and blood (7). Nickel is another dangerous element that can disrupt the biological activity of cells, delays growth, reduces hematopoiesis, and interferes with iron absorption (8). Symptoms of increased cobalt level in the bloodstream and in the human body include fatigue, weakness, peripheral neuropathy, and hypothyroidism (9). Chromium in food does not endanger a person; however, excessive consumption of chromium supplements can cause stomach problems, hypoglycemia, and irregular pulse and heartbeats, but the side effects of chromium supplements are rarely seen (10). Mercury poisoning is the result of excessive exposure to mercury, either through diet or the environment. This can cause severe symptoms and put the body at risk, which will be determined by measuring the level of mercury in the blood (11).

The most important complication of iron deficiency is anemia and high doses of iron can lead to poisoning and severe liver damages, coma, and death (12). Inorganic arsenic is a carcinogen to the skin, lungs, bladder, kidneys, and possibly the liver. In addition, chronic exposure to arsenic through drinking water increases the risk of several non-cancerous diseases (13). Copper is an essential nutrient for the body and, along with iron, enables the body to make red blood cells. Adequate copper in the diet can also prevent cardiovascular diseases and osteoporosis (14).

Several studies have examined the concentration of heavy metals in milk and rice. In the study by Chinikar *et al.* on four types of milk, the average concentration of lead, nickel, cadmium and copper was 1.2 ppm, 42 ppm, 14 ppm, and 34 ppm, respectively (15). The amount of lead and cadmium in milk has been determined in different regions of Iran (16). In the study by Shokrzadeh *et al.*, it was found that there was no statistically significant difference between the amounts of lead in the Iranian and Indian rices, but the levels of cadmium in Iranian rice was greater than Indian rice, and the level of chromium in Indian rice was higher (17). In another study, it was shown that the concentration of heavy metals in rice samples was as follows: Manganes e>zinc>copper>nickel>lead>chromium. Also, the metal content in husked rice was higher than rice (18). In a previous study, arsenic concentrations in rice samples in Bangladesh ranged from 2 to 557 mg/kg (dw: dry weight) (19). Liu *et al.* showed that there was a relationship between concentration of cadmium, mercury, lead and chromium in soil and rice (20).

Rice milk is known as one of the traditional and popular foods in Khuzestan province, which has a desirable nutritional value due to the use of milk and rice in its manufacture. One of the dangers threatening the health of consumers is the presence of heavy metals in rice milk. No study has been carried out to clarify the possible presence of heavy metals in the rice milk. Additionally, there is no standard value for this nutrient, and none of the regulatory agencies are considering the risk of heavy metals in rice milk in Iran. Since these foods are often prepared in local workshops with poor hygienic conditions and raw materials such as milk and rice come from unreliable suppliers, it is possible that these foods contain heavy metals. Therefore, the aim of this study was to investigate the concentration of heavy metals in rice milk samples in Ahvaz, Khuzestan province, Iran.

Materials and Methods

In this study, as a pilot, 50 samples of rice milks were analyzed from Ahvaz city including 25 samples from East Ahvaz and another 25 samples from West Ahvaz, Khuzestan province, Iran. To assess wet digestion, 2 g of each product with 5 mL of 65% nitric acid (w/w) and 2 mL of hydrogen peroxide (30% w/w) were placed in a special container. The vessels containing the sample were placed in a microwave for digestion. The sample container was then removed, brought to room temperature, and allowed to cool completely. The door and wall of the container have been washed so that materials adhering to the door and the wall can enter the contents of the container. The sample was transferred to a 25 mL vial, diluted with deionized water, and transferred to plastic containers in the same way as the control sample was prepared (21).

For metal analysis, first, the calibration curves were plotted using different concentrations of

Table 1: Concentrations of heavy metals in the rice milk samples in ppm.

Sample	Cadmium	Copper	Nickel	Arsenic	Mercury	Chromium	Iron	Lead	Cobalt
1	-	0.504	-	-	-	0.170	0.260	-	-
2	-	-	-	-	-	0.148	0.359	-	-
3	-	-	0.053	0.192	-	0.161	0.273	-	-
4	-	-	-	-	-	0.136	0.265	-	-
5	-	0.337	-	0.204	-	0.130	0.386	-	-
6	-	0.306	-	-	-	0.174	0.839	-	-
7	-	0.384	-	-	-	0.220	0.570	0.313	-
8	0.006	0.363	-	0.122	-	0.268	1.197	0.320	-
9	-	0.302	-	0.200	-	0.092	0.086	-	-
10	-	-	-	-	-	0.134	-	-	-
11	-	0.274	-	0.141	-	0.138	0.995	-	-
12	0.011	0.360	-	-	-	0.117	0.408	-	-
13	-	0.320	-	-	-	0.259	1.564	0.324	-
14	-	0.257	-	-	-	1.360	2.133	-	-
15	-	0.209	-	-	-	0.081	1.003	0.561	-
16	0.005	0.459	-	0.157	-	0.087	0.364	-	-
17	-	-	-	0.153	-	0.080	0.290	-	-
18	-	0.532	-	0.154	-	0.133	0.377	-	-
19	-	0.267	-	0.125	-	0.138	0.588	-	-
20	-	0.206	-		-	0.150	0.480	-	-
21	-	0.335	0.169		-	0.160	0.576	-	-
22	-	-	0.115	0.182	-	0.107	0.226	-	-
23	-	0.362	-	0.191	-	0.181	0.790	-	-
24	-	0.222	-	0.189	-	0.174	0.421	-	-
25	-	0.679	-	0.179	-	0.174	0.407	-	-
26	-	0.203	-	0.209	-	0.158	0.329	-	-
27	-	0.430	-	0.207	-	0.384	0.889	-	-
28	-		-	0.146	-	0.127	0.126	-	-
29	-		-	-	-	0.163	0.291	-	-
30	-		-	-	-	0.103	0.092		-
31	-	0.355	-	-	-	0.193	2.233	0.296	-
32	-	0.355	-	-	-	0.346	1.339	-	-
33	-	0.275	-	-	-	0.113	0.416	-	-
34	-	0.294	-	-	-	0.125	12.416	-	-
35	-	0.300	-	-	-	0.498	4.291	-	-
36	-	0.342	-	-	-	0.130	0.139	-	-
37	-	0.377	-	0.141	-	0.158	0.746	-	-
38	-	-	-	-	-	0.121	0.376	-	-
39	-	-	-	-	-	0.118	0.817	-	-
40	-	-	-	-	-	0.110	0.153	-	-
41	-	-	0.028	-	-	0.091	-	-	-
42	-	-	-	-	-	0.105	-	-	-
43	-	0.181	-	-	-	0.114	-	0.322	-
44	0.013	0.729	-	-	-	0.123	-	-	-
45	-	0.425	-	0.143	-	0.176	-	-	-
46	-	-	-	-	-	0.119	-	-	-
47	-	-	0.127	-	-	0.265	-	-	-
48	-	0.486	-	-	-	0.209	-	0.239	-
49	-	0.357	-	-	-	0.220	-	0.856	-
50	-	0.161	-	-	-	0.137	-	-	-

a standard solution of 0.05, 0.1, 0.5, 1, 5, and 10 microliters per liter. The samples were then evaluated by inductively coupled plasma optical emission spectrometric method (ICP-OES, Spectro Arcos, SPECTRO Analytical Instruments GmbH, Kleve, Germany) and the amount of rare elements was measured (22). ICP-OES analysis is one of the methods of atomic spectroscopy in which the atomization of elements is carried out with the help of a hot plasma environment. It can then be detected and quantified using OES or mass spectrometry (MS). The metal concentration was measured by ICP-OES under the experimental conditions summarized as follow: Type of spray chamber cyclonic cross: Flow; type of detector solid state: Charge coupled device (CCD); radio-frequency generator (W): 1400; plasma torch: Auxiliary; nebulizer gas: Argon; frequency of RF generator: 27.12 MHz; element (λ/nm): As below; initial stabilization time (S): Preflush 60; plasma gas flow rate (L/min): 14.5; auxiliary gas flow rate (L/min): 0.9; and nebulizer gas flow rate (L/min): 0.85. The results of this study were expressed as the mean±standard deviation.

Results

This study examined 50 samples of rice milk collected in the city of Ahvaz, of which 25 samples were taken in the east of Ahvaz and the remaining's in the west of the city. Concentration of heavy metals in all samples was presented in Table 1 and the statistical analysis of heavy metals results was presented in Table 2. Table 2 shows an average concentration of 0.168 ppm for metallic arsenic in 18 samples; while according to the announcement of the National Organization for Standardization of Iran, the allowable concentration of arsenic in rice should be 0.15 ppm and this organization does not have a standard for arsenic in milk too. With comparing the concentrations of the samples assessed in this study, arsenic concentration was above the permissible limit. The study also found no mercury in the tested samples, so there is no concern about the presence of mercury and its possible poisoning in humans. According to the findings of Table 2, the average concentration of chromium in the samples was 0.187 ppm in all samples.

As Table 2 illustrates, metallic cadmium with an average concentration of 0.008 ppm was identified in 4 out of 50 samples of this study, while the ratio is within the range of the lowest concentrations reported in the available surveys. It should be noted that the maximum permissible cadmium concentration according to the Iran National Standards Organization should be 0.06 ppm for rice and 0.01 ppm for milk and the average concentration in this study was below the allowable limit. In addition, the presence of copper was confirmed in 34 out of 50 samples with an average concentration of 0.351 ppm. The FAO, WHO, and the Iranian National Organization for Standardization do not impose a virtual limit value for copper in milk or rice, but the Brazilian National Agency for Health Surveillance has set a limit value of 30 ppm for copper in milk.

In addition, 10 samples in the present study contained nickel at an average concentration of 0.093 ppm. On the other hand, as announced by the National Organization for Standardization of Iran and the Codex Committee for Milk, the allowable limit value for nickel is 0.5 ppm, while the permissible limit value for nickel in rice has not been disclosed; however, according to the Codex Committee, the maximum permissible nickel for cereals is 10 ppm. However, the average nickel concentration in our samples was less than the allowable limit value.

Table 2: Analysis of heavy metals in the rice milk samples in ppm.

Descriptive Statistics					
Element	**N**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Al	50	0.15	6.75	2.1178	1.81748
As	50	0.12	0.21	0.1686	0.02916
Cr	50	0.080	1.360	0.18756	0.186531
Cu	50	0.161	0.729	0.35141	0.127269
Fe	50	0.086	12.416	0.93398	1.836726
Mg	50	53.345	412.267	135.59320	54.260984
Mn	50	0.322	1.827	0.70402	0.266278
Ni	50	0.028	0.169	0.09340	0.042471
Se	50	0.234	1.431	0.66088	0.241073
Zn	50	1.971	16.336	6.66176	2.256163
Ca	50	535.003	5283.970	1716.19858	715.823573
P	50	508.5920	2805.8400	917.779000	352.4215887
K	50	106.112	4510.270	1457.44452	596.437553

Al: Aluminum, As: Arsenic, Cr: Chromium, Cu: Copper, Fe: Iron, Mg: Magnesium, Mn: Manganese, Ni: Nickel, Zn: Zinc, Ca: Calcium, P: Phosphorus, K: Potassium

Based on the findings in Table 1, there were no signs of cobalt in the examined samples, so there are no concerns about the presence of cobalt and possible poisoning in humans. Likewise, iron with an average concentration of 0.933 ppm was detected in 48 out of 50 samples. Of out of 50 samples, 8 contained lead at an average concentration of 0.403 ppm. It should be noted that the maximum permissible lead concentration according to the National Standards Organization of Iran is 0.15 ppm for rice and 0.02 ppm for milk. So the average concentration in our study was significantly different from the national standard.

Discussion

In studies on the rice samples supplied in various cities of Iran, the concentration of cadmium was reported 0.024 ppm in Golestan province (23) and 14.26 ppm in Shadegan city (24). In studies on milk samples from different areas of Iran, the cadmium concentration averaged 0.0005 ppm in Yazd province (25) and 0.002 ppm in Shahr-e-Kord (26). The presence of environmental pollutants such as soil and climate was demonstrated to cause the accumulation of heavy metals in the body, while through the consumption of products such as rice and cereals, these toxic metals get into the human body and have a negative effect on consumers. The possible contamination of rice with heavy metals in soil, irrigation water supplies, and pesticides can affect the quality of the rice and its nutritional properties. The comparison of the present study with previous ones showed discrepancies between these studies; and these differences were not unexpected due to differences in the brands and areas examined (27).

In the previous studies conducted on the rice samples supplied from different localities of Iran, the concentration of lead was determined to be 0.024 ppm in Golestan province (23) and 60.48 ppm in Shadegan city (24). In studies on milk samples in various regions of Iran, the lead concentration was 0.007 ppm in Yazd province (25) and 0.06 ppm in Shahr-e-Kord (26). Out of 50 samples examined in our study, 8 samples contained lead at an average concentration of 0.403 ppm. It should be noted that the maximum permissible lead concentration according to the National Standards Organization of Iran is 0.15 ppm for rice and 0.02 ppm for milk. The average concentration in our study was exhibited to be significantly different from the permissible limit of the National Standard of Iran. The reasons for the higher content of metallic lead in the examined samples may be due to the greater environmental pollution and because of the use of phosphate fertilizers in several industrial centers, and a large number of vehicles. In addition, several other factors can be mentioned including the contamination of food and water with heavy metals, healthy or sick animals, geographical location, climatic conditions, different seasons, soils of different regions, etc. that all can influence the contamination of raw milk with metallic lead (28). Based on the results of the average lead concentration in our samples, it can be determined that the exposure of animal products to heavy metals, including lead in large industrial cities due to the presence of industrial facilities such as leather and, battery production, and metallurgy have been much higher than in non-industrial cities, so it is imperative to regularly check and examine potential sources of contamination of food, especially milk and dairy products.

The concentration of arsenic has been reported 0.007 ppm in Golestan province, Iran (23), and 23.43 ppm in Shadegan city, Iran (24). In our rice milk samples collected in the city of Ahvaz, 18 samples contained arsenic with an average arsenic concentration of 0.168 ppm; however, according to an announcement by the National Organization for Standardization of Iran, the permitted arsenic concentration in rice is 0.15 ppm. Of course, this organization does not have a standard for arsenic in milk. In this study, we found that the arsenic concentration was more than the permissible limit. It is vital to mention that arsenic is one of the most important toxic elements in nature, while the origin of this metal in a region can be herbicides, insecticides, desiccants, plant and animal growth promoters, especially wood preservatives that can explain our findings (27).

Arsenic can enter the life cycle of people not only through the soil, but also directly via water to cause serious problems (29). When using water in the Karun River in Ahvaz, the entry of municipal waste water and effluent from industrial units into the river affects the water quality. On the other hand, these fields are directly irrigated by raw sewage and existing steel industries, which may be due to the high concentration of elements in locally grown rice (30). The contamination of the ground water with arsenic can also cause a gradual increase of this element in the rice grain (31).

In a study of rice grown in Mazandaran province, Iran, the average mercury concentration was found 0.044 ppm, while in imported rice to the province, it was 0.039 ppm (32). In our study conducted on rice milk samples in Ahvaz, all samples were mercury-free, so there is no concern about the presence of mercury and its possible poisoning in humans. Although no mercury was detected in the examined samples, the presence of mercury in the soil may be justified by various toxins and pesticides used in rice paddies at different stages of rice growth. The uptake of mercury by plants depends on several factors, including; chemical form of mercury, natural soil structure, organic and mineral substances, and the soil pH. The availability of mercury in soil is low for plants, and the high biological accumulation of mercury in plant roots indicates that the roots are a barrier to mercury uptake. In addition, the source of mercury exposure in livestock diets is usually a fish powder.

Several studies have evaluated the nickel concentration in rice. In the study by Malakootian *et al.* on imported Indian rice to Iran, the average nickel concentration was displayed 0.019 ppm (33). In the study of Abtahi *et al.* on Iranian rice, the average nickel concentration was reported 0.83 ppm (6). In the study by Eskandari *et al.* on imported and grown rice from Shadegan city, Iran, the average nickel concentration was shown 28.05 ppm (24). In a study on cow's milk in India, the average nickel concentration was demonstrated 0.03 ppm (33). Of the 50 rice milk samples examined in our study, 10 samples contained nickel at an average concentration of 0.093 ppm. On the other hand, according to the announcement by the National Organization for Standardization of Iran and the Codex Committee for milk, the permissible limit value for nickel is 0.5 ppm, while the permissible limit for nickel in rice has not been disclosed. According to the Codex Committee, the maximum nickel permissible for cereals is 10 ppm; however, the mean nickel concentration in the samples examined in this study was less than the permissible limit value. Nickel was shown to be produced from the atmosphere or by the combustion of petroleum products. The use of chemical fertilizers or waste water to irrigate plants can also be a risk factor for increased nickel concentration in milk produced on the farm that can explain the findings.

In a study on the imported Indian rice samples to Iran, the concentration of chromium was shown to be 0.653 ppm (33). In a study on milk samples, the chromium concentration averaged 2.631 ppm in Nigeria (34) and 0.026 ppm in Spain (35). In our study, the presence of chromium with an average concentration of 0.187 ppm was confirmed in 50 samples. It seems that the existence of industrial factories in the province can be one of the causes of contamination of cow's milk by heavy metals, including chromium. Of course, different sources of contamination such as food and the environment must be taken into account as chromium particles or vapors are released into the air in industrial processes; therefore, it can pollute the environment, and cattle and cow's milks.

The average copper concentration in rice has been reported 7.71 ppm in Isfahan province (36), while the permitted limit of copper concentration in rice grain should be 5 ppm. Also, the average copper concentration in milk was reported 0.142 ppm in Khorramabad, Iran (37). FAO, WHO and the National Organization for Standardization of Iran have not disclosed the permitted limit for copper concentration in milk, but the Brazilian National Agency for Sanitary Monitoring has announced that the permitted limit for copper in milk is 30 ppm. In 34 out of 50 rice milk samples in our study, the average concentration of which was 0.351 ppm (38).

Study on iron content of rice showed an average iron concentration of 134 ppm in Isfahan province (36), while the allowable limit of iron concentration in rice grain should be 40 ppm. When investigating the average copper concentration in milk in Egypt, it was found to be 16.38 ppm (39) and in Estonia was 0.778 ppm (40). In our study, 48 out of 50 rice milk samples, the presence of copper was verified to be 0.933 ppm. Therefore, it is advisable to implement the correct instructions to evaluate different rice varieties for quality and safety. Periodic measurement of heavy metals in water resources and rice grains from different regions, and comprehensive planning to implement an appropriate system to reduce the content of metal pollutants in water resources and rice crops can result in realization of food security in agricultural products, and a promotion in organic farming. So conducting similar researches in other areas and agricultural lands, especially in paddy fields are recommended to achieve a sustainable food security.

Conclusion

In this study, the chromium was found to be present in all rice milk samples, but no evidence of mercury or cobalt was found in any of the samples. The average concentration of arsenic and lead was above the permissible limit set by the National Organization for Standardization of Iran and the average concentration of cadmium and nickel was below the permitted limit set by the National Organization for Standardization of Iran. Ultimately, our results showed that the consumption of rice milk does not pose a direct and serious threat to the consumers' health.

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Conflict of Interest

None declared.

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