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## CROP PRODUCTION

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### PHYTOPROTECTIVE ROLE OF HUMUS ACIDS UNDER SALINITY

Research article

#### Abstract

The investigation of phytoprotective properties of humus acids is of interest nowadays due to the growth of anthropogenic soil salinity. The influence of peat humus acids preparation on wheat (*Triticum aestivum* L.) tolerance to chloridic salinity was studied under vegetational experiment in aquatic culture. The plants were grown on Hoagland nutrient mixture, the experimental variant of solution being enriched by NaCl in 25, 50, and 100 mmol/l concentrations as well as by humus acids preparation (0.005%) in accordance with the experiment scheme. Under mild salinity - 25 and 50 mmol/l - the protecting function of humus acids was shown to act with Na content increase, while under 100 mmol/l the protecting role of humus acids is caused by the decrease of sodium ions contents in plants shoots. Sodium ions accumulation in plants shoots in the presence humus acids under mild salinity (25 and 50 mmol/l of NaCl) can be used in phytoremediation.

**Keywords:** humic acids, salinity, wheat.

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### ФИТОПРОТЕКТОРНАЯ РОЛЬ ГУМУСОВЫХ КИСЛОТ ПРИ ЗАСОЛЕНИИ

Научная статья

#### Аннотация

В связи с ростом антропогенного засоления почв представляет интерес изучение фитопротекторных свойств гумусовых кислот. В условиях вегетационного эксперимента в водной культуре исследовали влияние препарата гумусовых кислот торфа на устойчивость пшеницы (*Triticum aestivum* L.) к хлоридному засолению. Растения выращивали на питательной смеси Хогланда, на опытных вариантах в раствор добавляли NaCl в концентрациях 25, 50 и 100 ммоль/л, а также препарат гумусовых кислот (0.005%) в соответствии со схемой опыта. В результате исследований установлено, что при умеренном засолении – 25 и 50 ммоль/л – протекторная функция гумусовых кислот реализуется при увеличении содержания Na, тогда как при 100 ммоль/л NaCl защитная роль гумусовых кислот обусловлена снижением содержания ионов натрия в побегах растений. Усиление накопления ионов натрия в побегах в присутствии гумусовых кислот при невысоком засолении (25 и 50 ммоль/л NaCl) может быть использовано в фиторемедиации.

**Ключевые слова:** гумусовые кислоты, засоление, пшеница.

#### 1. Introduction

The area of salinated soils covers about 25% of land surface and is constantly growing under the influence of different anthropogenic factors. Secondary salinity caused mainly by irrigation reaches thousands of hectares a year [1]. Technogenic salinity resulting from oil extraction and mineral fertilizers production progresses rapidly [2]. The application of anti-ice mixtures in winter leads to their spread in the form of aerosols to great distances from highways as well as their accumulation in soils. High concentrations of salts cause metabolic disturbances as well as plant physiological processes changes resulting in decreasing of their productivity [3], [4]. The main reason of crops damage is excessing salt ions accumulation [5], [6].

Nowadays there are some experimental proofs of adaptogenic properties of humus acids - humic and fulvic acids (HFA) - to plants under high salt concentration conditions [7]. The solution application of HFA was shown to result in partial salt stress releasing in wheat seedlings [8]. The protecting action of HFA under salinity is attributed to their anti-oxidant action [9] as well as to cytokinins amount maintenance in plants [10]. HFA seem to act as physiological plant protecting and detoxication

systems inductor [11]. HFA are known both to decrease [12], and to increase accumulation of metals in plants [13], [14], due to soluble and insoluble salts formation.

Humic preparations containing watersoluble humus acids salts possess the greatest physiological efficacy. The chief raw material for humats production are brown coal and peat, the latter being the most perspective renewable resource with stores in Russia estimated as high as 160 bil.tons [15]. These reserves are computed to grow by 250-280 mln.tons each year.

The above mentioned data prove the necessity of special researches aimed at the investigating of character and mechanisms of influence of humic preparations on toxicity and salinating ions accumulation in plants. Such investigations are necessary both for plant stability processes obviating and for phytoremediational technologies development.

## 2. Materials and methods

### 2.1. Materials and conditions of experiments

Peat humus acids preparation in the form of HFA watersoluble salt was chosen on the basis of preliminary experiments and special literature analysis. Peat HFA preparations are known to show the greatest activity in oxydizing-restoring processes and to have high superficial and antioxidant activity [16]. The preparation was obtained from low-lying land peat of wood-sedge group with decomposition rate 35-40% [17]. Being a relatively new geological formation, low-lying land peat contains a great amount of biologically active substances (aminoacids, carbohydrates, ferments, antibiotics, vitamins and phytohormons), mostly in humic acids. The preparation production technology provides the most complete transformation of all biologically active substances into the state available for plants, humic acids changing into physiologically active watersoluble salts – humats.

Spring wheat (*Triticum aestivum* L.) of Priokskaya variety was chosen as the object of investigation. As it is known from special literature, cereals are responsive to HFA stimulating application [18]. The aim of the research work was also the study of HFA influence on the salinating ions accumulation by a typical plant- root ions “excluder”. Wheat can be used in phytoremediation due to its high growth rate and ability to absorb and accumulate contaminating ions.

The vegetational experiments were carried out on aquatic culture which allowed to create proper concentration of both humic acids and sodium chloride as well as to observe the plant root system. The seeds were germinated on filter paper with NaCl solutions (25, 50, 100 and 200 mmol/l) with and without HFA addition. Humic preparation was used in concentration 0.01% which is considered to be the most physiologically effective in seeds germination. In 7days the seedlings were moved into 1 litre vessels with 0.25 of Hoagland solution rate [19]. The rate of nutrient mixture was increased to 0.5 and 1 as the plants grew. Plants grown without NaCl and HFA in the medium were used as control. NaCl in concentrations 25, 50 and 100 mmol/l and HFA (0.005 %) in accordance with the scheme were applied in case of experimental plants. The seeds practically didn't germinate at 200 mmol/l so this variant was excluded from the further experiment. The plants were grown with artificial light, the length of light day being 16 hours. The nutrient solution was changed every 7 days. The temperature was kept at 20-22 degrees, the solution pH being 5.3-5.6. The repetition in experiments was 4-times, in observations-3-times.

### 2.2. Research methods

Phases of plants development, external signs of root and shoot damage were written down in research process. The length of roots and shoots was measured and the dry weight was estimated. Plant reaction on salinating ions and HFA action was estimated by morphological characteristics of shoots on the seventh day of the experiment as well as by biomass accumulation in phases of tillering, stalk shooting and ears formation. The content of ions in plants was determined on atomic absorbtional spectrometre in the Lobachevsky State University Chemistry Research Institute.

The rate of plants salt tolerance was expressed in the form of correlation of plant shoots dry weight [20] (or seedlings root length) of control and experimental variants. The coefficient of HFA protectional action was computed [21]: 1) by plants mass accumulation – correlation of plant shoots dry weight (or seedlings root length) grown with and without HFA was computed 2) by ions accumulation – the correlation of sodium content in plants grown with and without HFA was computed. The correlation of sodium ions accumulation in plants grown with and without HFA (mg/plant) was taken as the coefficient of HFA fitoremediation efficacy. Sodium ions accumulation per one plant was found to reflect the remediational capacity of plants most accurately due to weight decreasing consideration.

The statistical data processing was carried out with the help of Excel program using dispersion and regressive methods of analysis.

## 3. Results and discussion

High concentrations of NaCl decreased the shoots and roots length of wheat (table 1, figure1). Seedlings salt tolerance rate was high at 25 mmol NaCl, there being practically no difference between control and experimental variants. At 50 mmol shoots length decreased considerably (by 45%), while at 100 mmol the shoots length decreased by as much as 49% and root length by 65% to control. The influence of salinity on seedlings shoots length at 50 and 100, and on roots at 100 mmol NaCl was statistically proved. At 200 mmol NaCl the seeds were not vital.

Table 1 – Seedlings salt tolerance rate (%)

| Variants               | Shoots | Roots  |
|------------------------|--------|--------|
| 1. NaCl 25 mmol        | 96.92  | 98.02  |
| 2. NaCl 25 mmol + HFA  | 114.71 | 148.32 |
| 3. NaCl 50 mmol        | 65.60  | 94.26  |
| 4. NaCl 50 mmol + HFA  | 70.34  | 137.57 |
| 5. NaCl 100 mmol       | 51.36  | 35.14  |
| 6. NaCl 100 mmol + HFA | 54.57  | 80.62  |
| 7. NaCl 200 mmol       | -      | -      |
| 8. NaCl 200 mmol + HFA | 26.93  | 23.07  |

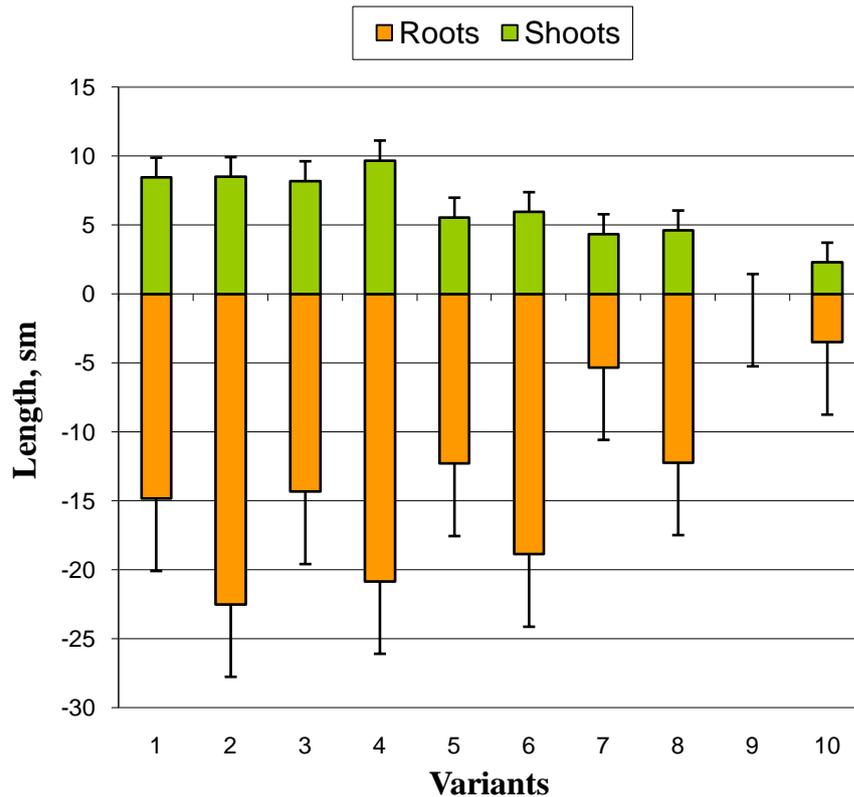


Figure 1 – Influence of salination and HFA on wheat seedlings growth

1. Control 2. HFA 3. NaCl 25 mmol 4. NaCl 25 mmol + HFA 5. NaCl 50 mmol 6. NaCl 50 mmol + HFA 7. NaCl 100 mmol 8. NaCl 100 mmol + HFA 9. NaCl 200 mmol 10. NaCl 200 mmol + HFA. Bars on the diagram show least significant difference for 5% significance level (LSD<sub>05</sub>)

The inverse linea correlation of wheat seedlings root length and NaCl concentration was stated (figure 2)

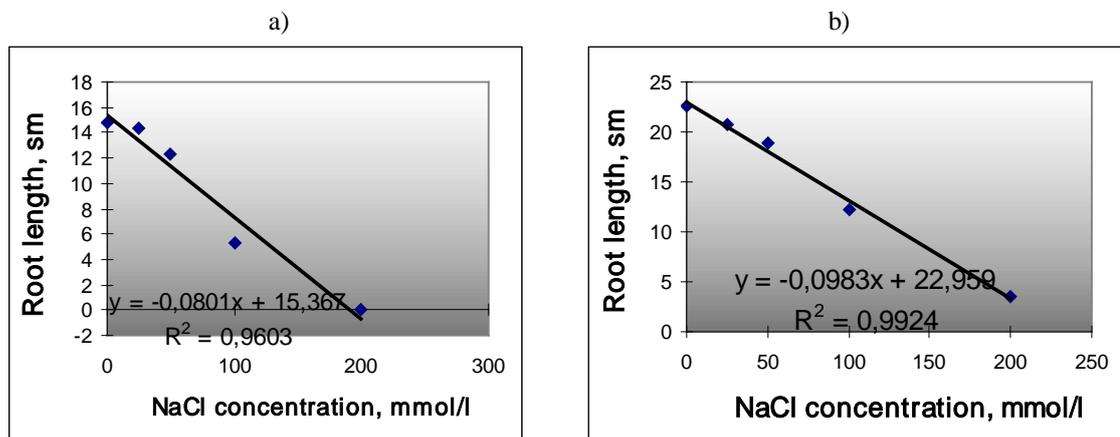


Figure 2 – Correlation of wheat seedlings root length and NaCl concentration  
 a) without HFA ( $r=-0.98$ ;  $p=0.003$ ) b) with HFA ( $r=-1.00$ ;  $p<0.001$ )

HFA preparation decreased the damaging action of salinity on wheat seedlings due to 1.5-2.3 times root length increasing as compared with variants without HFA (table 1, figure 1, 3). In presence of 200 mmol of NaCl HFA preparation stimulated seeds germination. The protecting influence of HFA on seedlings root length was seen in all the variants, on shoots length – in only at NaCl concentration 25 mmol.

Table 2 – HFA protecting action coefficient (by seedlings length)

| Variants        | Shoots | Roots |
|-----------------|--------|-------|
| 1. HFA          | 1.01   | 1.52  |
| 2.NaCl 25 mmol  | 1.18   | 1.45  |
| 3.NaCl 50 mmol  | 1.07   | 1.53  |
| 4.NaCl 100 mmol | 1.06   | 2.29  |

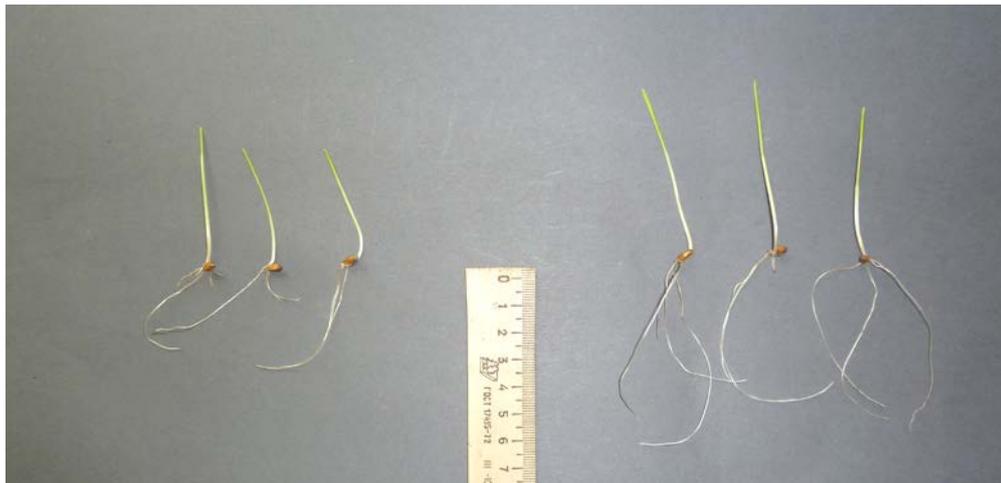


Figure 3 – HFA influence on the wheat seedlings root growth in presence of 50 mmol of NaCl  
To the left-without HFA, to the right –with HFA

Shoots and roots mass decreased with salt concentration increase (table 3, figure 4). It should be noted that at 25 mmol plants shoots mass to control decreased only in bushing phase, while in tube formation phase and ears formation phase NaCl influence is not proved statistically.

Table 3 – Shoots and roots dry weight (g)

| Variants  | Development phases (for control plants) |                |                |
|---|---|----------------|----------------|
|   | Tillering                               | Stalk shooting | Ears formation |
| shoots  |   |                |                |
| 1. Control  | 0.67                                    | 3.00           | 10.2           |
| 2. HFA  | <b>0.80</b>                             | <b>3.50</b>    | 11.3           |
| 3.NaCl 25mmol   | 0.60                                    | 2.70           | 9.00           |
| 4. NaCl 25mmol + HFA  | <b>0.66</b>                             | <b>3.10</b>    | 10.54          |
| 5.NaCl 50 mmol  | 0.27                                    | 1.20           | 4.00           |
| 6.NaCl 50 mmol + HFA  | <b>0.53</b>                             | <b>2.77</b>    | <b>8.20</b>    |
| 7.NaCl 100 mmol   | 0.05                                    | 0.10           | 0.52           |
| 8.NaCl 100 mmol + HFA   | <b>0.09</b>                             | 0.28           | 0.98           |
| LSD <sub>05</sub>   | 0.02                                    | 0.33           | 2.07           |
| roots   |   |                |                |
| 1. Control  | 0.10                                    | 0.68           | 2.00           |
| 2. HFA  | 0.11                                    | <b>0.80</b>    | <b>2.20</b>    |
| 3.NaCl 25mmol   | 0.08                                    | 0.52           | 1.50           |
| 4. NaCl 25mmol + HFA  | 0.10                                    | 0.64           | <b>1.86</b>    |
| 5.NaCl 50 mmol  | 0.04                                    | 0.30           | 0.89           |
| 6.NaCl 50 mmol + HFA  | 0.05                                    | 0.32           | 0.97           |
| 7.NaCl 100 mmol   | 0.01                                    | 0.08           | 0.23           |
| 8.NaCl 100 mmol + HFA   | 0.02                                    | 0.11           | 0.32           |
| LSD <sub>05</sub>   | 0.03                                    | 0.10           | 0.17           |
| Notes: - statistically proved – HFA influence is printed in bold type;<br>– LSD <sub>05</sub> - least significant difference for 5% significance level. |   |                |                |

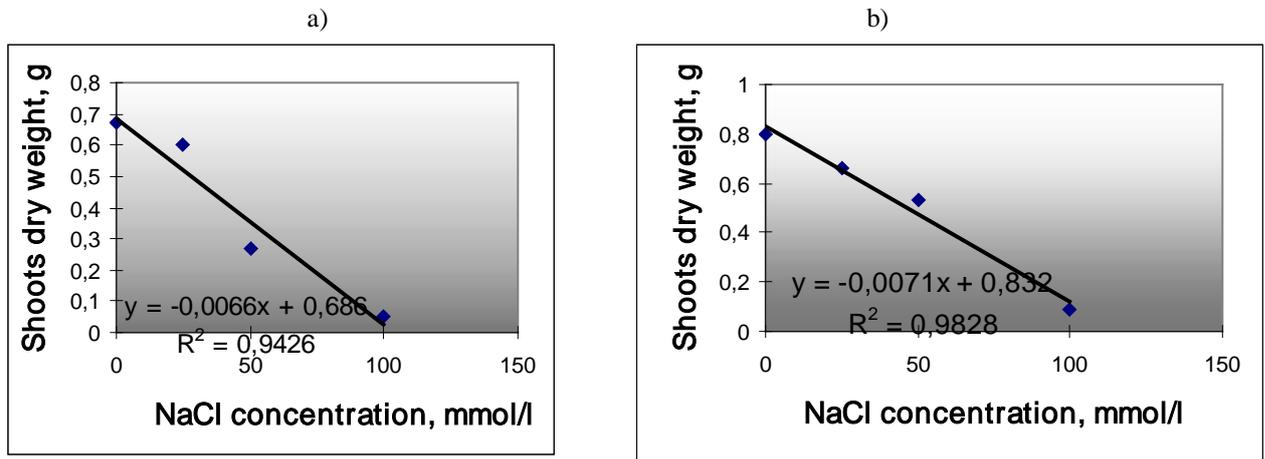


Figure 4 – Correlation of shoots dry weight and NaCl concentration in bushing state: *a)* without HFA ( $r=-0.97$ ;  $p=0.029$ ), *b)* with HFA ( $r=-0.99$ ;  $p<0.008$ )

Correspondingly, plants salt resistance rate was high at 25 mmol of NaCl (table 4). At 50 mmol plants salt resistance decreased by 60%, and at 100 mmol was as little as 3-7%.

Table 4 – Plants salt tolerance rate, %

| Variants                     | Development phases (for control plants) |                |                |
|------------------------------|---|----------------|----------------|
|                              | Tillering                               | Stalk shooting | Ears formation |
| 1. NaCl 25mmol               | 89.55                                   | 90.00          | 88.24          |
| 2. NaCl <sub>25</sub> + HFA  | 98.51                                   | 103.33         | 103.33         |
| 3. NaCl 50mmol               | 40.30                                   | 40.00          | 39.22          |
| 4. NaCl <sub>50</sub> + HFA  | 79.10                                   | 92.33          | 80.39          |
| 5. NaCl 100mmol              | 7.46                                    | 3.33           | 5.10           |
| 6. NaCl <sub>100</sub> + HFA | 13.43                                   | 9.33           | 9.61           |

HFA preparation decreased damaging action of NaCl on wheat plants (table 5, figure 5). It should be noted that HFA protecting role increases with salt concentration growth, being the highest in stalk shooting phase (figure 6). HFA contributed to plants weight growth more than two times at 50 and 100 mmol of NaCl as compared with variants without HFA. In presence of 25 mmol of NaCl HFA efficacy was as little as 10-17 % possibly due to low toxicity of this salt concentration.

Table 5 – HFA protecting action coefficient (by plants weight)

| NaCl concentration, mmol/l | Development phases (for control plants) |                |                |
|----------------------------|---|----------------|----------------|
|                            | Tillering                               | Stalk shooting | Ears formation |
| 1. 25                      | 1.10                                    | 1.15           | 1.17           |
| 2. 50                      | 1.96                                    | 2.31           | 2.05           |
| 3. 100                     | 1.80                                    | 2.80           | 1.88           |



Figure 5 – HFA influence on wheat plants growth at 50 mmol/l of NaCl  
To the left – without HFA, to the right – with HFA

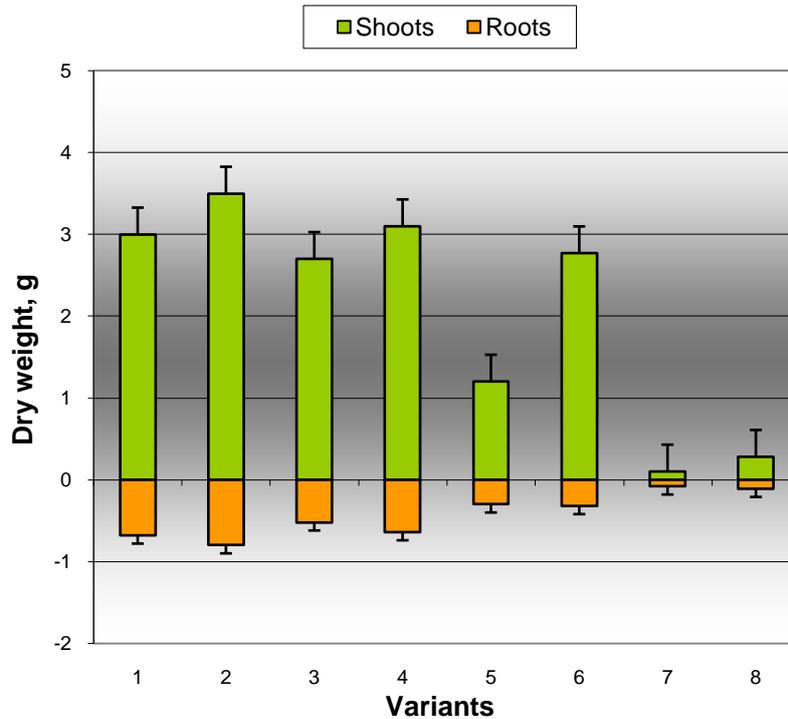


Figure 6 – HFA influence on wheat plants growth under chloride salinity in stalk shooting (tube formation) phase  
 1. Control 2. HFA 3. NaCl 25 mmol 4. NaCl 25 mmol + HFA 5. NaCl 50 mmol 6. NaCl 50 mmol + HFA 7. NaCl 100 mmol 8. NaCl 100 mmol + HFA . Bars on the diagram show least significant difference for 5% significance level (LSD<sub>05</sub>)

The damaging action of salinity is possibly caused by sodium ions accumulation. The inverse correlation of plants shoots weight and sodium contents coefficient is 0.72, of roots – 0, 84. Sodium ions contents in plants shoots and roots increased with NaCl concentration growth (table 6). At 25 mmol/l of NaCl sodium contents in shoots grew in 7.8 times, at 50 mmol/l – in 17 times, at 100 mmol/l – in 31.5 times to control.

Table 6 – Sodium ions contents, mg/kg of dry weight (Stalk shooting phase)

| Variants               | Shoots        | Roots         |
|------------------------|---------------|---------------|
| 1. Control             | 306.6         | 2970.8        |
| 2. HFA                 | 266.4         | <b>3695.5</b> |
| 3. NaCl 25 mmol        | 2400.4        | 7790.2        |
| 4. NaCl 25 mmol + HFA  | <b>2920.3</b> | <b>2750.5</b> |
| 5. NaCl 50 mmol        | 5220.8        | 18555.2       |
| 6. NaCl 50 mmol + HFA  | <b>6124.8</b> | <b>9199.8</b> |
| 7. NaCl 100 mmol       | 9646.5        | -             |
| 8. NaCl 100 mmol + HFA | <b>3863.6</b> | -             |
| LSD <sub>05</sub>      | 339.42        | 444.38        |

Notes:  
 - Statistically proved sodium contents growth under HFA influence is printed in bold type;  
 - Statistically proved sodium contents decrease under HFA influence is printed in bold and italic type;  
 - LSD<sub>05</sub> - least significant difference for 5% significance level.

HFA preparation increased sodium contents in shoots in 1.2 times and decreased it in plants roots in 2-2.8 times at 25 and 50 mmol of NaCl (table 7), shoots weight being 92 and 103 % at 50 and 25 mmol correspondingly. At 100 mmol of NaCl the protecting role of HFA increased, which contributed to shoots sodium contents decrease in 2.5 times as well as to plants stability increase in 2.8 times. So the protecting role of HFA was observed at presence of 25 and 50 mmol of NaCl at sodium contents increase, and at 100 mmol of NaCl - at sodium contents decrease.

Table 7 – HFA protecting action coefficient (sodium contents)

| NaCl concentration, mmol/l | Shoots | Roots |
|----------------------------|--------|-------|
| 1. 25                      | 0.82   | 2.83  |
| 2. 50                      | 0.85   | 2.02  |
| 3. 100                     | 2.50   | -     |

The strategy of glycophyts adaptation to excessive salt concentrations on organism level is known to be the regulation of ions concentration in over ground organs and avoiding salinating ions high concentrations in young growing leaves and especially in reproductive organs [22]. Roots are capable of detaining part of absorbed sodium, accumulating it in vacuoli, a certain part being transported to the environment and absorbed in cell walls outside the absorbing zone [23]. So under the condition of salinity 100 mmol of NaCl humus acids strengthened the regulating function of wheat plants root system.

Salinating ions accumulation per one plant better reflects the remedial capacity of plants taking into consideration their mass decrease. Accumulation of sodium by wheat plants was higher in shoots than in roots in 1.3-1.8 times and decreased markedly at 100 mmol of NaCl due to toxicity growth and plants weight decrease ( Table 8).

Table 8 – Sodium ions accumulation (mg/plant)

| Variants               | Shoots | Roots  |
|------------------------|--------|--------|
| 1. Control             | 3.127  | 5.942  |
| 2. HFA                 | 3.010  | 8.130  |
| 3. NaCl 25 mmol        | 21.604 | 11.685 |
| 4. NaCl 25 mmol + HFA  | 30.780 | 5.116  |
| 5. NaCl 50 mmol        | 20.883 | 16.514 |
| 6. NaCl 50 mmol + HFA  | 50.223 | 8.923  |
| 7. NaCl 100 mmol       | 5.016  | -      |
| 8. NaCl 100 mmol + HFA | 3.786  | -      |

HFA preparation increased sodium ions accumulation by wheat plants shoots at 25 and 50 mmol of NaCl in 1.4 and 2.4 times correspondingly in comparison with variants without HFA having increased their remedial potential (Table 9).

Table 9 – HFA phytoremediation efficacy coefficient

| NaCl concentration, mmol/l | Shoots | Roots |
|----------------------------|--------|-------|
| 1. 25                      | 1.42   | 0.44  |
| 2. 50                      | 2.40   | 0.54  |
| 3. 100                     | 0.75   | -     |

#### 4. Conclusion

So the present investigations proved the suggested phytoprotective role of humic acids under salinity. HFA preparation decreased the damaging action of salinity on wheat seedlings increasing root length in 1.5-2.3 times at 25, 50 and 100 mmol of NaCl as compared with variants without HFA. HFA preparation stimulated seeds germination in presence of 200 mmol of NaCl. In conditions of salinity 100 mmol of NaCl humus acids contributed to plant root system regulating function strengthening – sodium contents in wheat shoots decreased in 2.5 times at plant stability increase in almost 3 times as compared to variant without HFA .

Intensification of sodium ions accumulation by wheat plants shoots in presence of HFA at low salinity (25 and 50 mmol/l of NaCl) prove the perspectiveness of humus acids preparations use in phytoremediation as the effectors of phytoextraction of salinating ions.

#### Conflict of Interest

None declared.

#### Конфликт интересов

Не указан.

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