Health risk assessment of heavy metals (Lead and Cadmium) in transgenic corn in Tehran

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ABSTRACT

Corn plant and the products made from corn is one of the major transgenic concerns in the world, and due to public concerns about the presence of heavy metals in this product; the present study investigated the food safety in transgenic corn samples due to the presence of lead and cadmium. Iran has the highest exchange of agricultural products. Five packaged popular brands of raw corn samples and one unpackaged sample were purchased from Tehran markets in 2021, and after confirming transgenicity by quantitative real-time PCR and then wet digestion method. The heavy metal contents in all studied samples were analyzed by inductively coupled plasma spectrometer. Bulk samples had the highest content of lead and cadmium compared to other packaged studied samples. The comparison of lead and cadmium to the maximum permissible levels set by the National and international standards revealed that the toxic heavy metals ‘contents in 100% of studied raw corn samples were higher than the maximum limit. The results of the current study indicate that the Lead and Cadmium contents in the studied samples were 18 and 2.7 times higher than the Maximum Permissible Limit (MPL).

Keywords
Cadmium
Heavy metals
ICP
Lead
Transgenic corn

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Introduction

Nowadays, due to the continuous increase in the world population, providing food security and the contamination of food and agricultural products with heavy elements has become one of the concerns of human societies. The appearance of transgenic plants in agriculture to provide food with desirable traits has helped a lot to solve this problem. On the other hand, soil contamination with heavy metals is considered a global challenge, and the absorption of heavy metals such as cadmium, lead, mercury, and other heavy metals in agricultural fields has challenged human health. Excessive accumulation of these metals leads to changes in cellular components such as lipids, proteins, enzymes, and DNA and destroys human life. This concern
is not only limited to third-world countries, but developed countries face this problem\textsuperscript{3}. Environmental pollution with heavy metals affects soil quality and crop yield\textsuperscript{4}. Cultivation of transgenic plants improves food security in the world and more food can be obtained for humans.

Oilseeds are among the strategic and valuable agricultural products that have undergone changes in the transgenic process and have allocated the most area under the cultivation of transgenic plants. In between, we can refer to the oil seeds in which the pattern of fatty acids has been changed by conventional mutagenesis methods or recombinant DNA methods, or the oil seeds that have become resistant to herbicides\textsuperscript{5}. Since 1996, genetically modified corn has been commercially produced and mass-produced in many countries. Almost all transgenic corn in the world has traits such as the ability to resist insects and pesticides, etc.\textsuperscript{6}.

Iran is one of the largest consumers of oil and oilseeds with a high per capita consumption compared to the world average, and it supplies more than 80% of its oilseeds needs through imports, and most of these imports come from countries that have the most surface under the cultivation of transgenic plants. The import of corn seeds to the country is the largest number of imports after soybeans and cotton seeds. According to statistics extracted from the Chamber of Commerce, Iran's corn import in 2019 was about 9.8 million tons, and comparing this number with previous years, we can see an increase in the volume of corn imports in the last 9 years by 5 million tons, and this is even though Despite the increase in corn production in Iran from 6.2 tons to 8 tons per hectare in the past 9 years, the area under corn cultivation in Iran has decreased by 150 thousand hectares, equivalent to 52\%\textsuperscript{7}. Iran's corn consumption will increase to about 14 million tons and corn production will increase to 1.6 million tons per year in the next four years, as a result of which the amount of corn imports will experience an increase in the next four years\textsuperscript{8,9}.

The noteworthy point is the contamination of these products with heavy metals\textsuperscript{10,11}. Heavy elements are one of the most important sources of pollution of natural resources. Every year, thousands of tons of these elements enter the soil system on a global scale\textsuperscript{12}. The contamination of agricultural products with heavy metals on the one hand leads to a decrease in the quality of agricultural products and on the other hand, it is a serious threat to human health, therefore, from the environmental aspects, heavy metals accumulate and increase their concentration and reach the danger range. It can threaten health by entering the human food chain\textsuperscript{13}. Heavy metals in the soil enter the groundwater through leaching and osmosis and cause human disease\textsuperscript{14}.

Lead constitutes 0.002\% of the earth's crust. Lead is considered one of the most dangerous elements for human health, although research shows that the risk of arsenic is greater than lead\textsuperscript{15-17}. Apart from natural sources, lead enters nature mainly through human activities in several ways, including ore smelting, coal burning, battery industry storage effluents, automobile exhausts, metal plating, leather tanning, fertilizers, pesticides, and additives. Therefore, the introduction of lead into nature has led to environmental concerns and risks related to human health\textsuperscript{18}.

Lead remains stable in soil for long periods and is difficult to separate, so it is transferred to humans through food. Although plants absorb a significant part of lead ions from the soil, the largest amount is concentrated in the roots and has a limited transfer to the stems and leaves\textsuperscript{19}. The limited transport of Pb from root to stem and leaf is mainly due to transport barriers in the root epidermis and endoderm\textsuperscript{16}. Despite these barriers, significant transfer of Pb to the aerial parts of plants is observed in contaminated land, where aerial parts including leaves, fruits, and seeds are restricted for human consumption\textsuperscript{20}. Direct inhalation of lead-laden dust, skin exposure to lead-contaminated dust and soil, and consumption of lead-contaminated water and food produced in lead-contaminated areas are important ways of lead entering the human body\textsuperscript{21}. Lead affects the renal, cardiovascular, hematopoietic, nervous, and reproductive systems\textsuperscript{22}. In addition, lead increases the risk of cancer by increasing estrogen\textsuperscript{23}. In addition, elevated blood lead levels affect behavior, cognitive function, postnatal development, delayed
puberty, and hearing ability of infants and children. Brain damage, anemia, anorexia, vomiting, and circulatory and nervous system diseases are examples of the adverse effects of lead pollution.

Cadmium is one of the heavy metals that are usually found in low concentrations in soil. This metal is toxic to living organisms and carcinogenic to humans. Plants can accumulate large amounts of cadmium without being harmed. Relatively high concentrations of cadmium can accumulate in the edible parts of the plant without revealing the symptoms of the disease and affecting the plant. The accumulation of cadmium in plants can increase the absorption potential of this element by humans, and this happens in the case that these plants are part of the diet. Cadmium is absorbed in the human body through food, especially grains, plants, water, or air, and accumulates in the body over time and causes health problems. Cadmium accumulates in the liver and kidney with a half-life of about 17 to 30 years. Different types of agricultural products differ greatly in their ability to absorb, accumulate and tolerate cadmium. Among agricultural products that are important in terms of cadmium in the human diet, pasta, corn, wheat, oats, barley, rice, and peas have shown a higher concentration of cadmium than the permissible limit. As the world's population and the demand for food are increasing over time, a larger area is needed for agricultural use. Regulations for food products containing GMOs are required in many countries to inform consumers. Due to the lack of strong evidence on the safety of genetically modified foods, some GM products should be consumed with caution.

With the current emphasis on healthy diets and public concerns about the presence of heavy metals in food or agricultural products, it is very necessary to evaluate the chemical composition and check the presence of heavy metals in the products that we use in abundance in our diet. In general, the absorption of heavy metals from contaminated lands by plants and especially agricultural products is one of the most important ways of entering these elements into the food chain, also because these two metals are carcinogenic elements. They are known especially in the digestive system. Therefore, according to the importance of the topic, the purpose of this research is to measure the level of heavy metals lead and cadmium in the samples of transgenic corn available in the Tehran market.

**Method**

**Collection of Samples**

5 Packaged corn popular brands (A, C, D, E, Q) and one bulk brand (F) were purchased from recognized stores in Tehran in 2021. Before any experiment on the corns, they were first checked for transgenicity by polymerase chain reaction (PCR) and after confirming the transgenicity by quantitative real-time PCR, 200 grams of each brand was weighed and poured into a zip cap to prevent air from entering. Then the steps for preparing, drying, and drying the corns, wet digestion, and finally measuring the composition of the heavy metals were continued.

**Sample preparation**

At first, the purchased packaged and bulk corn samples were washed with deionized water and dried at a temperature of 80°C for 20 minutes in the oven reaching the constant weight.

**Wet digestion method**

2 grams of dried samples were ground in a mortar and then placed in an oven to be dried. After weighing, the samples must be placed in the oven for the necessary time to remove the excess moisture and dry and reach a constant weight. It should be noted that the use any container for wet digestion must be acid washed and all steps of wet digestion must be done under a hood.

Then, according to AOAC wet digestion protocol, nitric acid, and perchloric acid were added to each completely dried sample in a ratio of 1:1:3. Added 1 to 2 ml H₂O₂ 30% (Merck) due to organic compounds of tomato to stop enzymatic reactions would not be affected to the
analyses\(^{37,38}\), as solvents must be strong enough to absorb zinc from the samples\(^{39}\). Then it was left for a few days until the samples were completely digested before being injected into the ICP device after complete digestion and then dissolved. The digested sample was poured into a 5-gauge flask and made up to volume with deionized water.

The digested samples were filtered with a membrane filter (pore size 0.45 μm) and swelled with double distilled water to the volume of 100 ml. The number of heavy metals in samples was then measured by the ICP-OES system and standard solutions\(^{39-41}\), which include a mixture of two heavy metals, cadmium, and lead. Heavy metals were determined using the ICP method based on the AOAC standard\(^{42}\).

**Preparing the device**

The settings of the device for measuring lead and cadmium elements were made based on the instructions provided by the device manufacturer, and then the digested samples were injected into the ICP device, and finally, the concentration of heavy metals was read in mg/kg.

**Table 1. Setting device parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma gas</td>
<td>Argon</td>
</tr>
<tr>
<td>Plasma gas flow rate</td>
<td>20 L min(^{-1})</td>
</tr>
<tr>
<td>Auxiliary gas flow rate</td>
<td>0.5 L min(^{-1})</td>
</tr>
<tr>
<td>Frequency of RF generator</td>
<td>40 MHz</td>
</tr>
<tr>
<td>RF generator power</td>
<td>1/100 W</td>
</tr>
<tr>
<td>Observation height</td>
<td>8 mm</td>
</tr>
<tr>
<td>Nebulizer pressure</td>
<td>20 psi</td>
</tr>
<tr>
<td>Eluent</td>
<td>Deionized water</td>
</tr>
<tr>
<td>Elution rate</td>
<td>4 mL min(^{-1})</td>
</tr>
<tr>
<td>Wavelength</td>
<td>Pb: 220.3-Cd:226.5</td>
</tr>
</tbody>
</table>

**Statistical methods**

State differences based on the states: Smoking and not smoking filter samples and their tobacco were determined by student t-tests. The changes were calculated by one-way ANOVA and for analysis of the role of multiple factors univariate analysis was used by SPSS 18. Probability values of < 0.05 were considered significant.

**Results and Discussion**

**Lead contents in transgenic corn samples**

The results of measuring lead concentrations in the samples of raw corn by ICP-OES in 5 brands and bulk samples were reported as a mean concentration in mg/kg in Table 2, respectively. All concentrations were expressed as (mg/kg DW ± SE). The amount obtained for each sample is the average of 3 repetitions of the measurement.

According to Table 2, the contents of lead in bulk samples showed the significantly highest contamination (p<0.01), while the packaged brand D and E, had significant contamination of lead (p <0.05) in comparison to other studied brands.

**Table 2. Mean concentration of lead (mg/kg DW±SE\(^{*}\))**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>(^{a}2.115±0.002 )</td>
</tr>
<tr>
<td>C</td>
<td>(^{a}2.116±0.001 )</td>
</tr>
<tr>
<td>D</td>
<td>(^{a}3.002±0.003 )</td>
</tr>
<tr>
<td>E</td>
<td>(^{b}2.907±0.001 )</td>
</tr>
<tr>
<td>Q</td>
<td>(^{a}2.878±0.002 )</td>
</tr>
<tr>
<td>F</td>
<td>(^{a}3.517±0.003 )</td>
</tr>
</tbody>
</table>

\(^{*}\text{SE= Standard Error}\)  
Different letters in the columns indicate a statistically significant difference (P<0.05)
Cadmium contents in transgenic corn samples

The amount of cadmium determined in corn samples is shown in Table 3. The amount obtained for each sample is the average of 3 repetitions of measurement. As the same as the lead contents, the results indicate that cadmium content in bulk corn samples had a significant difference from the other packaged ones, while in brand D significantly ($p < 0.05$) the highest level among packaged samples was detected.

Table 3. Mean concentration of Cadmium (mg/kg DW±SE*)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.102±0.001</td>
</tr>
<tr>
<td>C</td>
<td>0.103±0.001</td>
</tr>
<tr>
<td>D</td>
<td>0.107±0.001</td>
</tr>
<tr>
<td>E</td>
<td>0.103±0.001</td>
</tr>
<tr>
<td>Q</td>
<td>0.102±0.001</td>
</tr>
<tr>
<td>F</td>
<td>0.112±0.001</td>
</tr>
</tbody>
</table>

*SE= Standard Error

Different letters in the columns indicate a statistically significant difference (P<0.05)

Comparison of lead values in the studied transgenic corn samples with national standard values

Table 4. Mean concentration of lead and cadmium in all studied corn samples

<table>
<thead>
<tr>
<th>Mean cadmium (mg/kg)</th>
<th>Mean lead (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed corn</td>
<td>0.103</td>
</tr>
<tr>
<td>Bulk corn</td>
<td>1.112</td>
</tr>
<tr>
<td>ISIRI allowed level</td>
<td>0.15</td>
</tr>
</tbody>
</table>

National standard of maximum Permissible level of lead and cadmium heavy metals in transgenic corn (Based on the maximum tolerance of heavy metals with national number 12968, ISIRI)

Based on the results obtained from the comparison of lead concentration in 6 transgenic corn samples, it was reported that bulk corn (F) has the highest amount of lead content to other brands available in the Tehran market. The amount of lead in all brands of corn was reported to be much higher than the maximum permissible limit of the national standard. In Fig 1., we can observe that in a study carried out in 2021, the level of Pb in all samples was higher than the maximum permissible limit (MPL), as well as the reality of major contamination in the bulk sampling of corn.

Fig 1. Comparison of measured lead concentration values (mg/kg) in transgenic corn samples available in the market and the national standard amount of lead for corn (S= National Maximum Permissible Level)
Comparison of cadmium levels in the studied transgenic corn samples with the national standard level

Based on the results obtained from the comparison of cadmium concentration in 6 corn samples, bulk corn (F) had the highest amount of cadmium compared to other brands available in the Tehran market (Fig 2). Also, these results, comparing the amount of the national standard for cadmium, showed that except for bulk corn, other brands had a relatively similar amount of cadmium compared to the standard amount.

A study conducted in 2015 by Anjum et al., investigated the effect of cadmium stress at different concentrations on antioxidant activity, reactive oxygen species, cadmium accumulation, and reproduction of two types of corn. Significant changes were observed in both types of corn. It was shown that these changes were dependent on the concentration of cadmium. Also, exposing the plant to cadmium stress significantly increased the concentration of cadmium in all parts of the plant, especially in the roots. The evaluation of the results showed that the corn yield was significantly reduced under the influence of different amounts of cadmium toxicity.

In another study in 2019, Choi et al., investigated heavy metals (lead, cadmium, and arsenic) in some cereals (oats, corn, barley, millet, sorghum, and Adlay) by ICP-MS method. The results showed that among cereals, barley had the highest amount and corn had the lowest amount of lead and cadmium. In terms of arsenic concentration, Adlay had the highest amount, and corn had the lowest amount of this element. The results of this study showed that the total amount of these elements under investigation was lower than the number of regulatory guidelines in Korea.

In 2019, Vaghar et al. investigated the effect of irrigation with wastewater on the accumulation of cadmium and lead metals in soil and cultivated vegetables. For this purpose, sampling was done from an area irrigated with well water and an area irrigated with the sewage water. After washing and drying, the plant samples were ground and ready for testing. After air drying, the soil samples were sieved and ready for testing. Then the samples were digested with a combination of acids and heavy metals and were measured using an atomic absorption device. The results showed that wastewater had a significant effect on the accumulation of lead and cadmium metals in soil and vegetables so the average concentration of these metals in soil and vegetables in areas irrigated with wastewater was higher than in areas irrigated with well water. It was also found that the amounts of lead and cadmium metals in the studied vegetables were equal to or more than the standard limit, the cadmium metal in the soil of both the control and
treatment areas was equal to the standard limit, and the lead metal in the soil of both control and treatment areas was less than the limit. It was the standard.

In 2022, Bamagoos et al., investigated the role of salinity and lead element concentration in quinoa plants and reported that the bioconcentration factor and lead transfer factor remained less than one in the absence or presence of salinity. Lead accumulation and plant tolerance potential showed that quinoa is resistant to the presence of lead in saline soil.

In 2022, Amjad and colleagues investigated the role of quinoa in resistance to cadmium and lead pollution and reported that the health risk assessment of cadmium and lead showed that the risk factor for both metals was greater than 1. However, the results of the total risk factor showed that the value was greater than 1 for lead and 1.19 for cadmium, which indicated the potential carcinogenicity of these two elements.

Rahdaraian et al., in 2022, studied the potential of *V. radiata* for remediation of cadmium content in contaminated soil or water in the West Karun Region, Iran. The Cd content in the soil played antagonistic roles in the uptake of Fe, Zn, and Cu from the soil by *V. radiata*, and their specific behavior is strongly affected by their interactions with other metals. Additionally, there was a substantial negative correlation between the Cd levels in soil and *V. radiata*.

The results obtained from the current study regarding the mean contents of lead in the samples of transgenic corn available in the market of Tehran showed that all the samples were contaminated with the element of lead, in addition to the fact that all these amounts have a lead amount higher than the permissible limit determined by the National Standards Organization of Iran, which is 0.15 mg/kg has been determined. Bulk corn (F) had the highest amount of lead compared to other brands available in the Tehran market. The maximum estimated amount of weekly intake of lead based on the standard of the World Health Organization is equal to 0.0256 mg/kg (body weight). Lead is the most important pollutant in the environment and many countries; it is even received through PT or WI diet. The amount is higher than that. The results obtained from the studies in this field show that the cultivation area, the presence of factories, the traffic of vehicles, as well as the deposition of metal from the air on plants are the main reasons for the accumulation of lead in agricultural products.

The results obtained from the concentration of cadmium in the corn samples showed that all the studied transgenic corn were contaminated with cadmium, but except for the bulk corn, all the cases were within the permissible limits determined by the national standard organization. Iran, which has determined the amount of 0.1 mg/kg, was located. Among all 6 available brands, bulk corn (F) had the highest amount of cadmium compared to other brands available in the Tehran market. The maximum estimated weekly intake of cadmium according to the World Health Organization is equal to 0.007 mg/kg. In some parts of the world, human exposure to cadmium through food and drinking water can reach 60% of PTWI. For some people, being exposed to cadmium with half the amount of PTWI can also cause kidney disorders. According to the results obtained from the studies, the use of chemical fertilizers, especially phosphorus fertilizers, the presence of urban and industrial effluents, as well as the presence of a lot of cadmium in the atmosphere are among the most important ways cadmium enters the life cycle. Atmospheric deposition can increase heavy metal levels in vegetables and pose potential health risks to consumers.

**Conclusion**

The current study examined the levels of lead and cadmium heavy metals in the samples of transgenic corn available in the Tehran market and prepared a report on the significantly high levels of these toxic metals. Considering the high contamination of vegetables and agricultural products with heavy elements, as well as taking into account the high per capita consumption of corn and the prevalence of digestive diseases, it is necessary to make an effort to reduce heavy metals in these products. The investigations carried out in this study proved the high
contamination of this transgenic product with lead and cadmium contents, therefore the responsible Organizations must screen and monitor the heavy metals concentrations in foodstuffs and crops, as screening tends to pick up slowly progressive conditions that are less aggressive and more amenable to treatment.

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Institute of Standards and Industrial Research of Iran. Vol. National standard NO. 12968 (Institute of Standards and Industrial Research of Iran, 2010).


**Author contributions**

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by [Soha Hatami], [Zahra Mousavi] and [Parisa Ziarati]. The first draft of the manuscript was written by [Parisa Ziarati] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.