

Thesis of doctoral (PhD) dissertation

**EFFECT OF DIFFERENT ARSENIC TREATMENTS ON THE
GROWTH AND ELEMENT UPTAKE OF SOME FIELD CROPS IN
THE EARLY PHASE OF THE PLANT DEVELOPMENT**

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1. INTRODUCTION AND AIMS

Contamination of agricultural soils and groundwaters by arsenic is a global environmental problem. Arsenic is toxic to humans, animals and plants. It occurs in soils and groundwaters predominantly as inorganic arsenic species, namely arsenite [As(III)] and arsenate [As(V)].

The aim of my study was to investigate the effect of As(III) and As(V)-treatments in hydroponic-plant and in soil-plant system. During my work three different types of experiment were carried out: hydroponic experiments, rhizobox experiments, pot experiments. The soil and plant samples which were obtained from the experiments were analyzed.

During my soil examination my objective was to determine how much percent of the soil's total arsenic content can be utilized for plants (soluble arsenic content) and I would like to know that there was any differences between the effect of As(III) and As(V)?

During the examination of plant samples my aim was to answer to the following questions:

- How the As(III) and As(V)-treatments interact with the dry mass product of certain parts of the plant?
- What is the As(III) or As(V) quantity which has a favourable effect on the plants?
- What is the As(III) and As(V) quantity which cause visible toxic symptoms?
- What is the As(III) and As(V) quantity which cause the death of the plant?
- How the As(III) and As(V)-treatments interact with the intensity of initial root growth?
- What kind of effect do As(III)- and As(V)-treatments have on the arsenic content of certain parts of the plant and in which part of the plant does arsenic accumulate the most?
- Is there any difference between the monocotyledon and dicotyledon plants with regard to the As uptake?
- How much As do the experimental plants subtract from the nutrient solutions and soils including As(III) and As(V) of increasing concentration and in what proportions is the As quantity divided among certain plant parts?
- How do the applied As(III) and As(V)-treatments affect the macroelement and microelement contents of plants?
- Is there a synergistic or antagonistic relationship between the As(III)/As(V) and the examined macro- and microelements?
- What are the differences between the effects of As(III) and As(V)-treatments on the parameters which were examined?

2. MATERIALS AND METHODS

I involved a monocotyledon (maize) and a dicotyledon plant (sunflower) in the hydroponic and rhizobox experiments which directly or indirectly play an important role in human nutrition while their economic role is also essential. I grew the plants in the Climate Chamber of Department of Agriculture and Botany Plant in the Institute of Plant Sciences under strictly regulated environmental conditions: light intensity: $220 \mu\text{Em}^{-2}\text{s}^{-1}$, periodicity of temperature: 25/20 °C (day/night), relative humidity (RH): 65-75 %, lighting/dark cycle: 16 hours/8 hours. The nutrient solution used for monocotyledon had the following composition: 2.0 mM $\text{Ca}(\text{NO}_3)_2$, 0.7 mM K_2SO_4 , 0.5 mM MgSO_4 , 0.1 mM KH_2PO_4 , 0.1 mM KCl , 0.1 μM H_3BO_3 , 0.5 μM MnSO_4 , 0.5 μM ZnSO_4 , 0.2 μM CuSO_4 . Nutrient solution of the following had to be prepared in order to grow dicotyledon: 2.0 mM $\text{Ca}(\text{NO}_3)_2$, 0.7 mM K_2SO_4 , 0.5 mM MgSO_4 , 0.1 mM KH_2PO_4 , 0.1 mM KCl , 10 μM H_3BO_3 , 0.5 μM MnSO_4 , 0.5 μM ZnSO_4 , 0.2 μM CuSO_4 . Iron was supplied in the form of 10^{-4} M Fe-EDTA. Potassium dihydrogen arsenate (KH_2AsO_4) and sodium arsenite (NaAsO_2) were used as a source of arsenate [As(V)] and arsenite [As(III)], respectively. Sunflower and maize seedlings were exposed to 1, 3, 10, 30 and 90 mg L^{-1} As in a form of As(III) and As(V) and there were no arsenic treatment in the case of control plants. The experiment ended 14 days after planting in the case of maize, and 21 days after planting in the case of sunflower.

Experiments in soil were carried out in rhizoboxes, which allowed us to easily monitor many aspects of root development, including overall growth, circadian rhythm of the growth as well as symptoms of phytotoxicity that might have been caused by increased concentrations of arsenic. The seedlings were planted with 2.5-3 cm coleoptiles.

The calcareous chernozem soil used in the experiment was collected from the Látókép Experimental Station of the University of Debrecen. The moisture content of the soil at field capacity was 60%. The arsenic was applied in a form of As(III) (NaAsO_2) and As(V) (KH_2AsO_4), respectively. Arsenic was supplemented to the soil as aqueous solution prepared with distilled water in six different concentrations: 0 (control), 1, 3, 10, 30, 90 mg kg^{-1} arsenic. The most important parameters of the experimental soil were demonstrated in the *Table 1*.

Table 1.: The most important parameters of the experimental soil

Depth	0-0.3 m
pH (KCl)	5.71
pH (H ₂ O)	6.58
Soil texture category	loamy clay
Total water-soluble salt	0.015%
CaCO ₃	0.202%
Humus	3.54%
KCl-soluble NO ₃ -N+NO ₂ -N	8.04 mg·kg ⁻¹
AL-soluble P ₂ O ₅	199 mg·kg ⁻¹
AL-soluble K ₂ O	451 mg·kg ⁻¹
AL-soluble Na	332 mg·kg ⁻¹
KCl-soluble Mg	176 mg·kg ⁻¹
KCl-soluble SO ₄ ²⁻ -S	6.04 mg·kg ⁻¹
KCl-EDTA-soluble Cu	5.79 mg·kg ⁻¹
KCl-EDTA-soluble Zn	7.9 mg·kg ⁻¹
KCl-EDTA-soluble Mn	262 mg·kg ⁻¹

To prevent the light interference on the root the transparent side of the rhizoboxes was covered with dark foil. The number of the replicates was 5. Water losses were determined gravimetrically every day and refilled by applications of de-ionized water, which allowed us to easily monitor many aspects of root development. The mass of rhizoboxes and the length of the roots were measured daily and water (evaporation, transpiration) was also replenished daily. The experiment was finished when the root of the seedlings reached the bottom of the box. In the case of the maize it was 6 days after the planting, and in the case of sunflower it was 5 day after the planting.

I set the pot experiments in the Greenhouse of Institute of Agricultural Chemistry and Soil Science in University of Debrecen, at April-June of 2016. In this experiment I chose green peas (*Pisum sativum* L.) as test plant since it is one of the vegetables grown in the largest area in Hungary. In the experiment was also used calcareous chernozem soil. Supplementary fertilization was used according to the next: NH₄NO₃ (0,568 g pot⁻¹), KH₂PO₄ (0,229 g pot⁻¹), K₂HPO₄ (0,079 g pot⁻¹) and K₂SO₄ (0,148 g pot⁻¹). The concentrations of the As(III) (NaAsO₂) and As(V)-treatments (KH₂AsO₄) were 0, 3, 10, 30, 90, 270 mg kg⁻¹. The determination of the element content of green peas was carried out in 4 different stages of development which are the following: four-node condition, the beginning of flowering, green ripening, and complete maturity.

During my research work I carried out the element analysis of plant samples with inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Wet digestion with nitric acid and hydrogen peroxide was applied

during sample preparation of these plants. The elemental analysis of soil samples can be divided to two main components which are the following: 1. The determination of the total element content of the samples with atmospheric wet digestion using concentrated nitric acid and hydrogen peroxide. 2. The determination of the samples' soluble arsenic content easily available for plants using the extraction tool by Lakanen-Erviö.

All data obtained from element analysis were subjected to one-way analysis of variance (ANOVA) and Duncan's test at 0.05 significance level to compare populations and arsenic treatments. Independent Sample T-test was used to determine the statistically significant differences at 0.05, 0.01 and 0.001 significance levels between the effect of As(III) and As(V) at the same level, used by SPSS statistics software version 22.0.

3. RESULTS AND DISCUSSION

3.1. Results of the soil samples

During my work I determined the calcareous chernozem soil's total and Lakanen Erviö's soluble As content for the rhiobox and the pot experiment. Based on the elemental analysis test results I found that as an effect of the As(III) or As(V) treatments both the total and soluble As content of the soil has evenly increased, but only a small part of the total As content of the soil was accessible for the plants (*Table 2*).

Table 2.: The total and the soluble arsenic content of the experimental soil (n=3±s.e.)

As-treatments (mg kg ⁻¹)	"Total" As content (mg kg ⁻¹) (A)	"Soluble" As content (mg kg ⁻¹) (B)	B/A
0	2.07±0.04 ^a	0.799±0.064 ^a	0.386
1	3.23±0.17 ^a	1.61±0.12 ^{ab}	0.497
3	5.20±0.28 ^a	2.54±0.78 ^{ab}	0.488
As(III) 10	12.4±1.7 ^b	4.84±0.12 ^b	0.390
30	31.9±1.8 ^c	19.3±1.6 ^c	0.607
90	93.4±1.2 ^d	53.3±3.2 ^d	0.570
270	273±6 ^e	162±10 ^e	0.593
0	2.07±0.04 ^a	0.799±0.064 ^a	0.386
1	3.20±0.60 ^a	1.57±0.20 ^{ab}	0.490
3	5.26±0.25 ^a	2.53±0.37 ^{ab}	0.481
As(V) 10	11.6±0.1 ^b	4.42±0.18 ^{ab}	0.382
30	32.1±0.5 ^c	15.7±1.1 ^b	0.488
90	92.0±4.3 ^d	39.1±2.6 ^c	0.425
270	269±4 ^e	153±17 ^d	0.569

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Duncan multiple range test ($P \leq 0.05$). Means followed by *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$) within columns were significantly different between the effect of As(III) and As(V) at the same level according to independent sample T-test.

Based on the elemental analysis results it can be stated that the control soil also contained arsenic ($2.07 \pm 0.04 \text{ mg kg}^{-1}$), and I proved that in the case of the different treatments arsenic was evenly distributed in the soil. I have also observed that 38.6% of the control soil's "total" arsenic content, in the case of As(V)-treatments 38.2 to 56.9%, while in the case of As(III)-treatments from 39 to 60.7% of it were present in a form accessible for plants. The soil analysis results also pointed out that in the case of treatments of the same concentration but different form [As(III) and As(V)], As content which was available to the plants was higher in the case of As(III)-treatments than in the case of As(V)-doses.

3.2. Results of the plant samples

3.2.1. Effect of the different As(III)- and As(V)-treatments on the plant's dry mass and root growth

By examining the test plants' dry matter in general it can be said that in the case of nutrient solution experiments, as an effect of increasing concentration As(III) and As(V)-treatments the dry weight of both the maize and the sunflower seedlings (*Tables 3-4*) has reduced.

Table 3.: Dry weight ($g\ plant^{-1}$) of shoot and root of maize seedling grown in nutrient solution depending on As(III)- and As(V)-treatments (0, 1, 3, 10, 30 $mg\ kg^{-1}$)($n=12\pm s.e.$)

As-treatments ($mg\ kg^{-1}$)	Dry weight of maize ($g\ plant^{-1}$)			
	Shoot		Root	
	As(III)	As(V)	As(III)	As(V)
0	0.3028±0.0666 ^a	0.3028±0.0666 ^a	0.0964±0.0227 ^a	0.0964±0.0227 ^a
1	0.2497±0.0353 ^b	0.2801±0.0805 ^a	0.0522±0.0175 ^b	0.0655±0.0207 ^b
3	0.0752±0.0209 ^c	0.0724±0.0192 ^b	0.0506±0.0164 ^b	0.0370±0.0112 ^c
10	0.0318±0.0103 ^c	0.0399±0.0134 ^b	0.0214±0.0051 ^c	0.0205±0.0032 ^{cd}
30	0.0296±0.0086 ^c	0.0334±0.0078 ^b	0.0200±0.0061 ^c	0.0149±0.003 ^d

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Duncan multiple range test ($P\leq 0.05$). Means followed by *($p<0.05$), **($p<0.01$), ***($p<0.001$) within columns were significantly different between the effect of As(III) and As(V) at the same level according to independent sample T-test.

Table 4.: Dry weight ($g\ plant^{-1}$) of shoot and root of sunflower seedling grown in nutrient solution depending on As(III)- and As(V)-treatments (0, 1, 3, 10 $mg\ kg^{-1}$)($n=12\pm s.e.$)

As-treatments ($mg\ kg^{-1}$)	Dry weight of sunflower ($g\ plant^{-1}$)			
	Shoot		Root	
	As(III)	As(V)	As(III)	As(V)
0	0.811±0.208 ^a	0.811±0.208 ^a	0.228±0.025 ^a	0.228±0.025 ^a
1	0.498±0.055 ^b	0.567±0.068 ^{b*}	0.191±0.018 ^b	0.205±0.026 ^{ab}
3	0.399±0.024 ^b	0.522±0.111 ^{b*}	0.167±0.005 ^c	0.215±0.031 ^{a**}
10	-	0.374±0.005 ^c	-	0.178±0.008 ^b

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Duncan multiple range test ($P\leq 0.05$). Means followed by *($p<0.05$), **($p<0.01$), ***($p<0.001$) within columns were significantly different between the effect of As(III) and As(V) at the same level according to independent sample T-test.

By analyzing the impact of increasing concentration of As treatments on mono- and dicotyledonous plants I also observed that the sunflower (dicotyledonous plant) responded to As-toxicity in a more sensitive way than the maize (monocotyledonous plant). I found that in the case of the maize seedlings's nutrient solution experiment the 90 $mg\ kg^{-1}$ treatment in respect of both inorganic As forms proved to be so toxic that it caused the destruction of the plants already at the beginning of the experiment. However, in the case of the sunflower seedlings in addition to the 90 $mg\ kg^{-1}$ treatment the 30 $mg\ kg^{-1}$ dose in the case of both types of treatments, while for a 10 $mg\ kg^{-1}$ concentration an As(III)-treatment caused destruction of the

experimental plants. This is not only draws attention to a different sensitivity of mono- and dicotyledonous plants, but also to the differences in As(III) and As(V)'s toxicity.

As long as in the case of the experiment with nutrient solution the full volume of As was available for the plants, in the case of the rhizobox experiment only a small part of the As allocated to the soil was accessible for the plants, due to which the toxic effects of arsenic manifested less in the case of the rhizobox experiment, even treatments of higher concentration did not cause destruction of plants. By evaluating the rhizobox experimental results I found that due to the treatments no significant change took place in the dry matter content of maize and sunflower plants, solely as an impact of the 10 mg kg⁻¹ As(V)-treatment the dry matter content of maize seedling has significantly increased. The development of arsenic treated maize and sunflower, as well as the differences between the impact of As(III) and As(V)-treatments show well by studying the rhizobox experiment's root grow results. I found that in the case of maize seedlings' As(III)-treatment the root grow of the control plant was the most intense. As(III)-treatments of lower concentrations (1-10 mg kg⁻¹) had no significant effect, however, the higher As(III)-doses (30-90 mg kg⁻¹) have already inhibited the root development. By contrast, regarding the plants treated with As(V) control plant's roots developed in the slightest extent, the increasing concentration of As(V)-treatments increased the maize seedling's root grow more and more. In the case of the sunflower seedling's rhizobox experiment carried out compared to the controls the As(V)-treatments increased the seedlings' root grow according to the following order: 30 mg kg⁻¹ > 3 mg kg⁻¹ > 10 mg kg⁻¹ > 1 mg kg⁻¹ > 90 mg kg⁻¹ > control. In contrast, the maize plant's root growth treated with As(III) was increased only by a 3 mg kg⁻¹ dose, and 1 and 10 mg kg⁻¹ doses had no significant effect on the root growth compared to the control, however the 30-90 mg kg⁻¹ treatments already strongly inhibited the root development. This again draws attention to the different impact of As(III)- and As(V)-treatments on plants, as well as to a different sensitivity of mono- and dicotyledonous plants.

The results of the pot experiment pointed out that the development of green peas was strongly determined by the forms of treatment used in the experiment. By analyzing the dry weight results of the pot experiment I found that as a result of As(III)-treatments, some parts of the pea plant's dry mass were decreased, during the progress of the plant's development a significant reduction of vegetative parts above the soil surface and the generative organs occurred, however, only as a result of treatments of higher and higher concentration. Furthermore, during the complete maturity phenophase even the smallest (3 mg kg⁻¹) As(III)-treatment had a positive effect on the leaf's dry matter accumulation.

In contrast, taking into account the pea plant's dry matter results treated with As(V) I noticed that the lower concentration treatments increased the dry weight, which I could also prove in a statistical way in the case of the stems and the root in the case of the 3 mg kg⁻¹ treatment at the I. phenological phase and in the case of the 10 mg kg⁻¹ treatment the leaf dry weight content in the II to IV. phenophases, the stem's dry weight content in the III-IV. phenophases, and the pod's dry weight content in the IV. phenophase. However, the higher doses of As(V) inhibited the development of green peas in all phenophases, which I proved statistically several times.

3.2.2. Effect of the different As(III)- and As(V)-treatments on the plant's arsenic uptake

By analyzing the As uptake of test plans, I have found that in the case of experiments with nutrient solution (Tables 5-6) and rhizobox the As(III) and As(V)-treatments had an effect on maize and sunflower shoots and roots that their As content has both increased, which in many cases I could prove statistically.

Table 5.: The concentration of arsenic of shoot and root in maize seedlings after grown in nutrient solution treated with As(III) and As(V)(0, 1, 3, 10, 30 mg kg⁻¹) (n=3±s.e.)

As-treatments (mg kg ⁻¹)	As content of maize seedlings (mg kg ⁻¹)		
	Shoot	Root	
As(III)	0	0.620±0.039 ^a	1.57±0.11 ^a
	1	8.51±2.95 ^{ab}	27.2±4.4 ^a
	3	30.05±3.23 ^b	146±17 ^b
	10	125±16 ^c	389±29 ^c
	30	131±21 ^c	448±64 ^d
As(V)	0	0.620±0.039 ^a	1.57±0.11 ^a
	1	5.01±0.30 ^a	15.8±3.5 ^a
	3	25.1±4.1 ^b	93.5±17.5 ^{a*}
	10	53.3±6.9 ^{c*}	400±66 ^b
	30	93.5±10.6 ^d	716±132 ^{b**}

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Duncan multiple range test ($P \leq 0.05$). Means followed by * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) within columns were significantly different between the effect of As(III) and As(V) at the same level according to independent sample T-test.

Table 6.: The concentration of arsenic of shoot and root in sunflower seedlings after grown in nutrient solution treated with As(III) and As(V)(0, 1, 3, 10 mg kg⁻¹) (n=3±s.e.)

As-treatments (mg kg ⁻¹)	As content of sunflower seedlings (mg kg ⁻¹)	
	Shoot	Root
As(III)	0	<k.h. ^a
	1	77.4±3.8 ^b
	3	97.3±1.7 ^c
As(V)	0	<k.h. ^a
	1	81.4±1.9 ^{b***}
	3	86.4±0.7 ^{b*}
	10	132±7 ^c

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Duncan multiple range test (P≤0.05). Means followed by *(p<0.05), **(p<0.01), ***(p<0.001) within columns were significantly different between the effect of As(III) and As(V) at the same level according to independent sample T-test.

I found that in case of both the nutrient solution and the rhizobox experiment As(III)- and As(V)-treatments resulted in the case of sunflower in a more intense As accumulation than for the maize seedlings. Thus, it can be proven that the sunflower in nutrient solution reacted more sensitively to the As, because its As-uptake was much higher than compared to the maize. However, the measurement results also verify that the sunflower seedlings compared to the maize accumulates arsenic in its tissues in a higher proportion without any visible symptoms of As toxicity appearing, which again stresses differences between monocotyledonous and dicotyledonous plants As uptake and sensitivity.

During the evaluation of the results I considered it important to determine the amount of As a plant subtracts from the solution/soil, and how this As amount is distributed the plant, taking into account the plant's different parts' dry weight. I found that the As amount present in the maize and sunflower seedlings's shoots, roots, and in the whole seedlings had gradually increased by increasing concentration of As(III)- and As(V)-treatments increases. I only observed in the case of the sunflower plant which grown in nutrient solution that the 10 mg kg⁻¹ treatment has resulted in a slight decline of the extracted As content for the whole plant. The nutrient solution and rhizobox experiment also showed that in both forms of treatment of maize and sunflower seedlings, As was accumulated mainly in the roots and a lower amount translocated to the shoot which, according to my view is part of the detoxification mechanism of the plant.

By studying the results of the nutrient solution experiment I observed that the sunflower seedlings had a lower ratio of arsenic translocated to the shoot as in the case of maize. The

experiments carried out in rhizobox, apart from the 1 mg kg⁻¹ doses' experience, in the case of both As(III)- and As(V)-treatments I found its opposite (*Tables 7-8*).

Table 7.: As amount (μg) uptake by maize seedlings which were grown in rhizobox, treated with As(III) and As(V) (0, 1, 3, 10, 30, 90 mg kg⁻¹)($n=3\pm s.e.$)

As-treatments (mg kg ⁻¹)	As amount (μg) uptake by maize seedlings			
	Shoot	Root	Whole plant	
As(III)	0	0.00229 \pm 0.00012 ^a (18)	0.0105 \pm 0.0004 ^a (82)	0.0127
	1	0.00680 \pm 0.00010 ^{ab} (27)	0.0182 \pm 0.0001 ^a (73)	0.0250
	3	0.00785 \pm 0.00009 ^{ab} (4)	0.175 \pm 0.003 ^b (96)	0.183
	10	0.00976 \pm 0.00002 ^{ab} (4)	0.208 \pm 0.001 ^b (96)	0.218
	30	0.0132 \pm 0.0005 ^b (2)	0.639 \pm 0.002 ^b (98)	0.652
	90	0.0247 \pm 0.0003 ^b (1)	3.26 \pm 0.01 ^b (99)	3.28
As(V)	0	0.00229 \pm 0.00012 ^a (18)	0.0105 \pm 0.0004 ^a (82)	0.0127
	1	0.00970 \pm 0.00047 ^{ab} (30)	0.0224 \pm 0.0005 ^a (70)	0.0321
	3	0.0134 \pm 0.0003 ^{b*} (5)	0.243 \pm 0.004 ^b (95)	0.256
	10	0.0171 \pm 0.0001 ^{b*} (5)	0.357 \pm 0.013 ^b (95)	0.374
	30	0.0174 \pm 0.0010 ^b (1)	1.15 \pm 0.04 ^{c*} (99)	1.17
	90	0.0338 \pm 0.0021 ^b (1)	4.23 \pm 0.15 ^c (99)	4.26

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Duncan multiple range test ($P \leq 0.05$). Means followed by * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) within columns were significantly different between the effect of As(III) and As(V) at the same level according to independent sample T-test. The value put in brackets shows percentage of the As-amount of the specific plant part and of the whole plant.

Table 8.: As amount (μg) uptake by sunflower seedlings which were grown in rhizobox, treated with As(III) and As(V) (0, 1, 3, 10, 30, 90 mg kg⁻¹)($n=3\pm s.e.$)

As-treatments (mg kg ⁻¹)	As amount (μg) uptake by sunflower seedlings			
	Shoot	Root	Whole plant	
As(III)	0	0.00938 \pm 0.00006 ^a (22)	0.0329 \pm 0.0001 ^a (78)	0.0423
	1	0.0140 \pm 0.0004 ^b (22)	0.0500 \pm 0.0001 ^a (78)	0.0640
	3	0.0203 \pm 0.0002 ^b (20)	0.0794 \pm 0.0007 ^a (80)	0.0997
	10	0.0289 \pm 0.0005 ^b (8)	0.322 \pm 0.007 ^b (92)	0.351
	30	0.0888 \pm 0.0022 ^b (11)	0.693 \pm 0.004 ^b (89)	0.781
	90	0.424 \pm 0.023 ^c (11)	3.57 \pm 0.11 ^c (89)	3.99
As(V)	0	0.00938 \pm 0.00006 ^a (22)	0.0329 \pm 0.0001 ^a (78)	0.0423
	1	0.0173 \pm 0.0004 ^{b*} (28)	0.0437 \pm 0.0012 ^{a*} (72)	0.0610
	3	0.0243 \pm 0.0007 ^b (20)	0.0940 \pm 0.0027 ^{a*} (80)	0.118
	10	0.0698 \pm 0.0006 ^{b***} (20)	0.274 \pm 0.013 ^{b*} (80)	0.344
	30	0.800 \pm 0.034 ^{c***} (40)	1.17 \pm 0.08 ^{c***} (60)	1.97
	90	0.361 \pm 0.008 ^{d*} (7)	4.46 \pm 0.05 ^{d*} (93)	4.82

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Duncan multiple range test ($P \leq 0.05$). Means followed by * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) within columns were significantly different between the effect of As(III) and As(V) at the same level according to independent sample T-test. The value put in brackets shows percentage of the As-amount of the specific plant part and of the whole plant.

In the pot experiment I have examined the As content of the plant in several stages of plant growth (4 nodes, begin of blooming, leaf expansion, full maturation). According to the results it was obvious in all stages of plant development that the applied As(III)- and As(V)-treatments have increased As content of various plant parts, and in most cases the increase could

statistically be proven. Based on the results from the pot experiments my earlier finding can be affirmed that arsenic can hardly in the plant, it is mostly present in the roots and only a small part of it is being transferred to other plant organs. I found that by the progress of the plant development as well as with increase of As(III)-treatments' concentration the absorbed As increasingly remained in the stem, however, in the case of the IV. phenophase the stem's As-retention capacity slightly declined. In contrast, in the case of As(V)-treatments the soil As concentration and the development stage of the plant greatly influenced that the As would be accumulated a greater rate in the stem or in the leaves.

Furthermore, in general we can say that in all stages of plant development the amount of As retained in the root increased as increased the treatments concentration, however, with the appearance of the generative organs the rate of the As extracted from the soil was higher in the leaves than in the root. By analyzing the As content of stem and leaves it was found that in the case of the I. phenophase the leaves's As content exceeded the stem's, however, along the development progress of the plant the stem's As retention capacity increased to such an extent that by the full maturation phenophase the stem's As proportion in the case of higher concentration doses exceeded the leaf's As proportion. The As uptake of the generative parts was much lower than the vegetative organs and the seed generally also accumulated less arsenic compared to the pod (*Tables 9-10*).

Table 9.: As amount (μg) uptake by green pea plants which were in complete maturity, depending on As(III)-treatments (0, 3, 10, 30, 90, 270 mg kg^{-1})($n=3\pm s.e.$)

As(III)- treatments (mg kg^{-1})	As amount (μg) uptake by green pea plants at complete maturity					
	Seed	Pod	Leaf	Stem	Root	Whole plant
0	0.0365 \pm 0.0019 ^a (5)	0.0665 \pm 0.0013 ^a (10)	0.18 \pm 0.003 ^a (26)	0.153 \pm 0.003 ^a (22)	0.261 \pm 0.001 ^a (37)	0.697
3	0.0873 \pm 0.0026 ^a (3)	0.203 \pm 0.011 ^b (8)	1.02 \pm 0.02 ^b (39)	0.608 \pm 0.058 ^b (23)	0.702 \pm 0.002 ^a (27)	2.62
10	0.0923 \pm 0.0007 ^a (2)	0.192 \pm 0.003 ^b (3)	1.65 \pm 0.02 ^b (28)	0.912 \pm 0.011 ^b (16)	3.02 \pm 0.06 ^b (51)	5.87
30	0.0733 \pm 0.0008 ^a (1)	0.164 \pm 0.008 ^b (1)	1.74 \pm 0.01 ^b (12)	2.11 \pm 0.01 ^c (15)	10.1 \pm 0.2 ^c (71)	14.1
90	0.0805 \pm 0.0022 ^a (0.4)	0.123 \pm 0.001 ^b (0.4)	3.68 \pm 0.15 ^c (13)	4.57 \pm 0.25 ^d (16)	20.6 \pm 0.5 ^d (71)	29.1
270	0.1306 \pm 0.0032 ^b (0.3)	0.437 \pm 0.007 ^c (1)	1.71 \pm 0.04 ^b (5)	4.42 \pm 0.04 ^d (12)	29.4 \pm 0.12 ^d (81)	36.1

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Duncan multiple range test ($P \leq 0.05$). Means followed by * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) within columns were significantly different between the effect of As(III) and As(V) at the same level according to independent sample T-test. The value put in brackets shows percentage of the As-amount of the specific plant part and of the whole plant.

Table 10.: As amount (μg) uptake by green pea plants which were in complete maturity, depending on As(V)-treatments (0, 3, 10, 30, 90, 270 mg kg^{-1}) ($n=3\pm\text{s.e.}$)

As(V)- treatments (mg kg^{-1})	As amount (μg) uptake by green pea plants at complete maturity					
	Seed	Pod	Leaf	Stem	Root	Whole plant
0	0.036 \pm 0.002 ^a (5)	0.0664 \pm 0.0013 ^a (10)	0.18 \pm 0.003 ^a (26)	0.153 \pm 0.003 ^a (22)	0.261 \pm 0.001 ^a (37)	0.696
3	0.170 \pm 0.001 ^b (7)	0.162 \pm 0.002 ^b (6)	0.845 \pm 0.002 ^b (33)	0.786 \pm 0.011 ^a (30)	0.623 \pm 0.011 ^a (24)	2.59
10	0.182 \pm 0.008 ^b (3)	0.259 \pm 0.003 ^b (4)	2.64 \pm 0.01 ^c (44)	1.53 \pm 0.01 ^b (25)	1.41 \pm 0.01 ^b (23)	6.02
30	0.140 \pm 0.01 ^b (1)	0.198 \pm 0.001 ^b (2)	2.36 \pm 0.01 ^c (19)	1.98 \pm 0.01 ^b (16)	7.82 \pm 0.07 ^c (63)	12.5
90	0.18 \pm 0.01 ^b (0.5)	0.408 \pm 0.007 ^b (1)	2.9 \pm 0.01 ^c (7)	5.19 \pm 0.04 ^c (13)	30.42 \pm 0.09 ^d (78)	39.1
270	0.07 \pm 0.001 ^b (0.1)	0.79 \pm 0.017 ^c (1)	5.63 \pm 0.17 ^d (10)	10.8 \pm 0.2 ^d (18)	41.23 \pm 0.21 ^e (70)	58.5

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Duncan multiple range test ($P \leq 0.05$). Means followed by * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) within columns were significantly different between the effect of As(III) and As(V) at the same level according to independent sample T-test. The value put in brackets shows percentage of the As-amount of the specific plant part and of the whole plant.

I have noticed in most cases that the augmentation of the whole plant's As content decreased As content of generative organs. Furthermore, a significant human health finding is that in the case of treatment levels, where the stem's As-retention capacity amplified, out of the As amount taken up by the entire plant only less than 1% reached the seed.

3.2.3. Effect of different As(III) and As(V)-treatments on the nutrient uptake of plants

During my work, I also examined how increasing concentrations of As(III)- and As(V)-treatments affect plants' of macro- and micro elements uptake. I considered the test plants' **phosphorus** concentration specifically important, because the phosphates and arsenates are similar in their chemical behavior, because of that one part of the research has identified an antagonist connection between them. I could demonstrate the antagonist relation between arsenate and phosphate in the case of maize both under nutrient solution and rhizobox conditions, however, I have found in the case of sunflower and peas that as a result of As(V)-treatments an increase in the P concentration of plants occurred. In my opinion, this may be related to the fact that transporters playing a role in the P absorbing can be phosphate transporters of high and low affinity. The high-affinity phosphate transporters have a greater affinity towards phosphate and especially not towards arsenate, so in case of plants with such an uptake system, P-uptake does not decrease even by the growth medium' increased As content. I believe that while in the case of maize the low, thus in the case of the sunflower and pea, the high-affinity phosphate transporter's occurrence is dominant. I have observed similar changes in the P concentration of plants treated with As(III), which proves that mono and dicotyledonous plants differ from each other also in this regard.

Examining the test plants' **sulfur** content in the case of the sunflower or pea test plants I observed an increase in most of the cases, in the case of the maize seedlings As(III) and As(V)-

treatments have tended to inhibit the incorporation of S. In the case of a nutrient solution experiment the sunflower's both part, while in the case of a rhizobox experiment the shoot's S content in the case of both As(III)- and As(V)-treatments was increased. In the case of peas I observed an increase in the S concentration in the case of As(V)-doses for the seed and the root as an effect of the 270 and the 3 mg kg⁻¹ treatment, for the pod the 3 and 90-270 mg kg⁻¹ treatment, for the leaf the 10-270 mg kg⁻¹ treatment, and for the stem the 270 mg kg⁻¹ treatment. The 90 mg kg⁻¹ As(III)-treatment increased the seed's, the 3-10 and 90-270 mg kg⁻¹ treatments the pod's, the 90 mg kg⁻¹ treatment the leaf's, the 90-270 mg kg⁻¹ treatments the stem's and the 30-270 mg kg⁻¹ treatments the root's S concentration. Contrary to the before mentioned the S content of the maize seedling's root was decreased both in the nutrient solution and rhizobox experiment as an effect of increasing concentrations of As(III)- and As(V)-treatments, and also the S-concentration of the maize shoot raised in the rhizobox has fallen by the As(V)-dosage used. It can be concluded that in the case of plants which have a higher S need - as for the sunflower as an oil plant, S is essential for the biosynthesis of fatty acids, for the green pea, as protein crop, the S as the basic building element of proteins is essential for its quality and quantity - an increase in the content of sulfur occurred. However, in the case of the test plant (maize) with lower S claim the uptake of S decreased in several cases under both forms of treatment.

By studying the plants' **magnesium** content I found that in a decisive majority of cases the plant roots' Mg content decreased as a result of As(III)- and As(V)-treatments which in the case of maize and sunflower I could statistically prove for both the nutrient solution and the rhizobox experiment. However, in the above soil parts of plants an increased Mg concentration was present, a decrease was indicated in particular only by higher concentration. In the nutrient solution 3-10 mg kg⁻¹ As(V)-treatments and the 1-3 mg kg⁻¹ As(III)-treatments increased the maize shoot's Mg concentration, however, a 30 mg kg⁻¹ treatment has already inhibit the Mg incorporation in both types of treatments. In the case of a rhizobox experiment the sunflower seedling shoot's Mg content increased by the As(III)- and As(V)-treatments. I could detected in the case of pot experiments on several times that the Mg concentration increase in the case of As(V)-treatments, in all plant parts, in the case of As(III)-treatments in the pod, leaf, stem and root. However, the inhibitory effect of higher concentration also showed in the case of the highest (270 mg kg⁻¹) As(V)-dose in the leaf, and in the highest concentration (270 mg kg⁻¹) of As(III)-treatment in the pod.

The As(III) and As(V)-treatments also had an effect on the plant microelement uptake. By examining the micro element composition of plant samples I concluded that the As(III)- and

As(V)-treatments usually increased the test plants' **iron** content. In the case of nutrient solution experiment the Fe concentration of maize and sunflower root's and shoot's was increased under both As(III)- and As(V)-treatments. In a rhizobox experiment for a sunflower shoot all of As(V)-doses and the 1-30 mg kg⁻¹ As(III)-treatments caused the increase of the Fe concentration. Significant rise in the Fe concentration of the root was caused by a 10 mg kg⁻¹ As(III)-treatment. The Fe content of maize shoot grown in a rhizobox was significantly increased in several cases in both treatment forms, in the case of maize seedling's root the Fe uptake enhancing effect of arsenic I could prove only for As(V)-treatments. In the pot experiment I observed an increased Fe concentration in the following cases: in the case of plants treated with As(III) for the pea 30-90 mg kg⁻¹, for the pod, leaf and root the 3 mg kg⁻¹, for the stem the 90-270 mg kg⁻¹ doses resulted in significant increase of the Fe content. In the case of As(V)-treatments in the seed the 3-30 mg kg⁻¹, in the pod the 10-270 mg kg⁻¹, in the leaf and the stem the 270 mg kg⁻¹ dose caused significant growth. This may be explained in partly by that the arsenate forms with the Fe³⁺ ion a complex, and the Fe(III)-arsenate is then absorbed the same way as the phosphate, so that the increased uptake of arsenic also implies an increase of the Fe content. Another possible explanation is that plants' exposure to arsenic generate reactive oxygen species (ROS) during the As(V) → As(III) transformation, the binding of the free radicals is the task of the antioxidant enzyme system, and the Fe is an important component of this antioxidant enzyme system.

Between arsenic and **manganese** I could prove an antagonistic relationship particularly by testing on maize. In the case of nutrient solution experiment. as an effect of 10-30 mg kg⁻¹ As(III)- as well as each As(V)-treatments the maize shoot's Mn concentration has significantly decreased, in the case of the root a significant decrease in Mn content appeared in all treatment levels for both forms of arsenic. In the case of the rhizobox experiment, the antagonistic relationship of As and Mn in the maize root could be observed for both As(III)- and As(V)-doses, however, the shoot's Mn content only declined in the case of As(V)-treatments of higher concentration (30 to 90 mg kg⁻¹). However, contrary to as described above, in the case of the sunflower as a dicotyledonous plant I observed an increase in the Mn content as well as for the green pea (dicotyledon). Both the sunflower shoot's and root's Mn content increased in the nutrient solution experiment. Under rhizobox conditions an Mn concentration increase was observed in the shoot of the sunflower under both types of treatment. In the case of the pot experiment for the As(V)-dosage caused an increased Mn content for the generative and vegetative, in several cases. Under As(III)-stress it was obvious that the Mn content was

increased in the pod in the case of 3 mg kg^{-1} , in the leaf in the case of $10\text{-}90 \text{ mg kg}^{-1}$, in the stem and root in the case of $3\text{-}90 \text{ mg kg}^{-1}$ As(III)-treatment.

Further, I concluded that the applied As(III) or As(V)-dosage in many cases inhibited the test plants' **molybdenum** uptake. I found a decrease in the Mo concentration in the case of nutrient solution experiment for all treatment in the case of sunflower shoots, as well as in the root as an effect of 3 mg kg^{-1} As(III)- and As(V)-treatment. In the case of a rhizobox experiment the Mo content of maize shoot was decreased in the case of As(III) and As(V)-dosage. In the case of the sunflower, the concentration of the Mo was decreased in the plants' shoot which was treated with As(III). In the pot experiment it could also be observed that the As-treatments inhibited the pea plant's Mo uptake. I could prove a significant reduction in the case of $3\text{-}90 \text{ mg kg}^{-1}$ As(III)-treatment in the seed, in the case of As(V)-treatment in the plant's seed in the case of $3\text{-}30 \text{ mg kg}^{-1}$ doses, in the pods and leaf in the case of 90 mg kg^{-1} treatment. The recognition of Mo shortages caused by arsenic is of outstanding importance, since Mo is a main component of the nitrate reductase enzyme, so in its absence nitrate accumulation may occur since its reduction does not occur, which leading to plant physiology disorders, and the plant which collected from the arsenic contaminated area use as a food or a feed means more and more threat for the human and animals health.

According to the results the the plants **zinc** uptake usually was decreased in the case of As(III)- and As(V)-treatments, which proves the existence of an antagonistic relationship between the As and Zn. I observed a decrease in Zn content in the case of a nutrient solution experiment for both forms of treatment for the sunflower and the maizes shoot as well as for the As(III)-treated maize's root. In the case of a rhizobox experiment the maize seedlings root's Zn concentration decreased in both type of treatments, in the case of the sunflower I recorded a decrease in the Zn content for the 90 mg kg^{-1} As(III)-treatment in the case of the shoot. In the case of the pot experiment I observed a decline in the Zn concentration in the seed and the leaf in the case of $30\text{-}270 \text{ mg kg}^{-1}$ doses, in the case of the pod of 90 mg kg^{-1} doses, in the case of the stem of $10\text{-}90 \text{ mg kg}^{-1}$ doses, and in the case of the root of $3\text{-}90 \text{ mg kg}^{-1}$ As(V)-doses. I could prove the negative impact of As(III)-treatments on Zn content in the case of the leaf in the case of the $10\text{-}270 \text{ mg kg}^{-1}$ As(III)-treatments and for the root in the case of the $10\text{-}90 \text{ mg kg}^{-1}$ As(III)-treatments.

Several researchers have reported that the As content of the growing medium increases the plants' **copper** uptake which was obvious in several times in the case of the experiments. I observed an increase in the Cu concentration in the nutrient solution grown sunflower roots in the case of both As(III) and As(V)-doses. In the case of the rhizobox experiment the Cu content

was increased in the maize root and shoot. In the case of the pot experiment the concentration of Cu was significantly increased in several cases in the above ground parts of the plant which were treated with As(III). The amount of Cu was increased in the seed in the case of 3-10 and 270 mg kg⁻¹ As(V)-treatments, in the pod in the case of 3-30 and 270 mg kg⁻¹ As(V)-treatments in the leaf, stem and root in the case of 270 mg kg⁻¹ As(V)-treatment.

4. NEW SCIENTIFIC RESULTS

1. It was proved, that the the sunflower as a dicotyledon plant is more responsive to the arsenic toxicity than the maize (monocotyledon plant). In the case of the sunflower seedlings the 30 and 90 mg kg⁻¹ As(III)- and As(V)-treatments, and the 10 mg kg⁻¹ As(III)-treatment caused the death of the plant, nevertheless in the case of the maize from the 90 mg kg⁻¹ treatment was toxic enough to cause death of the plant.
2. Under hydroponic conditions it was proved, that less arsenic was translocated to the sunflower shoot, comparing with the maize seedling shoot.
3. Under hydroponic conditions, it was proved, that there is a considerable difference in the Mn accumulation capability of maize (monocotyledon plant) and that of the sunflower (dicotyledon plant) under As(III) and As(V) induced stress. The Mn content was decreased in the case of maize seedling, nevertheless the concentration of Mn was increased in the case of sunflower.
4. It was proved, that the plant available arsenic concentration was higher in the case of As(III)-treatments, than in the case of As(V)-treatments.
5. According to the data the As was accumulated mainly in the root system and just a lower amount is translocated to the shoot. It was proved, due to the increasing concentration of the As(III)- and As(V)-treatments the effectiveness of the filtering effect by the root was increased.
6. In pot experiment, it was proved that the distribution of arsenic in the plant is considerably determined by the plant development state and the concentration of the treatment, however the inorganic forms of the arsenic-treatment have less effect on it.

7. In the pot experiment, it was proved that in the case of the higher As(III)- and As(V)-treatments (90-270 mg kg⁻¹), the filtering effect of root and stem was increased, hence the As amount of the seeds was lower than 1% of the whole plant. When the plants were treated with 0-30 mg kg⁻¹ arsenic in the form of As(III)- or As(V)-treatments, the As amount of the seeds was approximately 5-7% of the whole plant.
8. It was proved that the As(III)- and As(V)-treatments had different effect on a monocotyledon (maize) and a dicotyledon (pea, sunflower) plant uptake of P. The As(III)- and As(V)-treatments increased the P content in the case of dicotyledon plant (pea, sunflower), however the concentration of P was decreased in the case of monocotyledon plant (maize) when the plants were treated with As(III) and As(V).

5. SCIENTIFIC RESULTS FOR PRACTICAL APPLICATION

1. In the case of the rhizobox experiment it was obvious that the Mo content was decreased in maize tissues resulted by the As(III)- and As(V)-treatments. As Mo is the component of the nitrate reductase enzyme, hence the lack of Mo causes intensive nitrate accumulation, which poses a new threat to the plant origin food and feed which sites contaminated with arsenic.
2. Major part of arsenic is accumulated in the vegetative parts of the green peas and only a small part of the arsenic content present in the generative organs which is greatly favourable for human nutrition.
3. In the pot experiment the dry masses of the green pea parts were decreased in the case of the As(III)-treatment, nevertheless the dry mass of peas was increased when the soil contaminated maximum 10 mg kg^{-1} arsenic in the form of As(V).



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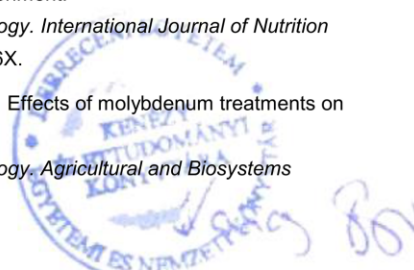
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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