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Food and feed safety assessment of green peas grown in an arsenic-contaminated area

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1. Summary

Both natural and anthropogenic arsenic contaminations of soil and groundwater are a global problem for all parts of the world, in terms of which, in Hungary, primarily the Great Plain region is affected. Through growing plants on arsenic-contaminated soil, arsenic can enter the food chain, which can mean a serious food safety risk. In Hungary, more than 70% of the vegetable growing areas are located in the Great Plain area affected by natural arsenic contamination. Green peas, the food industrial significance of which is due to the many forms of processing, among other things, are the second most common vegetable, in terms of the growing area. Based on this, the objective of our work was to determine the changes in the arsenic contents of the different plant parts (stem, leaf, pea pod, pea seeds) of green peas grown on arsenic-treated soil. Based on the results obtained, it was investigated how big a food and feed safety risk green peas grown in such areas pose. The effect of soil arsenic contamination on the arsenic uptake of green peas was investigated using pot experiments. During the experiments, arsenic was used separately as As(III) and As(V). In our work, arsenic treatments of 0, 3, 10, 30, 90 and 270 mg/kg were applied. Based on the results it can be concluded that, compared to the $BMDL_{0,5}$ value determined by the WHO, in none of the cases did the percentage contribution of the consumption of green peas from arsenic-contaminated areas to different tumor diseases (pulmonary, bladder and skin cancer) exceed 0.46%. The 2 mg/kg limit value of FVM decree 44/2003. (IV. 26.) on the compulsory regulations of the Hungarian Feed Code regarding undesirable contaminations of feeds [30] for arsenic was exceeded by the measured values for both arsenic forms and for all doses, with the exception of the control experiment in the case of the stem, and the control experiment and the lowest concentration treatment (3 mg/kg) in the case of the leaves. As for the pea pods, As contents above 2 mg/kg were found in the case of the 270 mg/kg treatments, however, in the case of the As(V) treatment, the As content of the pea pods, following a 90 mg/kg dose, was very close to the above mentioned limit value.

There has been no limit value designated for foods in the European Union regarding arsenic contamination, because currently there is no database describing the toxicities of organic and inorganic forms of arsenic in the different food groups [29].

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2. Introduction and literature review

Arsenic is one of the elements known since ancient times **[1]**, it was discovered by Albertus Magnus **[2]**. The opinion about arsenic has been ambiguous throughout history. Its toxicity has been known for thousands of years, but various arsenic compounds have been used for medicinal purposes. Arsenic was already used by Hippocrates to treat ulcer patients **[3]**, however, its regular use for medicinal purposes was started by a doctor of Swiss origin, Paracelsus. Later on, it was used as a medicine by several other doctors **[4]**. A solution containing small amounts of arsenic was used by Thomas Fowler to treat malaria, and this solution was later also commonly used in the case of psoriasis, anemia, rheumatism, tuberculosis and syphilis **[5]**. There was a time, when arsenic was regularly taken to preserve health, and it was also used in traditional Chinese medicine **[6]**. However, in the old days, arsenic was not only used for medicinal purposes. Various arsenic compounds were used in industry for wood preservation, among other things. Due to its toxicity, it was also used as a herbicide and insecticide **[7]**.

Arsenic poisoning has proved to be an effective means of eliminating unwanted persons. According to contemporary descriptions, the deaths of several monarchs could be related to arsenic, resulting in the expression “King of poisons, poison of kings” **[8]**. In Hungary, the most infamous arsenic killings were carried out between 1911 and 1929, later known as the “Tiszazug murders”. After it had been discovered by the midwife of Nagyrév that an arsenic-containing solution, suitable for killing living creatures, could be obtained by soaking flypaper in water, the method was used by several women to kill their relatives **[9]**, because arsenic is a colorless, odorless and highly toxic substance. Until the introduction of the Marsh test, it was an undetectable element **[10]**. Arsenic was mixed into the food or drink of the victims, thus causing their deaths **[9]**. Today, arsenic can still primarily enter the human body with food and drinking water **[11]**. According to the study of the European Food Safety Authority (EFSA), in addition to drinking water, different cereals, coffee, beer, rice, fish, and also vegetables contribute to the arsenic load of consumers **[12]**. It can also enter our bodies through inhalation and absorption through the skin, however, the extent of these is negligible **[13]**.

Arsenic has been shown to be carcinogenic, it causes liver, pulmonary, bladder and skin cancer **[1]**. Symptoms of arsenic poisoning can differ, depending on the amount of arsenic ingested. In the case of acute poisoning, symptoms are similar to those of indigestion: stomach pain, vomiting, chills, weakness, diarrhea. The first symptoms of chronic arsenic poisoning occur on the skin, first light and then dark spots develop, mainly on the skin of the limbs. The resulting syndrome is also called blackfoot disease, because of the ulcerous black wounds that develop on the lower limbs. Symptoms of chronic poisoning mainly occur in areas where the population has to consume drinking water with a high arsenic content. Arsenic is primarily accumulated in the integumentary system (hair, skin, nails) **[10]**.

The daily arsenic intake of the body depends largely on the food consumed. Foods contaminated with arsenic have been reported in many parts of the world, such foods including soy sauce (Japan), milk powder (Japan), and among drinks, wine (England, Germany) **[2]**. The arsenic content of the human body, although it increases with age, does not exceed 3 to 4 mg. Some of the arsenic entering the body is excreted in the urine. The normal arsenic content of urine is 5 to 40 μg/day, however, in the case of acute or subacute poisoning, the value can exceed 100 μg/day **[14]**. Primarily anionic and soluble arsenic species present are capable of being absorbed by the human body **[2]**.

Arsenic is present in nature in soil and groundwater, among other things, where regional enrichment of arsenic can be of natural or anthropogenic origin **[15]**. Arsenic contamination of natural origin can primarily develop through disintegration in the parent rock of soil, and we can also find this type of contamination near warm and thermal springs. In addition to geological events, arsenic contamination of soil is increased by the use of arsenic-containing herbicides and insecticides, various mining activities, as well as the agricultural use of arsenic-containing groundwater **[16], [17]**. Arsenic is primarily present both in the soil **[18], [19]**, and in the groundwater in inorganic forms **[2]** as arsenite [As(III)] and arsenate [As(V)], which forms are more toxic than organic ones **[20]**. In certain areas, the arsenic contamination of the soil can be as high as 2553 mg/kg, however, such high values are mainly due to human activity **[21]**. Arsenic contamination of soil and groundwater is a global problem **[2], [22]**, which is present in Hungary mainly in the Great Plain area, here the biggest concern is the arsenic content of geological origin of the groundwater **[23], [24]**. The problem of arsenic contamination of the soil and the groundwater is also present in certain areas of China, India, Thailand, the United States and Bangladesh **[2]**.

Through irrigation with arsenic-contaminated water and plant growing on soil containing such contamination, arsenic can enter the food chain. Between 1988 and 2011, according to data provided by the WHO, the provisional tolerable weekly intake (PWTI) for inorganic arsenic was 0.015 mg/kg body weight/week. Since the risk of developing cancerous diseases is increased by even smaller amounts of inorganic arsenic compounds, therefore, in the 2011 assessment of the expert committee of the FAO and the WHO, the previously established limit value was no longer considered to be appropriate since, due to the cancer development mechanism, in most cases, no safe intake value can be given for carcinogenic substances. In the case of such substances, the so-called benchmark dose level (BMDL) approach is used for the assessment of health risk. In its 2011 assessment, a $BMDL_{0,5}$ value was given by the WHO for inorganically bound arsenic for various cancerous diseases **[25]**. The $BMDL_{0,5}$ value is the lowest level of the smallest carcinogenic dose, given with a 95% probability, causing cancerous disease in 0.5% of the test animals in carcinogenicity tests.

The objective of our research was to assess the food and feed safety of green peas grown in arsenic-contaminated areas, since more than 70% of the vegetable growing areas in Hungary are located in the Great Plain area, and among the vegetables grown in Hungary, peas are present in the largest area. In order to assess the health risk due to the consumption and use in the feed industry of green peas grown in areas increasingly contaminated with arsenic, pot experiments were conducted.

3. Materials and methods

In the experimental building of the Institute of Soil Science and Agrochemistry of the Faculty of Agricultural and Food Sciences and Environmental Management of the University of Debrecen, pot experiments were performed, using green peas (*Pisum sativum* L.) as the test plant. The Avola green peas are one of the most popular early-ripening, marrow type peas, due to their suitability for canned and refrigerated industrial use.

3.1. Description of the soil used in the experiment

The most suitable soil type for green peas is calcareous chernozem soil formed on loess.

During the experiment, calcareous chernozem soil from the Látókép Experimental Plant of the Faculty of Agricultural and Food Sciences and Environmental Management of the University of Debrecen, located on the Hajdúság loessland, was used, the characteristics of which are the same as those of the soil used in the experiment of Kovács et al. **[26]**. Taking into consideration the NPK supply of the soil used in the experiment, the specific nutrient requirement of the peas and the planned average yield, NPK fertilization was applied, during which nitrogen was introduced into the soil as NH_4NO_3, phosphorus as KH_2PO_4, and potassium as KH_2PO_4 or K_2SO_4.

3.2. Arsenic treatments applied in the experiment

In our experiment, arsenic was applied as sodium arsenite [As(III)] ($NaAsO_2$) or potassium arsenate [As(V)] (KH_2AsO_4) dissolved in deionized water, because arsenic mainly occurs in soil in these two inorganic forms. In the course of our work, 0, 3, 10, 30, 90 and 270 mg/kg treatments were applied both in the case of arsenite and arsenate, during which the necessary concentrations were calculated for arsenic on the soil. All treatments were performed in triplicate.

3.3. Description of the pot experiment

During the experiment, 11 kg of air-dried soil, sieved through a sieve with openings of 1x1 cm, was placed in each pot, to which were added the fertilizer and the arsenite or arsenate as solutions (100 cm^3 each per pot). In the experiment, in addition to the control treatments, 5 treatment levels in triplicate were set up for both arsenite and arsenate, resulting in a total of 33 pots.

During the preparation of the soil mixtures (April 05, 2016), particular attention was paid to homogeneity. Following the loosening of the soil surface and irrigation using 200 cm^3 of distilled water, 25 pea seeds per pot were sown at a depth of approximately 3 to 4 cm. After compressing the surface of the media, pots were placed on the carts in a random-block arrangement, and were kept under a roof in the case of rain and at night. Following the germination of the seeds, the number of plants was reduced to 16 per pot, and the moisture content of the soil was adjusted to 60% of the maximum water capacity of arable land. Water loss due to evaporation and the controlled water release (transpiration) of the plants was compensated daily by weight supplementation. The experiment was concluded in the fourth phenophase of pea evolution (complete ripening), during which 4 plants were isolated from each pot for elemental analysis. Plants were separated into stalk, leaves, pea pod and pea seeds, and they were dried in a drying oven at 65 °C to constant weight.

3.4. Analysis of plant samples

Elemental analyses were carried out at the Institute of Food Science of the Faculty of Agricultural and Food Sciences and Environmental Management of the University of Debrecen.

Plant samples dried to constant weight were homogenized, and approximately 0.1 g of the adequately prepared samples were weighed into heat-resistant test tubes. 1 cm^3 of nitric acid (65 m/m%, Scharlau Chemie, Spain) was added to the samples, and they were allowed to stand overnight. The next day, test tubes were placed in the heating block of a LABOR MIM OE-718/A block digestion apparatus, which was used for pre-digestion at 30 °C for 60 minutes. 0.3 cm^3 of 30% hydrogen peroxide (Darmstadt, Merck, Germany) was then added to the sample solutions. Acid-peroxide main digestion was then carried out at 120 °C for 90 minutes. Digested samples were cooled to room temperature, and then were filled to 10 cm^3 using deionized water with a resistance of 18.2 MΩ /cm **[27]**. In parallel with the digestion of the samples, blank samples were also prepared.

3.5. Elemental analyses

To determine the arsenic content of the samples, a Thermo Scientific X-Series 2 Quadrupole type inductively coupled plasma mass spectrometer (ICP-MS) was used. Measurement parameters of the instrument are shown in ***Table 1***.

To eliminate interferences that occur during the analysis of low concentrations, the collision cell technology (CCT) measurement method and helium CCT gas containing 7% of hydrogen were used. During the analyses, application of an internal standard was necessary to eliminate non-spectral interferences. Internal standard means an element or elements with a low probability of occurrence in the sample. The element used by us as an internal standard was Rh (40 μg/L).

3.6. Statistical method

For the statistical evaluation of the results, the SPSS 22.0 statistical program was used. For the statistical analysis of the relationship between the parameters and the individual factors, single-factor variance analysis and Tukey test were used. Differences were considered significant if the P value was less than 5%. To compare the effects treatments at the same level but using different forms of arsenic, two-sample T-tests were performed.

4. Results and evaluation

4.1. The effect of arsenite and arsenate treatments on the arsenic content of green peas

Changes in the arsenic content of pea seeds due to arsenite and arsenate treatments of increasing concentrations are shown in ***Table 2***.

Based on the results of the analyses of the arsenic content of pea seeds it can be concluded that the arsenic concentrations of green pea seeds increased steadily with increasing soil arsenic content. In the case of As(V) treatment, even the smallest dose increased the arsenic content of the pea seed significantly, however, in the case of As(III) treatment, a significant increase was only observed due to the 90 mg/kg treatment. It can also be concluded for As(V) treatments that there was no significant difference in pea seed arsenic concentrations in the case of the 10 and 30 mg/kg treatments. There was no statistically significant difference between treatments at the same level using different forms of arsenic.

Of course, the largest increase was caused by the 270 mg/kg treatment. If arsenic was added to the soil as arsenite [As(III)], the arsenic content of the seed was 15.5 times higher than the value measured for the control plant. When arsenate [As(V)] was used, the increase compared to the control was 11-fold.

4.2. Food safety assessment of the consumption of green peas grown in arsenic-contaminated areas

Following the elemental analysis of pea seeds coming from the pot experiments, it was investigated what kind of food safety risk growing peas on soil contaminated with more and more arsenic poses. Since there was a change in 2011 in the tolerable weekly intake of arsenic, based on the WHO's position, it was also investigated what kind of food safety risk was attributed, before and after 2011, to the consumption of peas coming from areas contaminated by arsenic at different levels. To determine the magnitude of the risk, the 2010 data (0.9 kg/person/year) were used on the one hand, and the most recent, i.e., the 2014 per capita green pea consumption data in the database of the Hungarian Central Statistical Office (0.8 kg/person/year) were used on the other hand. In 2010, the tolerable weekly intake of arsenic, based on the data published by the WHO, was 0.015 mg/kg body weight/week. Thus, in this case, we could determine the magnitude of the risk by dividing the tolerable intake by the estimated dietary intake (***Table 3***). The risk assessment was performed for a man with a weight of 70 kg.

The data presented in the table were calculated according to the following relationships:

Risk = a/b = a/(c*d), where

a = the tolerable daily intake by the WHO, calculated from the weekly value (mg/kg body weight/day)

b = the estimated daily dietary As intake for 1 kg of body weight, calculated for an average body weight of 70 kg (mg/kg body weight/day)

c = the estimated daily green pea consumption calculated from the 0.9 kg/person/year value (kg)

d = the As content of peas (mg/kg)

If the ratio of the tolerable intake and the estimated dietary intake exceeds 10 **[31]**, consumption of the food in question is not considered risky. Since, for the year 2010, all of the values obtained during the risk assessment were greater than 10, the risk of any kind of health damage caused by the consumption of peas grown on soil receiving even the highest concentration (270 mg/kg) of arsenic treatment is negligible.

For the year 2014, the magnitude of food safety risk was no longer based on the ratio of the tolerable intake and the estimated dietary intake, since there was no longer a tolerable intake value for arsenic in 2014. In the assessment of the WHO, published in 2011, $BMDL_{0.5}$ values were given for inorganically bound arsenic with respect to various cancers as described below: in the case of malignant tumors of the lungs, 3 μg/kg body weight/day, in the case of bladder cancer, 5.2 μg/kg body weight/day, and for skin cancer, 5.4 μg/kg body weight/day is the $BMDL_{0.5}$ value **[25]**. The food safety effect of the consumption of green peas grown on soils receiving increasing concentration arsenite and arsenate treatments was estimated based on the contribution of its consumption to the $BMDL_{0.5}$ values determined for certain cancers.

The percentage contribution ($BMDL_{0.5}$) of the amount of arsenic absorbed due to the consumption of green peas to the development of different cancers was calculated as follows:

Contribution% = b/$BMDL_{0.5(i)}$

b = the estimated daily dietary As intake per kg of body weight, calculated for an average body weight of 70 kg (mg/kg body weight/day), as mentioned for the values of Table 3;

$BMDL_{0.5(i)}$ = values for the given diseases (μg/kg body weight/day), for each disease:

$BMDL_{0.5(lungs)}$ = 3.0 μg/kg body weight/day

$BMDL_{0.5(bladder)}$ = 5.2 μg/kg body weight/day

$BMDL_{0.5(skin)}$ = 5.4 μg/kg body weight/day

Results obtained are shown in ***Table 4***.

In the case of bladder and skin cancer, the consumption of the seeds of green peas grown on the control soil contributed to the $BMDL_{0.5}$ value by 0.02%, while in the case of lung cancer, the value was 0.03%. Since the arsenic concentration of the pea seeds increased steadily with the increasing concentrations of arsenite and arsenate treatments, thus the consumption of green peas grown on soils subjected to increasing arsenite and arsenate loads contributes more and more to the development of malignant tumors of the skin, bladder and lungs. However, it is important to note that the highest arsenic content in the seeds were measured in the case of the As(III) treatment, and the contribution of the consumption of pea seeds coming from soil with such contamination to the $BMDL_{0.5}$ values determined for the cancers is as follows: 0.46% in the case of lung cancer, 0.26% in the case of bladder cancer, and 0.25% for skin cancer.

Overall, the likelihood of the development of cancer in 0.5% of a given population due to the consumption of green peas coming from a contaminated area is extremely small.

4.3. The use of green peas grown in arsenic-contaminated areas as feedstuff

After harvesting the peas, the remaining plant parts such as the stalk and leaves, worthless from a food industry point of view, as well as the pods remaining after food industrial processing, are excellent feeds. Consequently, analysis of the changes in the arsenic content of these plant parts in light of the arsenic load of the soil are of utmost importance. On the one hand, feeding livestock with feed contaminated with arsenic can endanger the health of the animals, because arsenic has been proven to be toxic not only to humans, but also to animals, and on the other hand, although its likelihood is low, it cannot be ruled out that arsenic can enter animal products from the feed. Thus, feeding different parts of green peas coming from arsenic-contaminated areas to animals may pose an indirect risk even to human health. For the undesirable contaminants of feeds, the provisions of FVM decree 44/2003. (IV. 26.) **[30]** about the compulsory regulations of the Hungarian Feed Codex are in effect, according to which the arsenic content of green pea parts intended for use as a feedstuff cannot exceed 2 mg/kg at a moisture content of 12%. In order to determine the arsenic content of the residual parts, to be utilized as feedstuff, of green peas grown for food industrial use in arsenic-contaminated areas, elemental analysis was performed on the stalk, leaves and pods of the peas. The results of the analyses are summarized in ***Table 5***.

Based on the results, it can be concluded that the arsenic content of the stalk and leaves of green peas increased monotonously with increasing soil arsenic concentrations. In the case of peas pods, a continuously increasing trend for the averages could only be observed for the As(V) treatments.

Based on the measurement results, the arsenic concentration of the pods was lower than the 2 mg/kg limit value of the relevant decree for feeds, in the case of As(III) treatments not exceeding 90 mg/kg. However, the largest treatment (270 mg/kg) resulted in a significant increase. The arsenic concentration measured for the 270 mg/kg treatment was 6.14 times higher than the value measured in the case of the 90 mg/kg treatment, while it was also 2.36 times higher than the limit value of the decree. In terms of the As(V) treatments, the arsenic content of the pea pods was below the limit value in the case of treatments with doses lower than 90 mg/kg. However, the arsenic content measured at the 90 mg/kg treatment (1.93±0.13 mg/kg) was already very close to the 2 mg/kg limit value. As a result of the largest As(V) dose (270 mg/kg), the arsenic content of the pods increased significantly. The arsenic content found in the case of the 270 mg/kg treatment was 8 times higher than the limit value, and it was more than 8.29 times higher than the value measured in the case of the lower dose (90 mg/kg) treatment.

Considering the values measured during the elemental analysis of the stalk of green peas grown in the pots, it can be concluded that, with the exception of the control plant, the arsenic content of the stalk exceeded 2 mg/kg for all treatments, both for As(III) and As(V) doses. As a result of the increasing concentration As(III) or As(V) load of the soil, the arsenic concentration of the stalk increased drastically. As a result of the highest dose (270 mg/kg) of arsenic, the arsenic content of the stalk was 25.5 times higher than the allowed value in the case of the as(III) treatment, and 90 times higher in the case of the As(V) treatment.

As a result of increasing concentration As(V) and As(III) treatments, the increase in the As content of the leaves was so severe, that the As content of the leaves exceeded the 2 mg/kg limit value for all treatments exceeding 3 mg/kg.

Based on the arsenic content of green pea parts that can be used as feedstuff, the following order could be determined: stalk > leaves > pods. As a result of the analyses aimed at the determination of the arsenic content, it can also be concluded that, in terms of the averages, the accumulated amount of arsenic in the leaves and the stalk was higher in the case of the As(V) treatments than it was in the case of the As(III) treatments. However, in the case of the pea pods, the same trend could only be observed in the case of the 90 and 270 mg/kg treatments. In the case of the lower concentration (0, 3, 10 mg/kg) treatments, the opposite was observed, with the exception of the 30 mg/kg treatment, where there was no observable difference, in terms of the averages, between the treatments using different forms of arsenic.

According to our measurements, the arsenic content of the stalk and leaves of peas does not exceed the limit value of the Hungarian Feed Codex **[30]**, therefore, the peas grown in our experiments could be used as feedstuff.

However, the magnitude of the food safety risk of using peas contaminated with arsenic as feedstuff could not be determined on the basis of the arsenic content of the feedstuff, it would require the carrying out of feeding experiments.

5. Conclusions

As a result of the increasing arsenic load of soil, there was an increase in the arsenic content of all plant organs of green peas. The percentage contribution of the consumption of green peas contaminated with arsenic to the $BMDL_{0.5}$ values determined by the WHO for various cancers did not exceed 0.46% in any of the cases, i.e., the risk of developing lung, bladder or skin cancer is negligible. Based on the arsenic content of the plant parts of green peas grown on soils receiving increasing concentration arsenite and arsenate treatments (stalk, leaves, pods), to be used as feedstuffs, it can be concluded that the As content of pea pods exceeded the 2 mg/kg limit value for the undesirable contaminants of feeds of FVM decree 44/2003. (IV. 26.)[1] **[30]** about the compulsory regulations of the Hungarian Feed Codex, both in the case of the 270 mg/kg As(III) and As(V) treatments, and the As concentration of the pea pods was very close to the above-mentioned limit value in the case of the 90 mg/kg As(V) treatment as well. As contents exceeding the limit value were measured for all doses, with the exception of the control plants, for the stalk, and for all treatments exceeding 3 mg/kg for the leaves.

Based on the experiments, it is expected that, when using raw materials of plant origin, the elements of the food chain will be contaminated only to a negligible extent by arsenic coming from environmental pollution.

We did not have the possibility to determine the chemical forms of arsenic absorbed by the plants using speciation analysis, but it is assumed that a significant part of the arsenic was bound organically. This is promising, because it is well-known that organic compounds of arsenic are a lot less toxic than the inorganic ones **[28].**

[1] *FVM abbreviation of Ministry of Agriculture and Rural Development, currently Ministry of Agriculture*

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