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EFFECT OF DIFFERENT ARSENIC-TREATMENTS ON THE DRY WEIGHT OF VEGETATIVE PLANT PARTS OF GREEN PEA IN THE DIFFERENT PHENOPHASE OF THE PLANT DEVELOPMENT

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Abstract. *The objective of our study was to investigate the effect of arsenate and arsenite on dry weight of root, stem and leaves of green pea in the four different growth phases (four-node condition, the beginning of flowering, green ripening, and complete maturity) of the plant developement. As a results of our experiment shows, in some cases there were significant differences beetwen the effect of As(III)- and As(V)-treatmnets. Based on the data, in the case of the all phenophase, As(III)-treatments has a negatvive effect on the dry mass of vegetative plant parts. According to the results, in the case of the first phenopase the 3 mg kg^{-1} As(V)-treatments, in the case of the further phenophase the 3 and 10 mg kg^{-1} As(V)-treatment increased, but the higher concentration of As(V) decreased the dry mass of leaves and stem. Hovewer, the dry weigth of root was sightly increased in the first and second phenophase of the plant develompent as a result of the 3 mg kg^{-1} As(V)-treatmnet, the dry mass of root was negatively effected by the As(V)-treatments.*

Keywords: arsenite, arsenate, green pea, dry weight, pot experiment

1. Introduction

Arsenic (As) is a toxic element that is found naturally in soils all over the world [1]. Most environmental arsenic problems are the result in mining activities, use of arsenical herbicides and insecticides, irrigation with arsenic contaminated groundwater and some other agricultural and anthropogenic factors [2, 3, 4, 5]. The pollution of soil and groundwater with arsenic is a serious environmental problem all over the world [4, 6]. Arsenic is non-essential moreover toxic to

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plants [5, 7]. The phytotoxicity of arsenic depends on the form of the arsenic speciation and just only secondarily depends on the total concentration [8]. Inorganic arsenic forms are usually more toxic to plants, than organic compounds [9]. Arsenic in soil mostly found as inorganic form, namely arsenate [As(V)] and arsenite [As(III)]. Both are easily taken up by the cells of the plant root [10, 11]. Excessive uptake of arsenic by plants have led to physiological changes [12, 13]. Arsenic could inhibit the normal growth of the plants with toxicity symptoms such as morphological changes [14, 15] reduction of the plants productivity [16, 17] and loss of biomass [18].
The objective of this study was to investigate the physiological response of green peas to different As-treatments, evaluated by the parameters of dry mass of shoot, root, leaves, peas and pods in the four different phase of plant development.

2. Materials and methods

The pot experiment was carried out in calcareous chernozem soil was collected from the Látókép Experimental Station of the University of Debrecen. The parameters of the experimental soil were published previously by Kovács et al. (2015) [19]. 11 kg soil was weighed into each pot. The soil was air dried, sieved and additional NPK fertilization was applied (Table 1).

Table 1) Doses of NPK-fertilizer was applied in the greenhouse experiment

Doses of NPK-fertilizer			
N	**P**	**K**	
NH_4NO_3 (g pot^{-1})	KH_2PO_4 (g pot^{-1})	KH_2PO_4 (g pot^{-1})	K_2SO_4 (g pot^{-1})
0.568	0.229	0.079	0.148

Arsenic was applied in the form of arsenite ($NaAsO_2$) and arsenate (KH_2AsO_4), separately in seven different levels respectively as follows: 0 (control), 1, 3, 10, 30, 90 and 270 mg kg^{-1}. The NPK fertilizer and the arsenate as well as arsenite was supplemented to the soil as an aqueous solution.
Green pea (*Pisum sativum* L.) was chosen for our research to study the effect of different arsenic treatments on the dry weight of plant since it is one of the vegetables grown in the largest area in Hungary. Twenty-five seeds were planted in each pots and after the germination the number of the plants was slowed to sixteen. The study was conducted in randomized complete block design with three replications. Pots were weighed daily and the water losses were refilled by applications of de-ionized water. Plants samples (four plant in each phenophase) were collected in the four different phase of the plant development (four-node condition, the beginning of flowering, green ripening, and complete maturity). The roots of the plants were washed to eliminate the soil by distilled water. The

dry weights were recorded after drying the root, stem, leaves, peas and pods at 65°C until a constant weight was achieved.
All data obtained from experiment were subjected to one-way analysis of variance (ANOVA) and Duncan's test at 0.05 significance level to compare populations and arsenic treatments, used by SPSS statistics software version 22.0. 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 arsenate and arsenite at the same level.

3. Results

3.1. Effect of As(III)- and As(V)-treatments on the dry weight of green pea in the first phenophase of the plant development

The effect of As(III)- and As(V)-treatments on the dry weight of individual 0plant's part of green pea plants which were in four-node condition is demonstrate in the Table 3.

Table 2) Dry weight (g plant^{-1}) of individual plant's part of green pea plants which were in four-node condition grown in the calcareous chernozem soil of Látókép depending on arsenic-treatments (0, 3, 10, 30, 90, 270 mg kg^{-1})

As-treatments (mg kg^{-1})		Dry weight (g plant^{-1})		
		Leaves	**Stem**	**Root**
As(III)	**0**	0.162±0.042^{a}	0.123±0.039^{a}	0.0223±0.0055^{a}
	3	0.135±0.01^{b}	0.0817±0.0087^{b}	0.0210±0.0046^{a}
	10	0.123±0.01^{b}	0.0813±0.0069^{b}	0.0178±0.0058^{a}
	30	0.117±0.007^{b}	0.0785±0.0053^{b}	0.0176±0.0059^{a}
	90	0.114±0.013^{b}	0.0711±0.0083^{b}	0.0107±0.0007^{b}
	270	0.0198±0.0056^{c}	0.0207±0.0058^{c}	0.0101±0.0034^{b}
As(V)	**0**	0.162±0.042^{a}	0.123±0.039^{ab}	0.0223±0.0055^{a}
	3	0.175±0.026^{a*}	0.151±0.051^{b*}	0.0312±0.0069^{b*}
	10	0.160±0.038^{a*}	0.111±0.014^{ab}	0.0215±0.0048^{a}
	30	0.121±0.028^{b}	0.0837±0.0154^{a}	0.0181±0.0051^{a}
	90	0.109±0.024^{b}	0.0740±0.0119^{ac}	0.0164±0.0016^{a*}
	270	0.0215±0.0052^{c}	0.0287±0.0071^{c}	0.0157±0.0053^{a}

Means followed by the same letter within columns, separately in the case of As(III) and As(V), were not significantly different according to Ducan's 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.

This values show that increasing amount of As(III)-treatment resulted statistically lower dry weight of the leaves and stem of experimental plants. Based on the data,

in the case of the root the 90 and 270 mg kg^{-1} As(III)-treatments also significantly reduced the dry weight.
According to the data the 3 mg kg^{-1} As(V)-treatment statistically increased the dry weight of root. When the plants were treated with more than 10 mg kg^{-1} As(V), the dry weight of leaves were reduced, moreover the dry biomass of stem was also reduced when the plats were treated with 270 mg kg^{-1} As(V). Nevertheless, the 3 mg kg^{-1} As(V)-treatment significantly increased the dry mass of stem and root.

3.2. Effect of As(III)- and As(V)-treatments on the dry weight of green pea in the second phenophase of the plant development

As a result of our experiments shows, the treatments of green pea with As(III) had a negative effect on the dry mass of stem. The dry weight of root and leaves were also reduced when the plants were treated with at least 30 mg kg^{-1} As(III).
In addition it was observed that the dry mass of root, stem and leaves were decreased when the plants were treated with 90 and 270 mg kg^{-1} As(V). Nevertheless, the 10 mg kg^{-1} As(V)-treatment increased the dry mass of leaves (Table 3).

Table 3) Dry weight (g $plant^{-1}$) of individual plant's part of green pea plants which were in the beginning of flowering grown in the calcareous chernozem soil of Látókép depending on arsenic-treatments (0, 3, 10, 30, 90, 270 mg kg^{-1})

As-treatments (mg kg^{-1})		Dry weight (g $plant^{-1}$)		
		Leaves	**Stem**	**Root**
As(III)	**0**	0.192 ± 0.048^{a}	0.255 ± 0.085^{a}	0.0304 ± 0.0077^{a}
	3	0.165 ± 0.014^{ab}	0.170 ± 0.027^{b}	0.0287 ± 0.0095^{a}
	10	0.145 ± 0.046^{ab}	0.156 ± 0.037^{b}	0.0255 ± 0.0053^{ab}
	30	0.133 ± 0.041^{b}	0.141 ± 0.036^{b}	0.0200 ± 0.0059^{bc}
	90	0.128 ± 0.030^{b}	0.126 ± 0.037^{b}	0.0159 ± 0.0051^{c}
	270	0.0298 ± 0.0106^{c}	0.0238 ± 0.0046^{c}	0.0130 ± 0.0044^{c}
As(V)	**0**	0.192 ± 0.048^{a}	0.255 ± 0.085^{a}	0.0304 ± 0.0077^{a}
	3	0.218 ± 0.06^{a}	0.266 ± 0.07^{a}	0.0335 ± 0.0068^{a}
	10	$0.311\pm0.078^{b**}$	$0.345\pm0.081^{a**}$	0.0299 ± 0.0079^{ab}
	30	$0.208\pm0.056^{a*}$	0.276 ± 0.07^{a}	0.0267 ± 0.0077^{abc}
	90	0.112 ± 0.039^{c}	0.122 ± 0.025^{b}	0.0202 ± 0.0069^{bc}
	270	0.0294 ± 0.0098^{d}	0.0323 ± 0.0079^{c}	0.0189 ± 0.0057^{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 Ducan's 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.

3.3. Effect of As(III)- and As(V)-treatments on the dry weight of green pea in the third phenophase of the plant development

In the case of the third phase of the plant development, dry weight of each plant part were decreased when the plant were exposed to 90 and 270 mg kg^{-1} As(V)-treatments. Nevertheless, the 30 mg kg^{-1} As(V)-treatments significantly increased the dry weight of leaves and stem.
In the case if the leaves similar tendency was observed when the plants were treated with As(III). The 90 and 270 mg kg^{-1} As(III)-treatment significantly decreased the dry weight of leaves. The dry mass of root was not vary significantly under the As(III)-stress, however the biomass of stem was negatively affected by all As(III)-treatments (Table 4.).

Table 4) Dry weight (g $plant^{-1}$) of individual plant's part of green pea plants which were in green ripening grown in the calcareous chernozem soil of Látókép depending on arsenic-treatments (0, 3, 10, 30, 90, 270 mg kg^{-1})

As-treatments (mg kg^{-1})		Dry weight (g $plant^{-1}$)		
		Leaves	Stem	Root
As(III)	0	0.226±0.072[a]	0.299±0.087[a]	0.0463±0.0127[ab]
	3	0.176±0.038[ab]	0.194±0.066[b]	0.0460±0.0149[b]
	10	0.174±0.049[ab]	0.192±0.061[b]	0.0394±0.0087[ab]
	30	0.173±0.059[ab]	0.185±0.041[b]	0.0341±0.0033[ab]
	90	0.159±0.046[b]	0.176±0.057[b]	0.0323±0.0104[ab]
	270	0.0379±0.0126[c]	0.0270±0.0074[c]	0.0293±0.0061[b]
As(V)	0	0.226±0.072[a]	0.299±0.087[a]	0.0463±0.0127[a]
	3	0.240±0.078[a]	0.306±0.068[a*]	0.0455±0.0035[a]
	10	0.356±0.049[b**]	0.391±0.113[b**]	0.0452±0.0067[a]
	30	0.248±0.055[a*]	0.299±0.046[a***]	0.0415±0.0105[ab]
	90	0.116±0.028[c]	0.133±0.028[c]	0.0300±0.0071[bc]
	270	0.0340±0.0106[c]	0.0399±0.0124[d*]	0.0238±0.0072[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 Ducan's 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.

3.4. Effect of As(III)- and As(V)-treatments on the dry weight of green pea in the fourth phenophase of the plant development

The effect of As(III)- and As(V)-treatments was also evident on the vegetative plant's part of green pea. The As(V)-treatments caused a similar tendency in the dry weight of leaves, stem and root as previously mentioned in the case of the third phenophase of the plant development. The 90 and 270 mg kg^{-1} As(V)-

treatments decreased the dry mass of the vegetative plant parts, however the 10 mg kg^{-1} As(V)-treatment increased the dry weight of leaves and stem. In the case of the leaves the dry mass was also positively affected by the 3 mg kg^{-1} As(III)-treatment. As in the case of the third phenophase of the plant development, the highest As(III)-treatment (270 mg kg^{-1}) also significantly decreased the dry weight of the vegetative parts of the green pea, moreover the 90 mg kg^{-1} As(III)-treatment also decreased the dry mass of the root compared to the control as demonstrated in the Table 5.

Table 5) Dry weight (g plant^{-1}) of vegetative plant's part of green pea plants which were in complete maturity grown in the calcareous chernozem soil of Látókép depending on arsenic-treatments (0, 3, 10, 30, 90, 270 mg kg^{-1})

As-treatments (mg kg^{-1})		**Dry weight (g plant^{-1})**		
		Leaves	**Stem**	**Root**
As(III)	**0**	0.24±0.061^{ab}	0.305±0.064^{ab}	0.0607±0.0119^{a}
	3	0.368±0.072^{c}	0.374±0.129^{b}	0.0565±0.0194^{ab}
	10	0.301±0.039^{bc}	0.322±0.036^{ab}	0.0527±0.0110^{ab}
	30	0.238±0.074^{ab}	0.263±0.032^{ab}	0.0450±0.0150^{ab}
	90	0.197±0.059^{a}	0.209±0.028^{a}	0.0430±0.0120^{b}
	270	0.0554±0.0182^{d}	0.065±0.0107^{c}	0.0249±0.0081^{c}
As(V)	**0**	0.24±0.061^{a}	0.305±0.064^{a}	0.0607±0.0119^{a}
	3	0.246±0.056^{a*}	0.316±0.085^{a}	0.0505±0.0097^{ab}
	10	0.379±0.052^{b*}	0.465±0.108^{b*}	0.0471±0.0092^{bc}
	30	0.262±0.037^{a}	0.307±0.048^{a}	0.0463±0.0119^{bc}
	90	0.127±0.036^{c}	0.153±0.043^{c}	0.0342±0.0074^{c}
	270	0.0435±0.0119^{d}	0.0525±0.0154^{d}	0.0341±0.0089^{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 Ducan's 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.

Conclusion

This study provides important information concerning the relationship between the levels of As in the soil and its impact on dry weight of the of individual plant's part of green pea plants.

According to the results, in the case of the all phenophase As(III)-treatments has a negative impact on the dry weight of the vegetative plant's parts, however is statistically not supportable in all cases. Based on the data in the case of the first phenophase the lower concentration of As(V)-treatment (3 mg kg^{-1}) increased, but the higher As(V)-treatments (10, 30, 90 and 270 mg kg^{-1}) inhibited the dry mass accumulation, however is statistically also not supportable in all cases. In the case of the second, third and fourth phenophase of the plant development, increasing tendency was observed in the dry weight of leaves and stem until the 10 mg kg^{-1} As(V)-treatment, nevertheless decreasing tendency was observed in the case of the higher As(V)-treatments (30, 90 and 270 mg kg^{-1}). However, in the case of the first phenophase as a result of the 3 mg kg^{-1} As(V)-treatment the dry weight of root was slightly increased and in the second phase also was slightly increased. Nevertheless, the dry mass of root was negatively affected by the As(V)-treatments in case of the other treatments and other phenophase of the green pea development. As a results of our experiment shows in some cases there were significant differences between the effect of As(III)- and As(V)-treatments. Some authors reported that the arsenic is toxic, for plant, however at very low concentration As(V) may be beneficial for plants [11, 18]. Our study confirms that the low concentration of As(V) has a positive effect on the dry biomass of plants. The reason of that is the next: As(V) chemically similar to the phosphate, which element is necessary to the plant growth. As(V) is able to replace the phosphate on the surface of the minerals in the soil, which mechanism increased the plant-available amount of phosphate.

R E F E R E N C E S

[1] P. L. Smedley and D. G. Kinniburgh, *A review of the source, behavior and distribution of arsenic in natural waters*, Appl. Geochem. **17**, 517 (2002).

[2] P. N. Williams et al., *Variation in arsenic speciation and concentration in paddy rice related to dietary exposure*, Environ. Sci. Technol. **41**, 6854 (2007).

[3] Y. G. Zhu et al., *Exposure to inorganic arsenic from rice: a global health issue?*, Environ. Pollut. **154**, 169 (2008).

[4] N. Stoeva et al., *Physiological response of maize to arsenic contamination*, Biol. Plant. **47**, 449 (2004).

[5] F. J. Zhao et al., *Arsenic uptake and metabolism in plants*, New Phytol. **181**, 777 (2009).

[6] J. M. Shaofen Wang and C. Wai, *Arsenic in Drinking Water - A Global Environmental Problem*, J. Chem. Educ. **81**, 207 (2004).

[7] D. J. Kim et al., *Arsenate tolerance mechanism of Oenothera odorata from a mine population involves the induction of phytochelatins in roots*, Chemosphere. **75**, 505 (2009).

[8] A. A. Meharg and J. Hartley-Whitaker, *Arsenic uptake and metabolism in arsenic resistant* and nonresistant plant species, New Phytol. 154, 29 (2002).

[9] C. I. Ullrich-Eberius et al., *Evaluation of arsenate - and vanadate-associated changes of electrical membrane potential and phosphate transport in Lemna gibba G1*, J. Exp. Bot. **40**, 119 (1989).

[10] P. M. Finnegan and W. Chen, *Arsenic Toxicity: The effects on plant metabolism*. Front Physiol. 3, 182 (2012).

[11] J. Y. Xu et al., *Arsenic in garden soils and vegetable crops in Cornwall*, *England: implications for human health*, Environ Pollut. 194, 105 (2014).

[12] B. R. Wells and J. T. Gilmor, *Sterility in rice cultivars as influenced by MSMA rate and water management*, Agron. J. 69, 451 (1977).

[13] S. E. Smith et al., *Arsenic uptake and toxicity in plants: integrating mycorrhizal influences*, Plant Soil. 327, 1 (2010).

[14] N. S. Mokgalaka-Matlala et al., *Toxicity of Arsenic (III) and (V) on plant growth, element uptake, and total amylolytic activity of Mesquite (Prosopis juliflora x P. velutina)*, Int. J. Phytoremediat. 10, 47 (2008).

[15] C. Tu and L. Q. Ma, *Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator ladder brake*, J. Environ. Qual. 31, 641 (2002).

[16] M. R. Shaibur et al., *Critical toxicity of arsenic and elemental composition of arsenic-induced chlorosis in hydroponic Sorghum*,Water Air Soil Pollut. 191, 279 (2008).

[17] S. Srivastava et al., *Comparative biochemical and transcriptional profiling of two contrasting varieties of Brassica juncea L. in response to arsenic exposure reveals mechanisms of stress perception and tolerance*, J. Exp. Bot. 60, 3419 (2009).

[18] A. A. Carbonell-Barrachina et al., *The influence of arsenite concentration on arsenic accumulation in tomato and bean plants*, Sci. Hortic. 71, 167 (1997).

[19] B. Kovács et al., *Effect of molybdenum treatment on molybdenum concentration and nitrate reduction in maize seedlings*, Plant Physiol. Biochem. **96**, 38 (2015).