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Influence Of Sodium Arsenate (V) On The Content Of Nitrogen-Containing Compounds In Soil

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Abstract- Arsenic is one of the most toxic elements, which natural content exceedance leads to serious changes in soil characteristics. It is known that the element affects the enzymatic activity of soil, the composition and amount of soil microorganisms and other biological properties. Influencing the soil microorganisms and their ability to mineralization of organic matter, arsenic can also cause a change in the content of nitrogen-containing compounds. The incubation experiment allowed us to determine the effect of arsenic, applied in the form of various doses of sodium arsenate (V) to the soil (0, 5, 50, 200, 1200 mg/kg) on the amount and activity of microorganisms, and the content of compounds such as free amino acids, the ammonium and nitrate nitrogen. As the content of these compounds depends largely on the activity of microorganisms, we also considered in our study such indicators as the amount of microorganisms and substrate-induced soil respiration. The content of free amino acids and nitrate nitrogen correlated significantly with indicators of the amount of microorganisms. The investigation results also showed that the vital activity of arsenic-tolerant microorganisms is probably one of the main factors influencing the content of free amino acids and nitrates in the arsenic-contaminated soil.
Keywords: arsenic, free amino acids, ammonia, nitrates, incubation experiment.

1. INTRODUCTION

A growing anthropogenic load increases the risk of soil contamination by toxic elements such as cadmium, lead, arsenic, mercury, chromium, etc. Passing through the food chain, these elements do serious harm to the health of humans and animals, causing serious diseases of the skin and internal organs [1]. In soils, first of all, they affect the vital activity of microorganisms and biochemical processes they carry out. The impact of one of the most toxic elements - arsenic - on soil characteristics has been extensively studied in many countries of the world [2, 3]. Depending on soil formation conditions, the content of the element in the soil ranges from 1 to 40 mg/kg [4]. As a result of anthropogenic load, its content can increase multiple times [5, 6], causing changes in the biochemical processes and influencing the chemical composition of the soil. Moreover, in some countries (China, Russia), there are regions with naturally high content of this element [7, 8].

The effect of arsenic on the biological properties of the soil (enzymatic activity of soils, the composition and amount of soil microorganisms) have been actively studied [2, 9], while insufficient attention has been paid to the influence of pollutant on the content of nitrogen compounds such as ammonia, nitrates and free amino acids important for plant and microbial nutrition. Therefore, the objective of this study was to investigate the effect of different doses of arsenic, applied to the soil in the form of sodium arsenate (V), on the content of ammonia, nitrates, and free amino acids. As the content of these compounds depends largely on the activity of microorganisms, we also considered in our study such indicators as the amount of microorganisms and substrate-induced respiration (SIR) of soil.

2. MATERIALS AND METHODS

Object of the study was the upper horizon (A) HaplicLuvisol sampled in the Tetyushsky district of the Republic of Tatarstan (55°68´ N, 49°13´ E). The samples have the following physical and chemical properties: organic carbon content - 4.8%; gross nitrogen content - 0.42%; pH - 6.0; the sum of fractions of <0.01 mm - 34%; the total content of iron - 2.8%, manganese - 0.6%. The main reason for choosing this soil is low content of gross arsenic - 3.4 mg/kg. To our view, most microorganisms of this soil is likely to be sensitive to the increasing concentration of the pollutant.This soil will be react more to the application of arsenic and better characterize the processes occurring after the soil contamination with this element.

The effect of different doses of As (V) on the studied parameters were determined in the incubation experiment. The air-dried soil samples were saturated with aqueous solutions of $Na_3AsO_4{*}12H_2O$ of different concentrations, so that the arsenic concentration in the samples were 0, 5, 50, 200, and 1200 mg/kg. Then, the samples were adjusted with bidistilled sterile water to 60-70% of maximum water-holding capacity and incubated for 10 days at constant humidity and temperature of 28°C [9].

After the incubation period, the investigated parameters were determined on the samples. To remove dissolved organic matter, 2 g soil samples were taken, poured with 20 ml of bidistilled water, and extracted in a rotator for 30 minutes. Then, the slurries were filtered through a folded paper filter. For amino acid analysis, 400 µl of the filtrate was further filtered through a membrane filter with a pore diameter of 0.2 µm and evaporated on a steam bath under vacuum at 60-70°C. Solid residue was modified with phenylisothiocyanate to produce amino acid derivatives [10], which were further chromatographed on HPLC Flexar (Perkin Elmer, USA) with the reverse phase column Brownlee Analytical C18 and a UV detector at a wavelength of 254 nm. Both quantitative and qualitative content was determined with the use of standard samples of individual amino acids (Sigma Aldrich, Belgium). The concentrations were calculated on peaks areas.

The amount of ammonifyingmicro-organisms and arsenic-tolerant ammonifiers was counted by the method of seeding the soil suspension on selective media: the meat-peptone agar (MPA), and the meat-peptone agar with 0.01M As (V) [11].Substrate-induced respiration (SIR) in soil samples was determined after enriching the soil with additional carbon and energy source - glucose. The content of soil-absorbed ammonium nitrogen was determined with the use of Nessler reagent, and the nitrates were determined by ionometric method [12].

Statistical processing of the results was performed with Statistica program. The data are presented in the text and diagrams as an average ± standard deviation (n = 3). Correlation analysis was performed according to Spearman. The significance of differences between the variants was determined by *ANOVA* test.

3. RESULTS AND DISCUSSION

Irrespective of the applied dosage of As(V), all samples had 15 free amino acids detected (Figure 1), including: non-polar - valine, isoleucine, leucine, phenylalanine, alanine; uncharged polar - asparagine, glycine, tyrosine, threonine, serine; positively charged polar - arginine, histidine, lysine; and negatively charged polar - aspartic acid, glutamic acid.

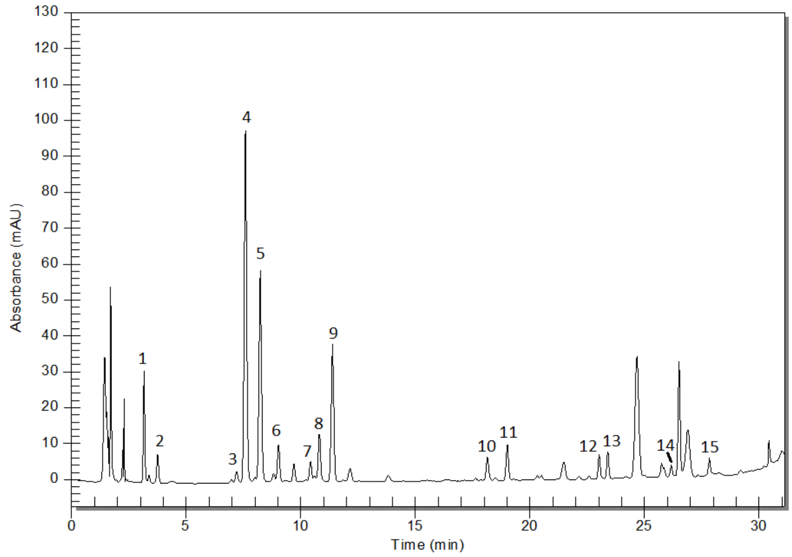

Fig. (1). A typical free amino acids chromatogram obtained after the analysis of the samples. Peaks: (1) aspartic acid, (2) glutamic acid, (3) asparagine, (4) serine, (5) glutamine, (6) histidine, (7) arginine, (8) threonine, (9) alanine, (10) tyrosine (11) valine, (12) isoleucine, (13) leucine, (14) phenylalanine, and (15) tryptophan.

A dispersive analysis showed that the total content of amino acids varies significantly under the influence of arsenic (Fig. 2a). The total amount of free amino acids (FAA) in the control sample free of this element is 68.2±4.8 mg/kg. At 5 mg/kg of As(V), the FFA value increases up to 131.8±6.2 mg/kg ($P<0.01$). The maximum concentration of amino acids is 248.5±40.6 mg/kg at 200 mg/kg of As(V), and at 1200 mg/kg of As (V) the content of amino acids is significantly decreases as compared with the maximum amount and makes 161.5±10.6 mg/kg. The obtained data indicate that the arsenic can increase the content of free amino acids in the soil.

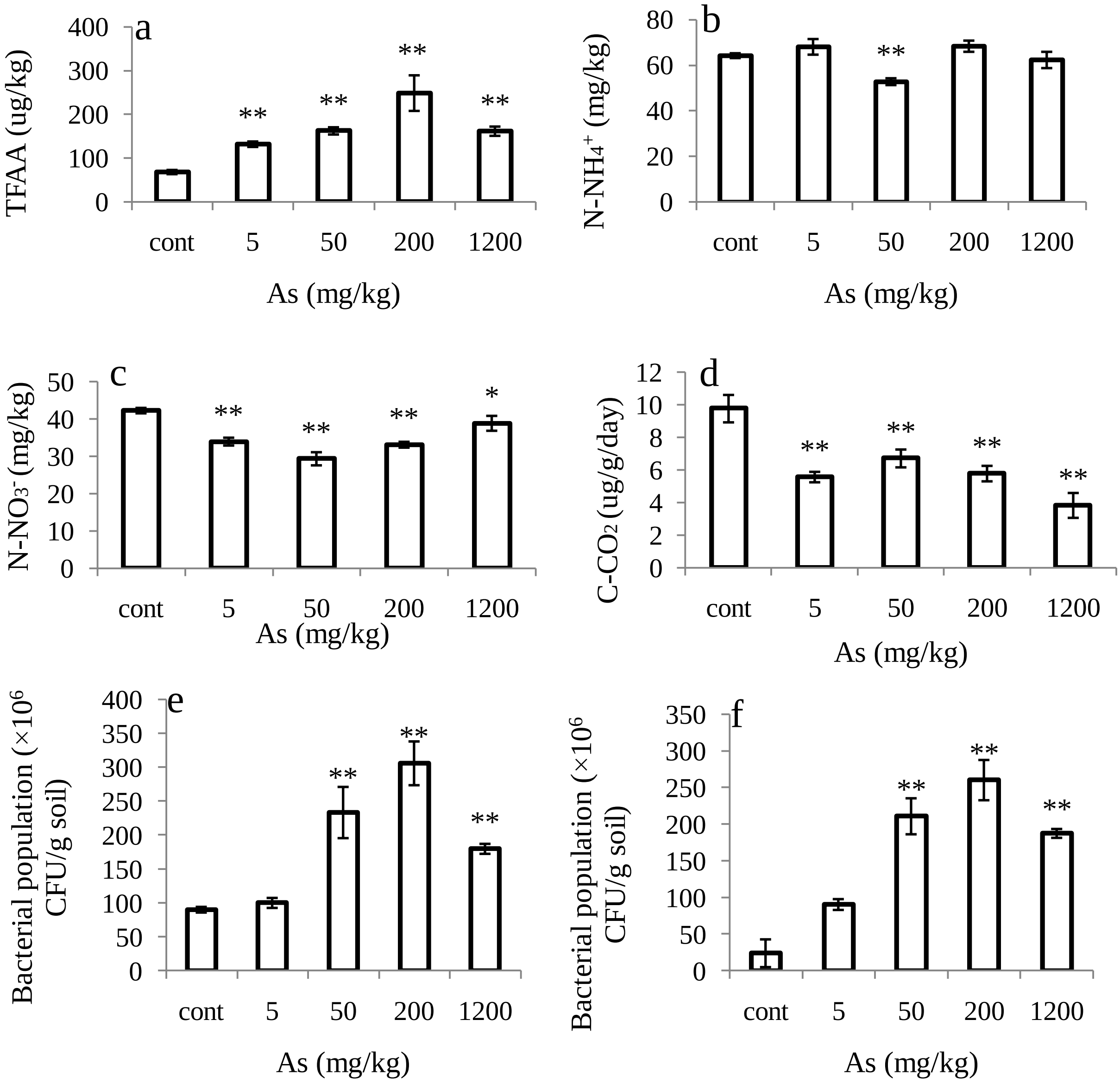

Fig. (2). Effect of different doses of As(V) on (a) the total content of amino acids (TFAA); (b) the content of ammonia nitrogen; (c) the content of nitrate nitrogen; (d) the substrate-induced soil respiration; (e) the number of microorganisms in the MPA medium; (f) the number of As-tolerant microorganisms in the MPA medium with 0.01 As (V). Note: * and ** are statistically significant differences as compared to the control (cont) at $P<0.05$ and $P<0.01$.

Regardless of the dose of As(V) in the samples, a profile of free amino acids is mainly represented by glycine and serine. Their content ranged 26.0 to 31% and 20.8 to 24.3% of TFAA, respectively. The lowest content was for arginine (1.5-2.6), phenylalanine (1.1-2.5%), and asparagine (0.5-1.1%). Due to the high content of glycine and serine, which total amount is about half of the TFAA, the nonpolar amino acids dominate in soils. The content of non-polar acids is slightly lower, and the lowest content was found for the basic and acidic amino acids. The percentage of amino acid groups depending on the content of As(V) has not substantially changed.

The test results showed that the content of ammonia nitrogen in the control sample was 61.1±2.3 mg/kg (Fig. 2b). Only the samples with arsenic dose of 50 mg/kg show a slight decrease in its content up to 52.7±1.5 ($P<0.01$). The content of nitrate nitrogen in the control was 42.3±0.7 mg/kg (Fig. 2c), which decreased in the samples with arsenic doses of 5, 50, 200 ($P<0.01$), and 1200 mg/kg ($P<0.05$). Its lowest content of 29.4±1.8 was detected at 50 mg/kg of As (V). The study showed low content of free amino acids in the soil as compared with inorganic forms of nitrogen ($N\text{-}NO_3^-$ and $N\text{-}NH_4^+$). The proportion of free amino nitrogen in the soil nitrogen pool was only 0.00024%, while the proportion of nitrate and ammonia nitrogen was 1.0 and 1.4%, respectively. The obtained data suggest that the mineralization of amino acids in this soil is much faster than the protein decomposition to individual amino acids [13].

The SIR value for control sample was 9.7±0.8 µg C/g per hour (Fig. 2d). In the samples contaminated with various doses of arsenic the SIR value decreased by 30.7-60.5% of control value ($P<0.01$).The lowest SIR value (3.8±0.8µg C/g per hour)was detected for samples with 1200 mg/kg of As(V).

The experiment has shown that the application of As(V) affects the amount of microorganisms. The amount of ammonifiers in the MPA medium varies in the range of 90.3±4.1 to 306.0±32.2 million CFU/g soil (Fig. 2e). Growth of count as compared with the control was detected at As(V) doses of 50, 200 and 1200 µg/kg ($P<0.01$). Thus, despite the element toxicity, there is an increase in the number of microorganisms, in general, in the arsenate-contaminated soil. We suggested that these changes could occur as a result of replacement of pollutant-sensitive species of microorganisms with the pollutant-tolerant ones. To identify the count of As(V)-tolerant microorganisms, seeding was carried out in the MPA medium with 0.01M As(V) [11]. The count of tolerant species in the control sample was 24.2±18.9 mil CFU/g of soil (Fig. 2e) and increased significantly ($P<0.01$) at As (V) doses of 5, 50, 200 and 1200 µg/kg.

Correlation coefficient for the count of microorganisms grown on MPA and 0.01M As(V) MPA media was 0.92 (Table. 1). As can be seen from Fig. 2e and Fig. 2f, the control sample contains lower count of tolerant cells counted on 0.01M As (V) MPA medium, than on the MPA medium, with their ratio of 0.24. However, this ratio is close to 1 for all variants of element-contaminated soils, i.e., the count of arsenate-tolerant cells and the count of cells grown in the arsenic-free MPA medium are similar. Therefore, we may assume that the community of microorganisms starts changing already with the As(V) dose of 5 mg/kg, and arsenic-tolerant species develop in the soil. Turpeinen et al. [11] came to the same result and showed that the soil contamination with arsenic leads to changes in the structure of microbial community and to the selective development of resistant species. As(V) dose of 1200 µg/kgacts, probably, as inhibitor even to the tolerant microorganisms, due to which their count at the given concentration of the pollutant decreases, as compared with the maximum at 200 µg/kg of As(V).

It is believed that the indicators of microbiological activity, in particular SIR, allow characterizing the mineralization of soil organic matter and serve as indicators of soil contamination [14, 15]. Indeed, the greater the soil is contaminated with As(V), the lesser the soil SIR, and, probably, the mineralization ability of soil microorganisms are. However, this indicator has no significant association with a content of either free amino acids, or nitrate and ammonium nitrogen (Table 1). Probably, this indicator hasno informative meaning in assessing the changes in nitrogen-containing compounds in the soil in the case of arsenic contamination.

The content of free amino acids correlates significantly with the count of microorganisms grown in the MPA medium both with 0.01M As (V) and without arsenic (r= 0.89 and 0.91, respectively), and also has a negative correlation with $N\text{-}NO_3$ (r=-0.56). The content of $N\text{-}NO_3$ is also in negative correlation with the count of microorganisms (r=-0.68 and -0.66). The content of NH_4-N, as well as the values of the substrate-induced respiration, has no correlation with any of the indicators.

Table 1.Correlations between the studied parameters

	CCA	$N\text{-}NH_4$	$N\text{-}NO_3$	$C\text{-}CO_2$	MPA	MPA As
CCA	1.00					
$N\text{-}NH_4$	0.16	1.00				
$N\text{-}NO_3$	-0.56**	0.18	1.00			
$C\text{-}CO_2$	-0.50	-0.21	0.08	1.00		
MPA	0.89**	-0.06	-0.66**	-0.33	1.00	
MPA As	0.91**	0.04	-0.68**	-0.36	0.92**	1.00

Note: TFAA - the total content of amino acids; $N\text{-}NH_4$ –the content of ammonia nitrogen; $N\text{-}NO_3$ – the content of nitrate nitrogen; $C\text{-}CO_2$ – the substrate-induced soil respiration; MPA - the number of microorganisms in the MPA medium; MPA As - the number of As-tolerant microorganisms in the MPA medium with 0.01 As (V). ** –$P<0.01$.

4. SUMMARY

We have established that the arsenic applied as sodium arsenate (V) in the soil in a dosage range of 5-

1200 mg/kg may affect the content of free amino acids and nitric oxide. The percentage of amino acid groups depending on the dose of As(V) has not substantially changed. The content of ammonia nitrogen reduced significantly only at the contaminant dose of 50 mg/kg.

5. CONCLUSION

The results of the study showed that the content of free amino acids might significantly increase upon soil contamination with arsenate in the As (V) dose range of 5-1200 mg/kg. The application of arsenic in the soil has also affected such indicators as the substrate-induced respiration, $N\text{-}NO_3^-$ content, the count of microorganisms in both the arsenic-free MPA medium and 0.01M As (V) MPA medium. It is shown that As-tolerant microorganisms start developing in the soil at a dose of 5 mg/kg of As(V). Among the studied parameters, the count of tolerant microorganisms in the MPA medium with 0.01M As(V) has the strongest correlation with the content of $N\text{-}NO_3$ (-0.68, $P<0.01$) and free amino acids (-0.68, $P<0.91$). The obtained data show that the vital activity of microorganisms is probably one of the main factors influencing the content of free amino acids and nitrates in the arsenic-contaminated soil.

6. ACKNOWLEDGEMENTS

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