Evaluation of the impact of technogenically polluted wastewater on the morphological and physiological parameters of phytoremediants in combination with various types of microorganisms

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Abstract. The article presents data on the study of the effect of technogenically polluted wastewater on digital biomass and NDVI parameters of phytoremediants. Analysis of plant growth and development was carried out on *Phragmites australis* (Cav.) Trin. ex Steud. (obtained by *in vitro* method), *Typha angustifolia* L. and *T. latifolia* L. (obtained from seeds). All species were grown in combination with various microorganisms under the influence of technogenically polluted wastewater. The most effective combinations of symbiotic microbial-plant complexes have been identified, which are able to function actively under pollution conditions and can be recommended for their use in wastewater treatment.

1 Introduction

In recent decades, there has been a growing interest in the development and use of effective technologies for phytoremediation of technogenically polluted environment with the help of plants [1-2]. It is noted in the literature that the interaction of plants with some symbiont microorganisms can significantly increase their resistance to a variety of unfavorable abiotic [3-4] and biotic factors [5-6], as well as anthropogenic impacts associated with technogenic wastewater pollution [7-8]. Of considerable interest is the creation of biological systems microorganism-plant, allowing as quickly and efficiently as possible to remove certain types of pollutants from the pollution zone without inhibiting the phytocomponent. The use of symbiotic microbial-plant complexes (SMPC) for solving problems of phytoremediation in Russia has not yet found wide application. This is evidenced by the low level of publication activity in this direction. At the same time, microorganisms can not only increase the resistance of plants [7], but also contribute to an increase in the accumulation of toxic substances in their tissues [8-10]. A more detailed study of the effect of the action of technogenically polluted wastewater (TPW) on symbiotic microbial-plant complexes can significantly increase the efficiency of the work of

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pollutant hyperaccumulator plants [11-13] or allow the use of species for phytoremediation that do not normally have pronounced hyperaccumulator properties.

The purpose of the work: to study the effect of technogenically polluted wastewater on the morphological and physiological characteristics of phytoremediant plants developing in combination with various types of microorganisms.

2 Materials and methods

The study was carried out on the basis of the laboratory of experimental botany of the REC "Botanical Garden of the National Research University" BelSU "using the equipment of a unique scientific installation (UNU)" Botanical Garden of the Belgorod State National Research University "(https://ckp-rf.ru/usu/200997/).

Phragmites australis (Cav.) Trin. ex Steud. [14] obtained by the in vitro method, as well as Typha angustifolia L. [15-16] and T. latifolia L. [17] grown from seeds. Plants were cultivated on phytoracks with LED lamps ECOLED-60-LX Fito IP 65 60W with a long wave of 450-730 nm (LED).

Plants were grown under the following conditions:

- Control.
- TPW.
- Bacillus subtilis consortium.
- TPW + Bacillus subtilis consortium.
- The strain of the bacterium Bacillus subtilis 26D.
- TPW+bacterium strain Bacillus subtilis 26D.
- Azotobacter vinelandii IB-4 bacterium strain. 8 TPW + bacterium strain Azotobacter vinelandii IB-4.

Primary inoculation of plants with cultures of microorganisms was carried out on the 72nd day from the emergence of seedlings and introduction into the culture: a consortium of bacteria *Bacillus subtilis* strain DSM 32424, *Bacillus amyloliquefaciens* strain VKPM B-10642 (DSM 24614) and *Bacillus amyloliquefaciens* strain VKPM B-10643 (DSM 24615) at a concentration not less than 1×106 CFU/g; bacterial strain *Bacillus subtilis* 26D at a concentration of at least 2×109 CFU/g; bacterium strain *Azotobacter vinelandii* IB-4 at a concentration of at least 2-3×109 CFU/g. The plants were watered with tap water for six days, and on the 7th day they were treated according to the experimental scheme.

The morphological and physiological parameters of plants were assessed every 3-4 days on 8 even-aged and morphologically similar specimens using a multispectral 3D PlantEye F500 setup (Phenospex B.V., the Netherlands). Since the setup used allows measuring 14 parameters, we have chosen the most important parameters reflecting the general trend of plant responses: Digital Biomass, cm3 and Normalized differential vegetation index (NDVI) (range of values from - 1 to 1). The PlantEye F500 HortControl software was used to process the obtained data. Statistical data processing was carried out using Microsoft office Excel with the calculation of the arithmetic mean (M) and confidence interval (±CI) at a significance level of $p = 0.05$.

3 Results and Discussion

Among the morphological parameters that have the highest degree of information content is the digital biomass, which is calculated by multiplying the plant height by the leaf area, and, therefore, allows taking into account these two parameters in combination. Figures 1-3

show the percentage of growth in the digital biomass of test plants, depending on the type of symbionts used, under the systematic action of technogenically polluted wastewater.

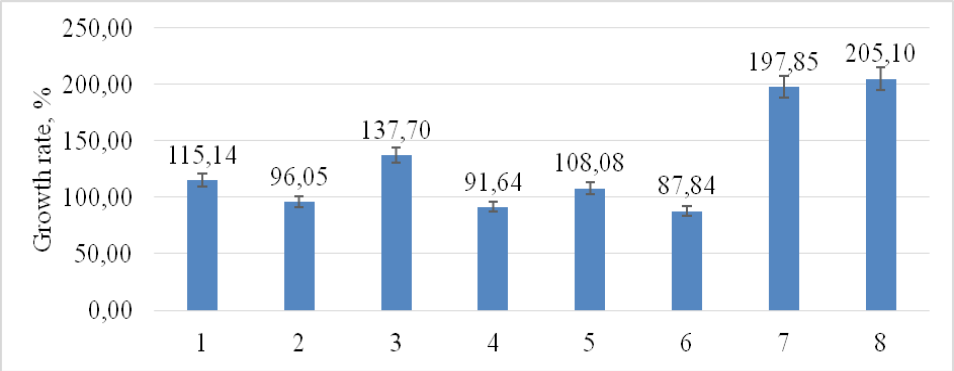

Fig. 1. The growth rate of the measured digital biomass of *Phragmites australis* (Cav.) Trin. ex Steud. depending on the strain of symbionts when using technogenically polluted wastewater.

Figure 1 shows that under the action of technogenically polluted wastewater (2) growth processes are inhibited compared to control (1), while the growth rate of symbiotic microbial-plant complexes, including the *Bacillus subtilis* consortium (3), is compared with higher control. However, the addition of technogenically polluted wastewater even during inoculation (4) leads to inhibition of growth processes, that is, the consortium used acts as an inhibitor when exposed to technogenically polluted wastewater. The use of the bacterium strain *Bacillus subtilis* 26D (5), even compared to the control (1), turned out to be ineffective, and additional exposure in the form of technogenically polluted wastewater (6) inhibited the growth of digital biomass even more. Thus, it can be stated that the studied strains of Bacillus subtilis with *Phragmites australis* (Cav.) Trin. ex Steud. under the influence of an anthropogenic factor in the form of technogenically polluted wastewater, they are ineffective for use in carrying out phytoremediation measures. The use of *Azotobacter vinelandii* IB-4 as symbionts (7) led to a significant increase in biomass compared to the control and even greater activity of growth processes in plants when technogenically polluted wastewater was introduced [8]. In this regard, this culture can be considered as a promising symbiont for intensifying the growth and development of *Phragmites australis* (Cav.) Trin. ex Steud. symbiotic microbial-plant complexes formed by this pair forms a functionally stable system to the impact of the anthropogenic factor in the form of technogenically polluted wastewater.

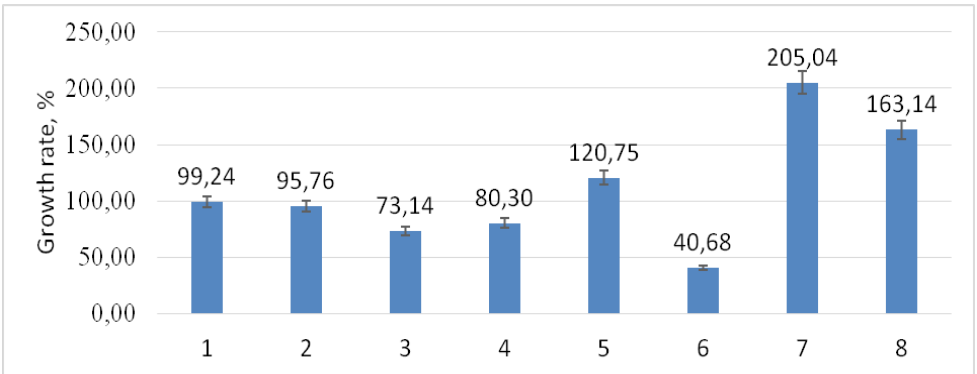

Fig. 2. Growth rates of the measured digital biomass of *Typha angustifolia* L. depending on the strain of symbionts when using technogenically polluted wastewater.

Figure 2 shows that under the influence of technogenically polluted wastewater (2), growth processes are inhibited in plants compared to control (1). The growth rates of symbiotic microbial-plant complexes that include *Bacillus subtilis* bacteria (3) are lower compared to the control, and the addition of technogenically polluted wastewater even during inoculation (4) leads to the intensification of growth processes, but to a lesser extent than in the control (1). Thus, it can be concluded that the consortium used during inoculation can increase the adaptability of *Typha angustifolia* L. plants to the action of toxicants in technogenically polluted wastewater. The use of the bacterium strain *Bacillus subtilis* 26D (5) compared to the control (1) proved to be effective, but the additional impact in the form of technogenically polluted wastewater (6) significantly reduced the activity of growth processes to the minimum values.

The use of *Azotobacter vinelandii* IB-4 as symbionts led (7) to an increase in plant biomass compared to the control, but somewhat less activity of growth processes in them when technogenically polluted wastewater was introduced [8]. Thus, this culture can also be considered as a promising symbiont of the phytoremediant *Typha angustifolia* L, which contribute to the formation of sustainable systems in relation to anthropogenic impact.

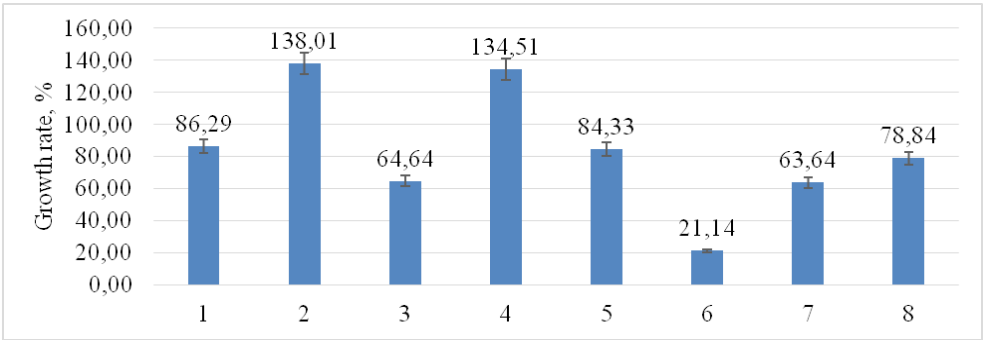

Fig. 3. Growth rates of the measured digital biomass of *Typha latifolia* L. depending on the strain of symbionts when using technogenically polluted wastewater.

Figure 3 shows that under the influence of technogenically polluted wastewater (2) in the only species *Typha latifolia* L., growth processes are intensified compared to control (1), while the growth rate of symbiotic microbial-plant complexes with the presence of the *Bacillus subtilis* consortium (3) compared to the control is lower. The addition of technogenically polluted wastewater during plant inoculation (4) leads to the intensification of growth processes to a much greater extent compared to the control (1), but at the same level as when using technogenically polluted wastewater (2).

Thus, the obtained results indicate that different plant species react differently both to anthropogenic impact and to the symbiotic component of the *Bacillus subtilis* consortium, which acted as an inhibitor of growth processes in the studied plants of the genus *Typha*. The use of the bacterial strain *Bacillus subtilis* 26D (5) and (6) in comparison with the control (1) was also ineffective for all three plant species. The growth of digital biomass for plants of the genus *Typha* is much greater than when using the *Bacillus subtilis* consortium, and for *Phragmites australis* (Cav.) Trin. ex Steud. growth dynamics was reversed. The use of *Azotobacter vinelandii* IB-4 (7) and (8) as symbionts contributed to an increase in the digital biomass of plants, as in Phragmites australis (Cav.) Trin. ex Steud. and *Typha angustifolia* L., while *Typha latifolia* L. had the opposite effect.

The results of measuring the NDVI index at the beginning and end of the experiment are shown in Figures 4-6.

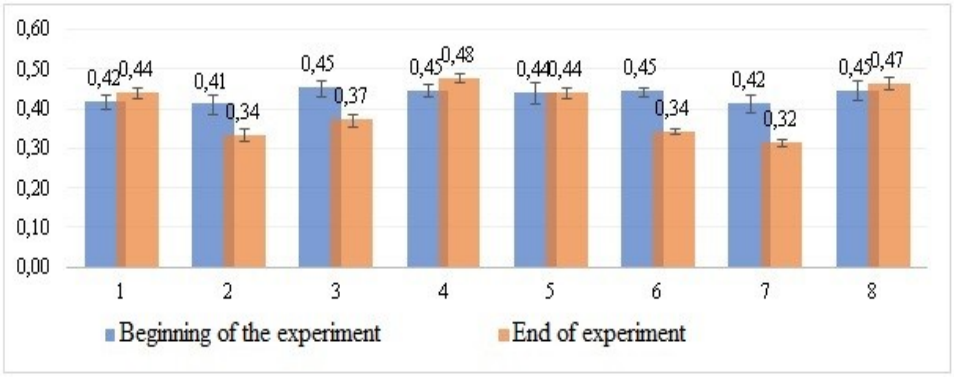

Fig. 4. Changes in NDVI indices in Phragmites australis (Cav.) Trin. ex Steud. depending on the action of the symbiont strain when using technogenically polluted wastewater.

In the course of the study, it was found that an increase in NDVI indices occurred only in three variants in *Phragmites australis* (Cav.) Trin. ex Steud: control (1), a consortium of *Bacillus subtilis* from industrially polluted wastewater (4) and *Azotobacter vinelandii* IB-4 from industrially polluted wastewater (8). In other species, there was a decrease in the index values, which indicates the inhibition of photosynthetic activity, which is apparently due to the establishment of symbiotic relationships and competitive relationships between plants and microorganisms. The photosynthetic systems of plants are the most sensitive to the action of toxicants and, therefore, are the key indicators of the early diagnosis of the inhibition of phytoremediants. At the same time, the data obtained demonstrate that the involvement of microorganisms of the *Bacillus subtilis* (4) and *Azotobacter vinelandii* IB-4 (8) consortium under the influence of technogenically polluted wastewater increases plant resistance.

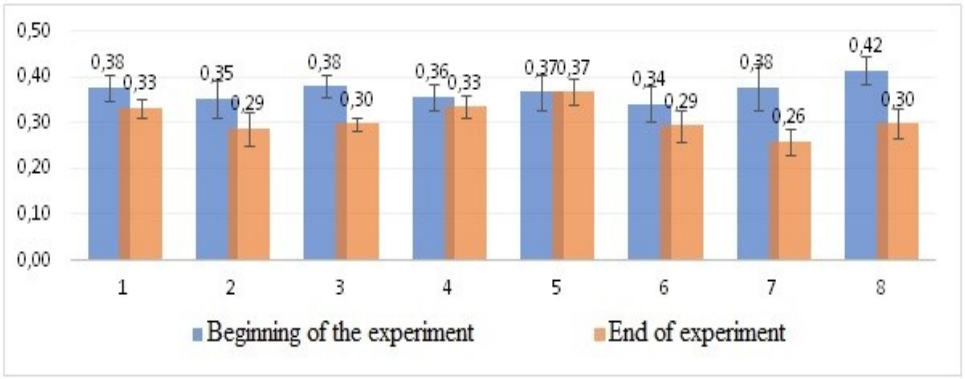

Fig. 5. Changes in the NDVI indices of Typha angustifolia L. depending on the action of the symbiont strain when using technogenically polluted wastewater.

In the process of growth and development in plants *Typha angustifolia* L. and *Typha latifolia* L. in all variants and even in the control, the values of the NDVI indices decreased. When using technogenically polluted wastewater with symbionts, the decrease was much greater than when using only symbionts (with the exception of *Azotobacter vinelandii* IB-4 (8)). Thus, it was found that the photosynthetic systems of plants of these species were constantly depressed during the experiment. This effect seems to be explained either by the LED light source used, which is apparently not efficient enough for growing these species, or by a more powerful root system that had formed by the beginning of the experiment in

plants grown from seeds, and therefore depleted the supply much faster. micronutrients necessary for the formation of pigment complexes.

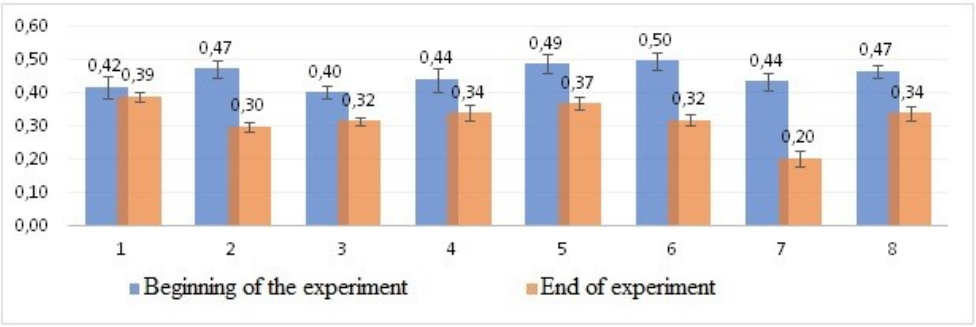

Fig. 6. Changes in the indices of NDVI Typha latifolia L. depending on the action of the symbiont strain when using technogenically polluted wastewater.

4 Conclusion

Summarizing the obtained experimental data, we can draw the following conclusions:

- The effect of different strains of symbiotic bacteria on different plant species differs even in closely related groups, and therefore it is necessary to continue creating new combinations of symbiotic microbial-plant complexes.
- The use of symbionts of the *Azotobacter vinelandii* strain IB-4 contributed to a significant increase in digital biomass by the end of the experiment in *Phragmites australis* (Cav.) Trin. ex Steud. and *Typha angustifolia* L. in comparison with the control, both during inoculation and during inoculation and treatment of technogenically polluted wastewater. In this regard, it seems possible to recommend this strain as an agent that promotes effective adaptation of plants to the action of toxicants when planting them in phytoremediation systems.
- Analysis of photosynthetic activity, assessed through NDVI indicators, indicates a decrease in the content of photosynthetic pigments in *Typha angustifolia* L. and *Typha latifolia* L. plants in all variants and even in control. In *Phragmites australis* (Cav.) Trin. ex Steud. the combined action of technogenically polluted wastewater and the consortium of *Bacillus subtilis* and Azotobacter vinelandii IB-4 contributed to an increase in the content of green pigments.
- The formation of stable and effective symbiotic microbial-plant complexes in the treatment of toxic wastewater components requires special studies that will reveal the features of the interaction between plants and microorganisms. The results obtained indicate that a successful search for combinations of different plant species and complexes of strains of world organisms can contribute to the synergy of the remediation properties of these groups of living organisms and allows in the future to create effective innovative phyto-purification systems of a new type, aimed at neutralizing the action of various types of pollution in technogenic ecosystems.

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References

1. M. Materac, A.Wyrwicka, Sobiecka El″bieta Phytoremediation techniques in wastewater treatment, Environmental biotechnology, **11**, **1**, 10-13 (2015)
2. R. Yuliasni, S.B. Kurniawan, B. Marlena, M.R. Hidayat, A. Kadier, P.C. Ma, M.F. Imron, Recent Progress of Phytoremediation-Based Technologies for Industrial Wastewater Treatment. Journal of Ecological Engineering, **24**, **2**, 208-220 (2023)
3. Harman Gary, Khadka Ram, Doni Febri, Uphoff Norman, Benefits to Plant Health and Productivity From Enhancing Plant Microbial Symbionts, Front. Plant Sci., 12 April 2021 Sec. Plant Pathogen Interactions, 11 (2020)
4. Averlane Vieira da Silva, Mayanne Karla da Silva, Emanuelly Beatriz Tenório Sampaio, Luiz Fernando Romanholo Ferreira, Michel Rodrigo Zambrano Passarini, Valéria Maia de Oliveira, Luiz Henrique Rosa, Alysson Wagner Fernandes Duarte, Chapter 4 - Benefits of plant growth-promoting symbiotic microbes in climate change era, Editor(s): Ajay Kumar, Joginder Singh, Luiz Fernando Romanholo Ferreira, Microbiome Under Changing Climate, Woodhead Publishing, 85-113 (2022)
5. E. Harman Gary, Uphoff Norman, Symbiotic Root-Endophytic Soil Microbes Improve Crop Productivity and Provide Environmental Benefits, Scientifica, **25**, 9106395 (2019)
6. G. Berg, M. Schweitzer, A. Abdelfattah, Missing symbionts – emerging pathogens? Microbiome management for sustainable agriculture, Symbiosis, **89**, 163–171 (2023)
7. Yao Liu, Guandi He, Tengbing He, Muhammad Saleem, Signaling and Detoxification Strategies in Plant-Microbes Symbiosis under Heavy Metal Stress: A Mechanistic Understanding, Microorganisms, **11**, **1**, 69 (2023)
8. Jianwu Wang, Yuannan Long, Guanlong Yu, Guoliang Wang, Zhenyu Zhou, Peiyuan Li, Yameng Zhang, Kai Yang, Shitao Wang, A Review on Microorganisms in Constructed Wetlands for Typical Pollutant Removal: Species, Function, and Diversity, Front. Microbiol., 05 April 2022 Sec. Microbiotechnology, 13 (2022)
9. C. Li, K. Zhou, W. Qin, C. Tian, M. Qi, X. Yan, A review on heavy metals contamination in soil: effects, sources, and remediation techniques. Soil Sediment. Contamination: Int. J., **28**, **4**, 380–394 (2019)
10. I.E. Mejias Carpio, A. Ansari, D.F. Rodrigues, Relationship of biodiversity with heavy metal tolerance and sorption capacity: a meta-analysis approach, Environ. Sci. Technol., **52**, **1**, 184–194 (2018)
11. Nicoletta Rascio, Flavia Navari-Izzo, Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting?, Plant Science, **180**, **2**, 169-181 (2011)
12. G.L. Sun,, E.E. Reynolds, A.M. Belcher, Designing yeast as plant-like hyperaccumulators for heavy metals. Nat Commun, **10**, 5080 (2019)
13. R.D. Reeves, A.J.M. Baker, T. Jaffré, P.D. Erskine, G. Echevarria, van A. der Ent, A global database for plants that hyperaccumulate metal and metalloid trace elements. New Phytol, **218**, 407-411 (2018)
14. S. Rezania, J. Park, P.F. Rupani, N. Darajeh, X. Xu, R. Shahrokhishahraki, Phytoremediation potential and control of *Phragmites australis* as a green phytomass: an overview. Environ Sci Pollut Res Int., **26**, **8**, 7428-7441 (2019)
15. T. Sricoth, W. Meeinkuirt, J. Pichtel, Synergistic phytoremediation of wastewater by two aquatic plants (*Typha angustifolia* and *Eichhornia crassipes*) and potential as biomass fuel, Environ Sci Pollut Res, **25**, 5344–5358 (2018)

16. Y. Lei, L. Carlucci, H. Rijnaarts, A. Langenhoff, Phytoremediation of micropollutants by Phragmites australis, *Typha angustifolia*, and *Juncus effuses*. Int J Phytoremediation, **25**, **1**, 82-88 (2023)
17. Hamed Haghnazar, Kourosh Sabbagh, Karen H. Johannesson, Mojtaba Pourakbar, Ehsan Aghayani, Phytoremediation capability of *Typha latifolia* L. to uptake sediment toxic elements in the largest coastal wetland of the Persian Gulf, Marine Pollution Bulletin, 188, 114699 (2023)