



# Examination of the arsenic accumulating capacity of lettuce growing in aggregate hydroponics under the influence of arsenic polluted nutrient solution

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**Abstract.** The arsenic polluted sprinkling water might appear in the southern regions of Hungary. Arsenic levels sometimes exceed the 200  $\mu\text{g/l}$  limit, allowed in underground water in Hungary.

In the teamwork of Soil and Plant Testing Laboratory and the Institute of Vegetable Growing (Kecskemét College, Faculty of Horticulture) we studied some of the effects of sprinkling water containing arsenic pollution on different vegetables since 2006.

In this work, lettuce in hydro-culture was used as an indicator plant. The aim of our examination was to clear up the effect of arsenic on the degree of arsenic accumulation. We used 25, 50, 75, 100, 200, 400 and 600  $\mu\text{g/l}$  arsenic pollution doses.

**Keywords:** arsenic pollution, lettuce, greenhouse, hydro-culture, hydroponically, ICP-AES

## 1 Introduction

Arsenic is a well known toxic element can be found in some waters and foods. High arsenic levels contribute to the development of serious disorders. According to laws in Hungary, drinking water may contain 10  $\mu\text{g/l}$  arsenic [1], whereas our food is able to have 200  $\mu\text{g/kg}$  maximal arsenic concentration [2].

Arsenic polluted drinking and sprinkling water appears at the southern parts of the country, in counties Bács-Kiskun, Békés, Csongrád and Szolnok. In these areas plants can accumulate arsenic easily and in high quantity. Based on the teamwork of faculty, we studied the effects of nutrient solution containing arsenic pollution on the growing of lettuce in hydro-culture.

Arsenic (As) is a well known toxic element found in Hungarian well waters due to natural geological conditions [3]. Underground waters in the southern and south-eastern parts of the Great Plain are polluted with 30-150  $\mu\text{g/l}$  arsenic concentration [4].

Due to these measures the impact of polluted water on the population can be reduced, but it must not be forgotten, that in the southern and south-eastern parts of the country fresh vegetables irrigated with arsenic water can threaten the consumers directly.

It is clearly known from geological research [4], that the southern and south-eastern parts of the Great Plain contain high arsenic water concentration. This area represents 80% of the irrigated vegetables territory.

The inorganic forms of arsenic are dangerous poisons noxious to the whole human body, reducing the activity of the nervous system, kidneys, respiratory organs and the liver, also resulting in reproductive and genetically anomalies and cancer [5].

Trial series were started in cooperation between the Ornamental Plant and Vegetable Crops Institute and Soil and Plant Analysis Laboratory of the College for Horticulture (Hungary, Kecskemét) to determine the concentration of this toxic element in some important vegetables irrigated with polluted water. Leaf-vegetables, pepper, tomato, carrot and parsley have been tested from 2006 onwards followed by hydroponic lettuce in 2009 and 2010. Lettuce is grown on about 2000 ha, half in the open and half in forcing. The water used for irrigation or for nutrient solutions is obtained from wells, 30-100 m deep [6].

Trial series aimed at finding out the effect of arsenic water characteristic of the region on the arsenic content of lettuce leaves grown in hydroculture when polluted water is used for the nutrient solution. Arsenic doses of 25, 50, 75, 100, 200, 400, 600  $\mu\text{g/l}$  were tested. The first five doses represent

concentrations found in nature, the extreme values ( $400\text{--}600\text{ }\mu\text{g/l}$ ) served for scientific observations or modelled extreme conditions.

## 2 Materials and Methods

Trials included lettuce in hydroponic culture in the greenhouse of the Ornamental Plant and Vegetable Crops Institute. There were three tables each containing three nutrient channels made of plastic plates, 4.3 m long, 15 cm high and 30 cm large (Fig. 1).



Figure 1: Hydroculture with nutrient channels

In each channel 25 l standard solution was circulated by a pump controlled by a time switch. An upper container (feeder) and a bottom container (collecting) facilitated the storage of the solution. The slight sloping of the channels furthered the solution flow. In the hydroculture roots developed in the solution and plants were fixed in a neutral agent, rock-wool, and cubes. Fig. 2 shows the cross section of the nutrient channel.

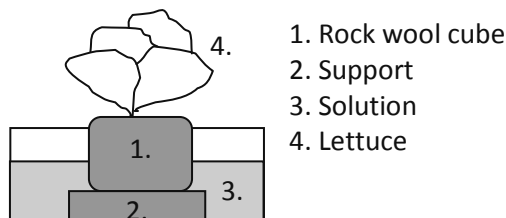


Figure 2: Cross section of the nutrient channel

The nutrient solution was prepared according to a recipe of the Ornamental Plants and Vegetable Crops Institute consisting of Ferticare IV fertilizer complex with 1.6-1.8 mS/cm EC-values and pH 5.5-6.5.

The arsenic solution used in the trials consisted of an arsenic stock solution and the nutrient solution describe above, in 250 mg/l concentration in the stock solution. The stock solution was prepared under laboratory conditions of the Soil and Plant Analysis Laboratory. Increasing doses of the stock solution (2.5; 5; 7.5; 10; 20; 40; 60 ml) were added to the containers (25 l).

The initial compound, arsenic acid ( $\text{H}_3\text{AsO}_4$ ) was made of arsenic trioxide. Thus, after dilution arsenic was present in the solution in form of arsenate ( $\text{H}_2\text{AsO}_4^-$ ). In the naturally polluted waters of the concentrated regions the same ion forms are found.

The hydroculture started 1st September 2009 and 29th March 2010. Two-four leaf lettuce was pricked into rock-wool cubes. The growing period lasted 6 weeks in both years. The nutrient solution was changed once a week. When adding the fresh solution great care was taken of precise dosing.

Evaporation required the replacement of the solution even during the weeks; great care was taken to maintain the initial concentration. Samples were taken and checked by the Laboratory.

At the end of the trial period the lettuce heads were removed from rock-wool cubes and weighed. Random samples were taken on the whole length of each channel (total 17 heads), fully developed healthy leaves were taken from the middle of the heads in four repetitions. Root samples were also collected by lifting the rock-wool cubes and disentangling the roots carefully (Fig. 3).



Figure 3: Rock-wool cube lifted at the end of the trial

The solids content in leaves and roots were determined by drying ( $70^\circ\text{C}$ ) and homogenizing samples in a mill in air dry stage. Samples were digested

in a microwave device by means of concentrated nitric acid and hydrogen peroxide using high pressure teflon bombs at 40-60 bar pressure, at 210°C for 20 minutes. For dilution pure, ion-free water was used and samples were filtered through quantitative filter paper.

Element contents were evaluated in an ICP-AES spectrometer, with radial plasma set, 12 l/min argon flow, 1000 W generator output, at 193.695 nm wavelength, 1 ml/min samples flow. Detector: High Dynamic Detection System (HDD). Limit of quantification: 0.300 mg/kg arsenic referring to samples solids. For quality control all samples were run in duplicates with blanks and certified IPE plant (International Plant Analytical Exchange, Wageningen University). Results from the certified samples were within  $\pm 10\%$  of the known value.

### 3 Results and discussions

According to classical analytical methods the arsenic content of samples was determined from the solids content. It must not be forgotten, however, that parts of vegetables (in lettuce the whole foliage) have very high water content. In our solids calculations the solids content of the samples varied between 3.05 and 5.82 m/m% with an average of 4.06 m/m%.

Relevant rules [2] allow 0.200 mg/kg arsenic in vegetables for fresh consumption at original water content. The value of arsenic concentration measured in lettuce solids should be divided by 25 to obtain the arsenic concentration of the plant at original water level.

The following two Figures represent arsenic concentrations in the two years and average of repetitions.

As shown by Fig. 4 the 200  $\mu\text{g/l}$  dose in 2009 did not result in measureable As-content in lettuce leaves. Doses 400 and 600  $\mu\text{g/l}$  increased As-content in leaves referring to control and the 200  $\mu\text{g/l}$  dose. Scattering among repetitions is high. The highest As value – 2.67 mg/kg – was found in the third repetition of the 400  $\mu\text{g/l}$  dose. Repetition averages in 400 and 600  $\mu\text{g/l}$  doses were contradictory as the mean of the 400  $\mu\text{g/l}$  dose surpassed that of the 600  $\mu\text{g/l}$  dose (1.56 and 0.952 mg/kg, respectively).

According to valid food decrees [2] the As content of vegetables with original water content can, at most, be as high as 0.200 mg/kg. Due to causes mentioned above the arsenic values of solids are to be divided by 25 to obtain the arsenic concentration of a sample with the original water content.

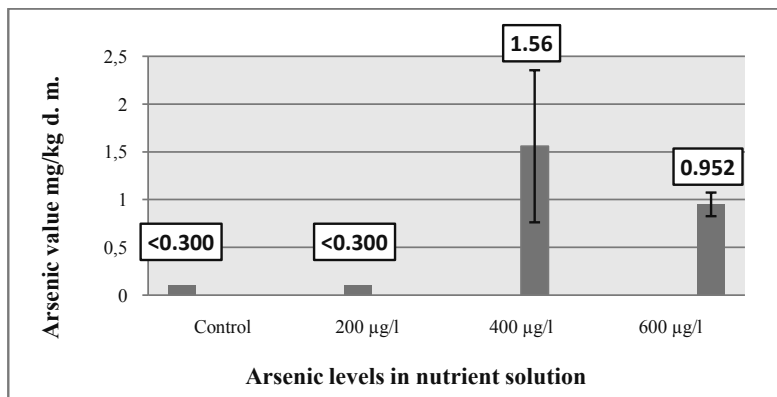


Figure 4: Arsenic levels in leaves referring to solids mg/kg (2009)

When the measured highest value, 2.67 mg/kg was divided by 25 we got the value 0.107 mg/kg which was nearly 50% lower than 0.200 mg/kg. That is, even the highest applied doses did not surpass the limit. Fig. 5 shows our results in 2010.

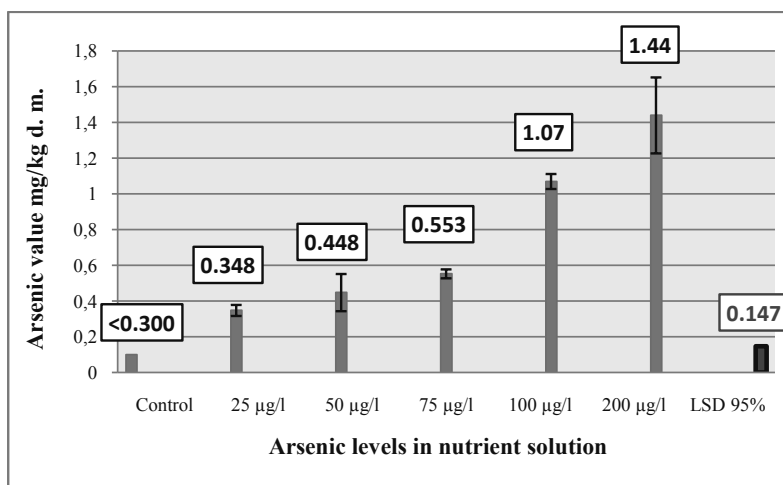


Figure 5: As levels in leaves referring to solids, mg/kg (2010)

Repetitions showed much less scattering than in 2009. Trials in 2010 indicated a more precise execution of trials. Between the same doses of the two years (200 µg/l) there was considerable difference despite similar conditions. To clear up the situation trial is going to continue in 2011 involving all the doses.

In 2010 increasing doses increased arsenic concentration in the leaves. Variance analysis [7] showed significant As content increase when applying 100

and 200  $\mu\text{g/l}$  doses referring to control and doses below 100  $\mu\text{g/l}$ . They also differed significantly from each other LSD 95% (Fig. 5).

The highest value was measured in the first repetition of the 200  $\mu\text{g/l}$  dose (1.62 mg/kg). When it was calculated back to the original samples the value of 0.1 mg/kg did not reach half of the limit.

Similar trends were observed in the increase of As content in roots in both years. Figs 6 and 7 represent As values in root samples.

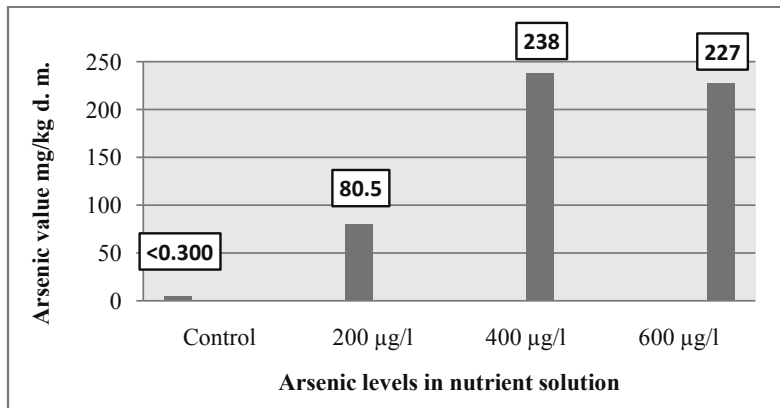


Figure 6: As levels in roots referring to solids (2009)

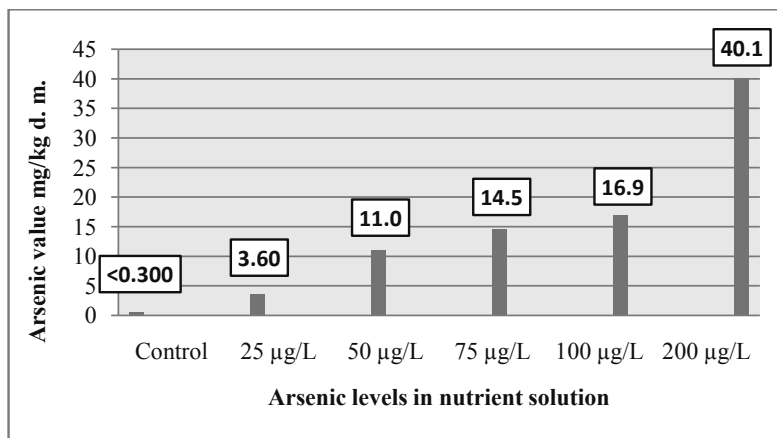


Figure 7: As levels in roots referring to solids (2010)

Increasing As doses increased As concentration in the roots, the low quantities of root samples did not allow repetitions and statistical analysis, yet the

physiological filtration effect of the roots is well expressed.

The ratio of As concentration between roots and foliage as affected by increasing doses was: 10.3; 24.6; 26.2; 15.8; 27.8; 152.6; 238.4. The widening As ratio may indicate the important accumulation function of the roots as affected by high doses of toxic elements. The As content in roots increased more rigidly than in leaves.

The accumulation rate in roots is also expressed by the As concentration in root solids which was 100-600 times higher than in the nutrient solution.

## 4 Conclusion

Trials show that the arsenic concentration of the nutrient solution affects the As content in the vegetative parts of lettuce. Even slight doses ( $200\mu\text{g/l}$ ) increased As level in the test plant.

According to [8] As poisoning symptoms in plants are as follow: Reddish-brown, necrotic spots on older leaves, brown discoloration on roots, developmental anomalies in the whole plant. In our trials no such symptoms could be observed. They might have been caused by higher doses than those applied by us.

Bowen stated that in nutrient solutions As belonged to the moderately toxic elements, hindering plant development between 1 and 100 mg/l concentration [9].

The As doses applied in our trials increased As concentration in lettuce leaves significantly from  $75\mu\text{g/l}$  upwards. The highest As concentration,  $2.67\text{ mg/kg}$  in the leaf solids was caused by the  $400\mu\text{g/l}$  dose.

Increasing As doses increased As concentration in the roots as well but the accumulation was more accentuated. In some doses As content in roots was 10-238 times higher than in leaves. Results are parallel to those of [10] who found 30 mg/kg in roots and 1-5 mg/kg in stems and leaves of the test plants, as affected by As doses.

Rofkar et al. proved in different phytoremediation trials that when comparing plant parts the highest As concentration was found in the roots both in soil and soilless cultures. They also found values between 200-600 mg/kg As in roots of test plants. Our trials confirmed the importance of roots in filtering toxic elements [11].

Smith et al. also studied lettuce in hydroculture adding 2 mg/l arsenic concentration to the nutrient solution. They found  $278\mu\text{g/kg}$  As in the roots and  $3.18\text{ mg/kg}$  in the leaves of the test plant which agrees with our results [12].



Summarizing it can be stated that the arsenic content of lettuce of original water content, grown in hydroculture, increases as affected by As application but it does not surpass the 0.2 mg/kg limit. According to our results even three times higher values than 200  $\mu\text{g/l}$  found in natural well water do not increase the As level above the limit in lettuce.

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