

## Change in Toxicity of Model Arsenic Contaminant in the Presence of Humates and Activated Zeolites

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Received: September 21 2014

Accepted: September 25, 2014

Online Published: December 29, 2014

doi:10.5539/mas.v9n1p223

URL: <http://dx.doi.org/10.5539/mas.v9n1p223>

### Abstract

Using the methods of chemical analysis and bioassays, we investigated the toxicity and detoxication of model solutions containing arsenic salts. The ability of zeolites to absorb arsenic from model solutions was examined. Commercially available humic substances (“Powhumus”, “Humate-80”), as well as zeolites, activated with their application were shown to reduce toxic effect of pollutants. Zeolite activated by degassing, decationization, thermal and chemical treatment showed the highest sorbent efficiency.

**Keywords:** detoxication, arsenic, arsenic pollution, humic substances, zeolite, activation of zeolites, bioassay

### 1. Introduction

To date, the negative effects of arsenic derivatives on the environment are fairly well known (Berg *et al.*, 2007). On the other hand, there is evidence that in many cases, after binding toxicants to humic substances (HS), their aggressive potential is significantly reduced (Stom *et al.*, 1992; Lin *et al.*, 2004; Buschmann *et al.*, 2006; Perminova *et al.*, 2006; Janoš *et al.*, 2010).

One of the most perspective methods of solid and liquid media detoxification, in particular for the elimination of arsenic contamination, is the use of various sorbents (Saada *et al.*, 2003; Jeon *et al.*, 2009; Jing *et al.*, 2012). Natural zeolites deserve special interest as absorbers of various pollutants. They are distinguished by sufficiently cheapness and availability (Mohan and Pittman, 2007; Yadanaparathi *et al.*, 2009).

Zeolites are microporous framework aluminosilicates. Voids in the frame occupied by various cations and water molecules. The volume of pores in the zeolite is up to 50% of the frame volume. They are capable of selectively binding cations of heavy metals, arsenic and several other substances due to the cation exchange properties (Wu and Zhou, 2009; Camacho *et al.*, 2011).

In this context, the aim was to study methods of activation of natural zeolites (in particular HS) and neutralization of arsenic contamination in model experiments by modified sorbents.

### 2. Method

Solutions Na<sub>3</sub>AsO<sub>4</sub>, reagent grade, were used as a model of arsenic contamination. Natural zeolite from deposit “Sokirnikskoe”, Transcarpathian Zeolite Plant (LLC “Etnakom”) was used as the adsorbent. Crystallochemical formula of used zeolite is:



Sorption capacity of the zeolite was evaluated by iodine (Kan *et al.*, 2013).

Sources of HS were commercial preparations of “Humate-80” and “Powhumus”. The first is a mixed humate

K/Na, manufactured by LLC “Agricultural Technologies”, Irkutsk. It is obtained by mechanochemical treatment of lignine and the mixture of  $K_2CO_3/Na_2CO_3$ . “Powhumus” is potassium humate (Humintech Ltd., Germany), produced by the standard wet alkaline extraction of oxidized coal (leonardite) (Perminova *et al.*, 2006).

Zeolite was activated according to (Kan *et al.*, 2013). For this purpose, 100 g of zeolite was washed with hot water (60-70°C) to remove the clay inclusions. The washed zeolite was calcined at 400-450°C and placed in a solution of nitric acid (60 g/dm<sup>3</sup>) for 24 hours for decationization. After decationization the zeolite washed with distilled water and calcined. Zeolite thus prepared was saturated with the respective solution (5 g of  $(NH_2)_2CO$ , 5 g of  $NH_4NO_3$ , 40 cm<sup>3</sup> of distilled water, 2.5 cm<sup>3</sup> of  $MnSO_4$ , 7,5 cm<sup>3</sup> HS “Powhumus”). Alternatively, zeolite pre-prepared and saturated with a solution “Powhumus” at 1 g/dm<sup>3</sup> was used (in some experiments, instead of “Powhumus” used “Humate-80” at the same concentrations). 100 g of zeolite was impregnated by 50 cm<sup>3</sup> of HS.

As control we evaluated the effectiveness of binding of arsenic by calcined zeolite without HS or other reagents added.

To determine the degree of absorption of the arsenic from the solution, the sorbents samples were placed in a column with a diameter of 20 mm and a height of 450 mm, through which the test solutions were passed through for 60 minutes. The amount of arsenic in the source and the outlet solution was measured colorimetrically with diethyldithiocarbamate of silver in chloroform (State Standard 26930-86).

The following test objects were used: seeds of *Lepidium sativum* L. variety “ordinary cress” (JSC “Irkutsk seeds”), microalgae (*Scenedesmus quadricauda* (Turp.) Breb.), ciliates (*Paramecium caudatum* Ehrenberg), dry baker's yeast *Saccharomyces cerevisiae* (cultivar “Saf-moment”, Lesaffre, France).

Toxicity of model solutions was evaluated by their effect on seed germination and seedling root length, by the change in fluorescence of chlorophyll of algae cells, by survival of ciliates, by bioluminescent of bacteria and by changes in foaming in the yeast slurry.

*L. sativum* seeds were placed in Petri dishes with filter paper “Blue Ribbon” (JSC “Plant Himreaktiv set”), to which 5 cm<sup>3</sup> of the test solution was added. The dishes were incubated overnight at 31°C. After counting, the seed plates were incubated for another day and the length of seedlings’ roots was measured (Stom, 1982; Guidelines ..., 2002).

Levels of chlorophyll fluorescence were measured on “Fluorat-02-3M fluorometer” in continuous measurement mode after three days of incubation under the luminescent light. For each sample, mean fluorescence value was calculated as a mean of two measurements (Methodology ..., 2007).

In experiments with ciliates mortality in the test sample of 50% or more individuals per day was used as a criterion of acute toxicity (Methodology ..., 2006).

The degree of exposure of the test sample was judged by yeast foaming rate. For this, 1.36 g dry baker's yeast was suspended in 20 cm<sup>3</sup> of the toxicant solution and 0.4 g glucose was added. The resulting suspension was placed in a thermostat at 30°C in test tubes containing 3 cm<sup>3</sup> of the suspension. After 15 minutes, the resulting foam volume was determined. As the control we used yeast suspension prepared in tap water, dechlorinated by aging (Vyatchina *et al.*, 2009).

All experiments were performed in at least 5 independent blocks with three replicate measurements in each. Statistical analysis of the data obtained was done using MS Excel data analysis package. Significance of the difference was determined by Student's t test.

### 3. Results and Discussions

The effects of different HS in the concentration range 0.1-10 g/dm<sup>3</sup>, as well as solutions  $Na_3AsO_4$  in the concentration range 0.1-10 mg/dm<sup>3</sup> on the germination of cress seeds are presented in Figure 1:

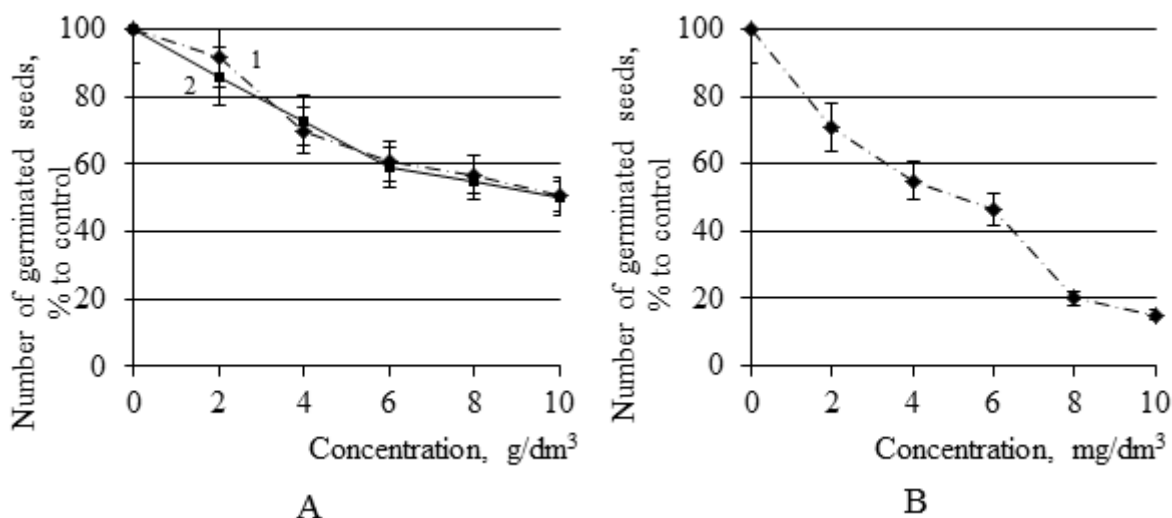


Figure 1. Influence of HS (A) and Na<sub>3</sub>AsO<sub>4</sub> (B) solutions of different concentrations on cress seeds germination (% to control - dechlorinated water)

1. "Humate-80"
2. "Powhumus"

The investigated HS ("Powhumus" and "Humate-80") did not have a negative effect on seed germination at concentrations ranging from 0.1 to 2 g/dm<sup>3</sup>. Although we observed a decrease in the number of germinated seeds of cress at concentration over 2 g/dm<sup>3</sup>, even at the HS concentration of 9 g/dm<sup>3</sup> 50.2 ± 4.8% of seeds germinated. Sodium arsenate in a concentration of 8 mg/dm<sup>3</sup> inhibited seed germination by 80.1 ± 7.9%. LC<sub>50</sub> of Na<sub>3</sub>AsO<sub>4</sub> for cress seeds was 5.3 mg/dm<sup>3</sup>. Based on the obtained results, in the further analysis HS in concentration range of 0.1 to 1.0 g/dm<sup>3</sup> was used.

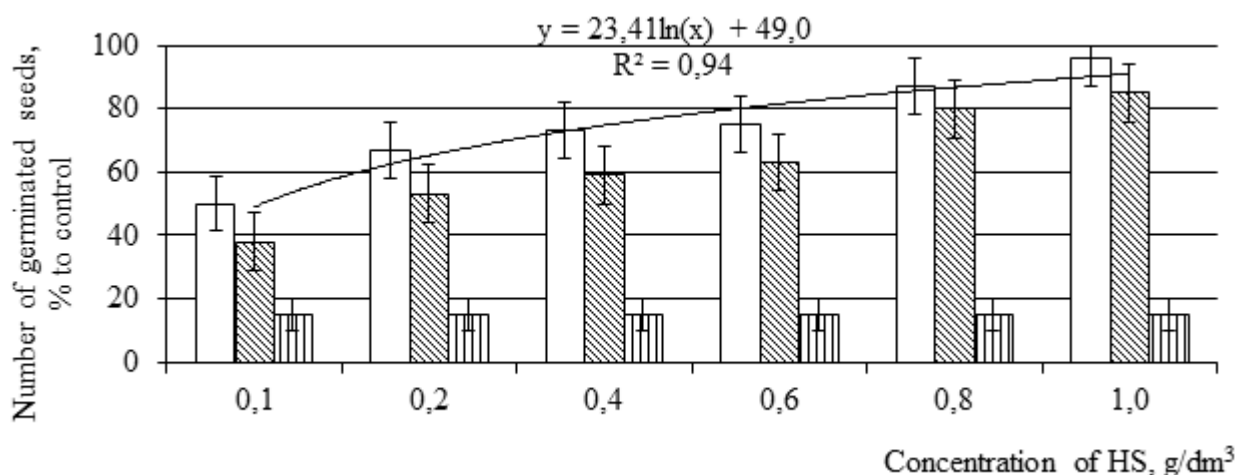


Figure 2. Combined effect of HS and Na<sub>3</sub>AsO<sub>4</sub> solutions on seed germination in cress (% to control-dechlorinated water):

- "Powhumus" + Na<sub>3</sub>AsO<sub>4</sub> 10 mg/dm<sup>3</sup>
- "Humate-80" + Na<sub>3</sub>AsO<sub>4</sub> 10 mg/dm<sup>3</sup>
- Na<sub>3</sub>AsO<sub>4</sub> 10 mg/dm<sup>3</sup>

Figure 2 shows that HS at concentration  $0.2 \text{ g/dm}^3$  reduced toxic effects of arsenic by  $25,3 \pm 2,7\%$ . The toxicity of the model arsenic contaminant fell even to a greater extent at  $1.0 \text{ g/dm}^3$  of “Powhumus”. In this case, the number of germinated seeds was  $96,1 \pm 8,7\%$ .

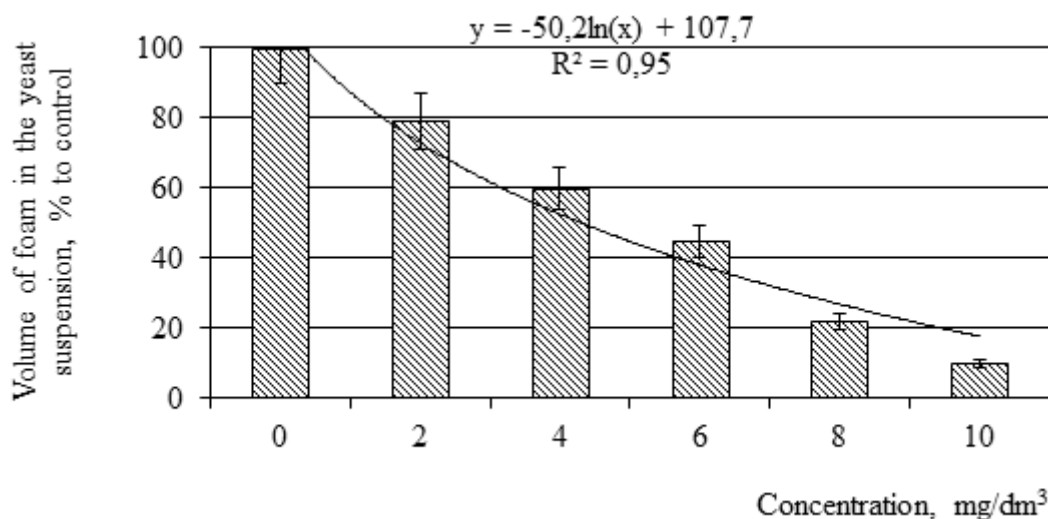


Figure 3. Influence of different concentrations of  $\text{Na}_3\text{AsO}_4$  solutions on the volume of foam in the yeast suspension (% to control - dechlorinated water)

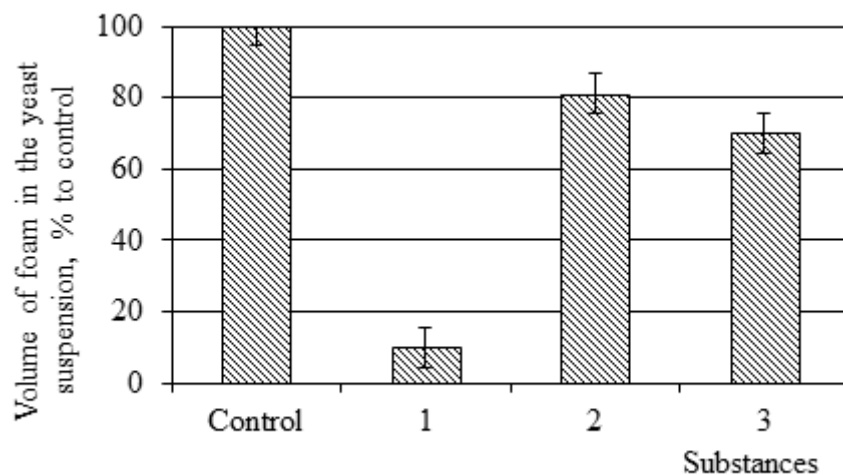


Figure 4. Combined effect of  $0.2 \text{ g/dm}^3$  HS and  $10 \text{ mg/dm}^3$   $\text{Na}_3\text{AsO}_4$  on the volume of foam in the yeast suspension (% to control - dechlorinated water)

- 1 –  $\text{Na}_3\text{AsO}_4$
- 2 – “Powhumus” +  $\text{Na}_3\text{AsO}_4$
- 3 – “Humate-80” +  $\text{Na}_3\text{AsO}_4$

Highly toxic concentration salt of arsenic for this bioassay was selected (Figure 3). “Powhumus” at a concentration of  $0.2 \text{ g/dm}^3$  was found to be the most effective in reducing the toxic effects of arsenic on the foam formation in yeast suspension. It reduced the toxic effect of  $\text{Na}_3\text{AsO}_4$  by  $70,3 \pm 7,1\%$  (Figure 4).

In chlorophyll fluorescence reduction bioassay using *S. quadricauda* cells the  $\text{LD}_{50}$  value was  $1.5 \text{ mg/dm}^3$  (Figure 5).

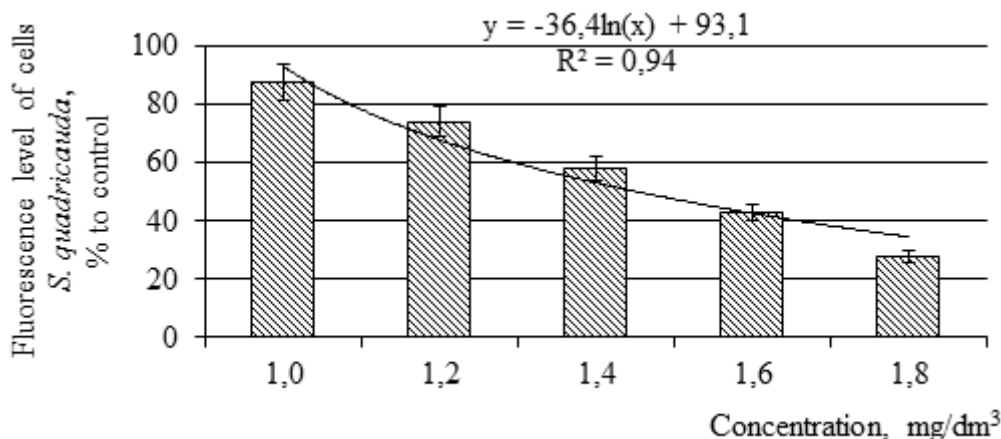


Figure 5. Influence of different concentrations of  $\text{Na}_3\text{AsO}_4$  solutions on fluorescence of cell algae *S. quadricauda* (% to control - dechlorinated water)

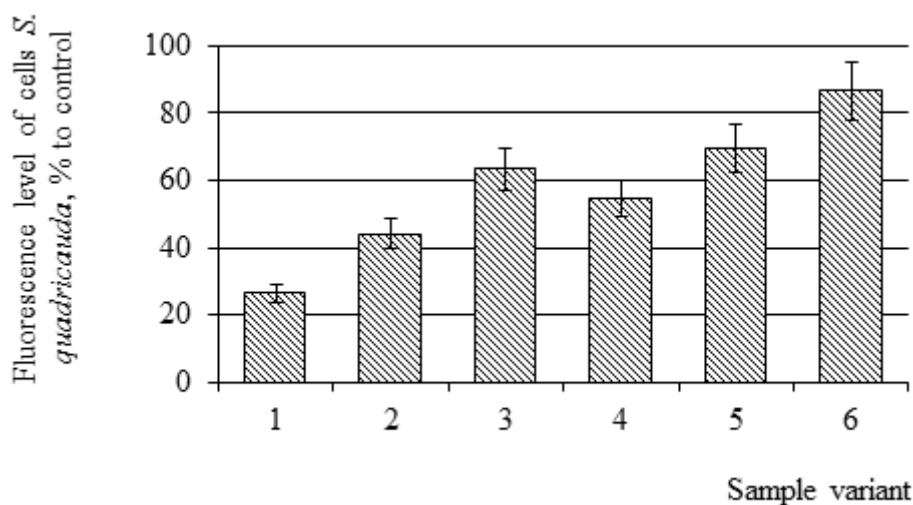


Figure 6. Combined effect of “Powhumus” and solutions  $\text{Na}_3\text{AsO}_4$  on the fluorescence of algal cells *S. quadricauda* (in% to control - dechlorinated water)

1. 1,8 mg/dm<sup>3</sup>  $\text{Na}_3\text{AsO}_4$ ;
2. 1,8 mg/dm<sup>3</sup>  $\text{Na}_3\text{AsO}_4$  + 0,01 g/dm<sup>3</sup> “Powhumus”;
3. 1,8 mg/dm<sup>3</sup>  $\text{Na}_3\text{AsO}_4$  + 0,05 g/dm<sup>3</sup> “Powhumus”;
4. 1,5 mg/dm<sup>3</sup>  $\text{Na}_3\text{AsO}_4$ ;
5. 1,5 mg/dm<sup>3</sup>  $\text{Na}_3\text{AsO}_4$  + 0,01 g/dm<sup>3</sup> “Powhumus”;
6. 1,5 mg/dm<sup>3</sup>  $\text{Na}_3\text{AsO}_4$  + 0,05 g/dm<sup>3</sup> “Powhumus”.

As seen in Figure 6, the intensity of chlorophyll fluorescence of microalgae cells treated with sodium arsenate at concentrations of 1.8 and 1.5 mg/dm<sup>3</sup> was  $26.4 \pm 3.2\%$  and  $54.5 \pm 6.1\%$ , while adding “Powhumus” at 0.05 g/dm<sup>3</sup> –  $63.1 \pm 6.4\%$  and  $86.4 \pm 8.8\%$ , correspondingly.

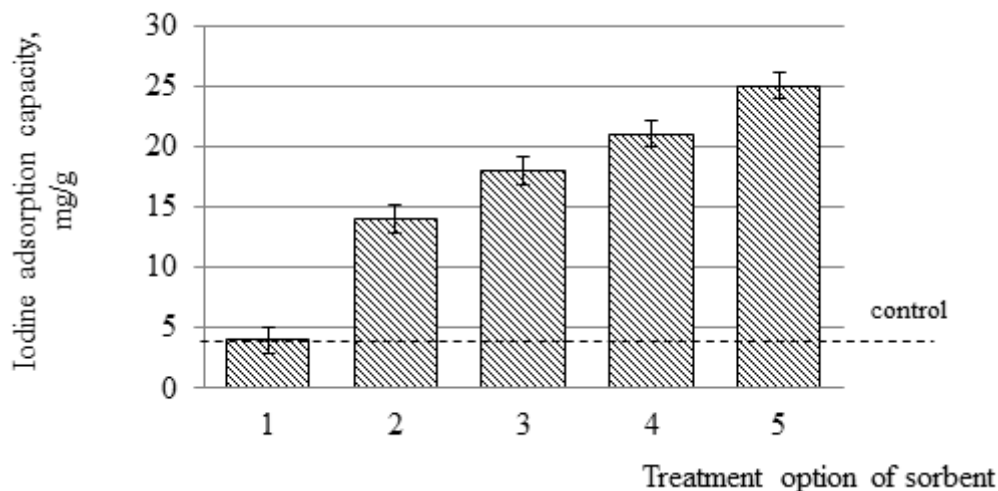


Figure 7. Sorption capacity of used zeolites depending on the method of processing:

1. Non calcined zeolite (control);
2. Calcined zeolite;
3. Zeolite calcined and saturated with HS “Humate-80”;
4. Zeolite calcined and saturated with HS “Powhumus”;
5. Zeolite modified by degassing, thermal and chemical treatment.

Figure 7 shows that the iodine sorption capacity of zeolite modified by degassing, thermal and chemical treatment was  $25.0 \pm 1.1$  mg/g. Sorption capacity of modified zeolite saturated by  $1 \text{ g/dm}^3$  “Powhumus” was  $21.1 \pm 1.3$  mg/g, and in the application of the same concentration of “Humate-80” –  $18.1 \pm 1.1$  mg/g.

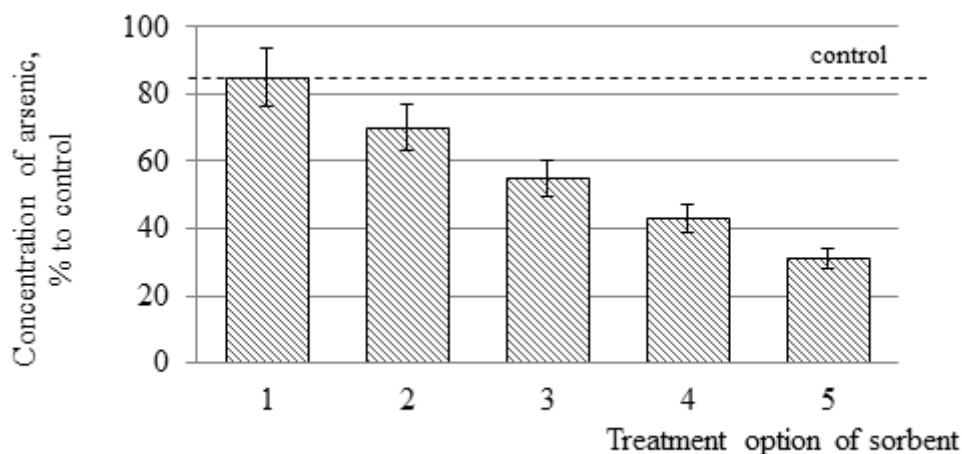


Figure 8. The effect of sorbent processing method on the uptake of  $\text{Na}_3\text{AsO}_4$  at concentration of  $100 \text{ mg/dm}^3$  (1-5 as on Figure 7)

Figure 8 displays the results of the study of the ability of zeolites to bind arsenic from a model solution. Zeolite activated by degassing, thermal and chemical treatment absorbs arsenic from a model solution  $41.2 \pm 3.9\%$  more intensely than the control zeolite. Zeolite saturated by “Powhumus” and by “Humate-80” showed  $27.2 \pm 3.1\%$ , and  $15.7 \pm 2.2\%$  higher binding ability than the control, correspondingly.

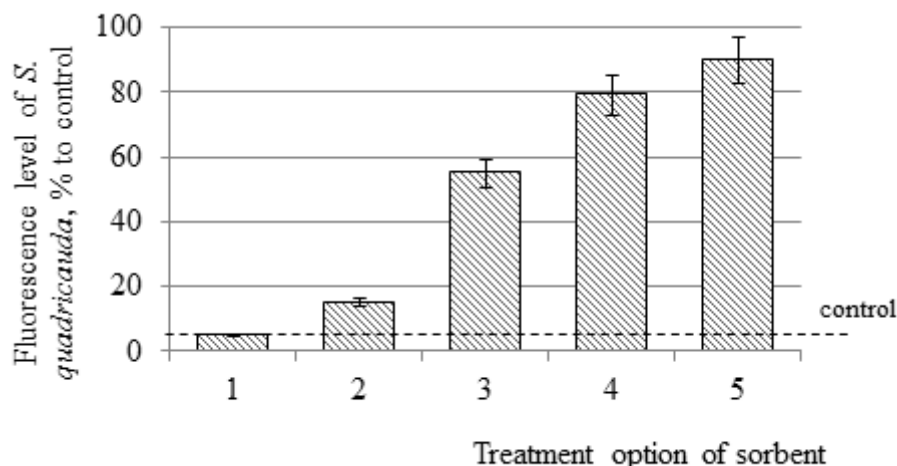


Figure 9. The effect of  $100 \text{ mg/dm}^3 \text{ Na}_3\text{AsO}_4$  solutions on fluorescence of *S. quadricauda* cells after passing through the modified zeolites (1-5 as on Figure 7)

As can be seen from figure 9, the  $100 \text{ mg/dm}^3 \text{ Na}_3\text{AsO}_4$  solution passed through a column of calcined zeolite without adding HS or other reagents suppressed the level of chlorophyll fluorescence by more than 80%. However, when zeolite, activated by degassing, thermal and chemical treatment (or saturated by “Powhumus”) was used, the toxic effect of arsenic was on 60% lower relative to the calcined zeolite. The intensity of fluorescence of the cells of algae *S. quadricauda* reached  $90.3 \pm 9.1\%$ ,  $79.5 \pm 8.4\%$  and  $15.5 \pm 2.1\%$  of the control, correspondingly. When bioassay solution passed through the zeolite saturated with HS “Humate-80”, the luminescence intensity of chlorophyll cells of microalgae was  $55.7 \pm 5.9\%$  relative to the control.

#### 4. Conclusions

Zeolite activated with calcining at  $400\text{--}450^\circ\text{C}$ , decationized and saturated with appropriate solution (5 g of  $(\text{NH}_2)_2\text{CO}$ , 5 g of  $\text{NH}_4\text{NO}_3$ ,  $40 \text{ cm}^3$  of distilled water,  $2.5 \text{ cm}^3$  of  $\text{MnSO}_4$ ,  $7.5 \text{ cm}^3$  HS “Powhumus”) the most effective in binding model arsenic contaminant ( $\text{Na}_3\text{AsO}_4$  salt aqueous solution at the concentration of  $100 \text{ mg/dm}^3$ ). The same zeolite preparation had the highest iodine adsorption capacity (7).

Zeolite processed according to the method proposed by us (7) resulted in the lowest suppression of chlorophyll fluorescence of *S. quadricauda* cells by arsenic solution passed through a layer of absorbent.

#### Acknowledgments

This work was financially supported by the Ministry of Education of the Russian Federation within the framework of the project of the state task in the field of scientific activity (Task # 13.1263.2014/K).

The authors are grateful to B.Stern, Humintech GmbH, LLC Agricultural technology for providing the HS samples and to N.I. Granina for the support work in this direction.

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