Features of accumulation of sulfur and phosphorus by phytoremediants from technogenically polluted wastewater of mining enterprises

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Abstract. The article presents data on the accumulation of phosphorus and sulfur in the tissues of phytoremediant plants *Phragmites australis* (Cav.) Trin. ex Steud., *Typha angustifolia* L., *Typha latifolia* L., used for the treatment of technogenically polluted wastewater from mining enterprises. The dynamics of changes in digital biomass and normalized chlorophyll ratio index during systematic exposure to mine wastewater is shown. Species have been identified that respond to anthropogenic influence by increasing leaf mass and activating the photosynthetic system of plants. The high degree of phytoextraction of phosphorus and sulfur by Phragmites australis allows us to recommend this species for the production of green fertilizers based on it.

1 Introduction

Currently, the world is paying more and more attention to the use of nature-like technologies that allow optimizing and minimizing production costs, creating sustainable closed-cycle systems that function effectively with minimal human intervention [1-6].

The problem of treating technogenically polluted wastewater from mining enterprises (TPWME) is currently being solved in many countries by using phytoremediant plants: *Phragmites australis (*Cav.) Trin. ex Steud. [7-10], *Typha angustifolia* L. [11-14] and *Typha latifolia* L. [15-18] –. The results of the operation of such systems show a significant reduction in pollutants in water, which indicates a high degree of efficiency of this approach [8, 10, 14, 19-20].

Despite this, among the disadvantages of such approaches, one can highlight the absence of a stage for removing elements accumulated in the vegetative mass of plants, which return to the system when they die. One of the ways to solve this problem is cuttings of plant mass, their processing and use to produce biofuel briquettes, for example, based on *Phragmites australis* [21-22]. The migration of valuable nutrients (phosphorus and sulfur) occurring from technogenically polluted wastewater from mining enterprises into the tissues of phytoremediants can become the basis for the creation of green fertilizers.

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Purpose of the work: to study the physiological state and degree of accumulation of phosphorus and sulfur in the tissues of *Phragmites australis*, *Typha angustifolia* and *Typha latifolia* under the influence of technogenically polluted wastewater from mining enterprises.

2 Materials and methods

The study was carried out on the basis of the laboratory of experimental botany and the laboratory of physical and chemical methods of plant research of the Botanical Garden of the National Research University Belgorod State University using the equipment of a unique scientific installation (Botanical Garden of the Belgorod State National Research University, https://ckp-rf.ru /usu/200997/).

Experience scheme:

- Control of *Phragmites australis*.
- *Phragmites australis* + TPWME.
- Control of *Typha angustifolia*.
- *Typha angustifolia* + TPWME.
- Control of *Typha latifolia*.
- *Typha latifolia* + TPWME.

In laboratory conditions, plants were cultivated on phytoracks with LED lamps ECOLED-60-LX Fito IP 65 60W with a wavelength of 450-730 nm (LED).

The experiment began on day 72 after the emergence of *Typha* seedlings and the introduction of *Phragmites australis* into the *in vitro* culture. The plants were watered with tap water for six days, and on the 7th day they were treated according to the experimental scheme for 28 days.

The determination of P and S was carried out on an AVIO 220 Max optical emission spectrometer after 6 weeks of the experiment; the results are presented as % of the dry weight of the sample.

The dynamics of the physiological state of plants was assessed every 3-4 days on 8 same-aged and morphologically similar specimens using the PlantEye F500 multispectral 3D installation (Phenospex B.V., the Netherlands) according to the following parameters:

- Digital Biomass, cm^3.
- Normalized pigment chlorophyll ratio index (NPCRI) range of values from -1 to 1.

PlantEye F500 HortControl software was used to process the obtained data.

Statistical 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 $p = 0.05$. The growth rate of digital biomass and normalized chlorophyll index was calculated using the formula:

$$\Delta TR = (Pk - Pn) / Pn \times 100\% \quad (1)$$

Where Pk – final values of the indicator; Pn – initial values of the indicator.

3 Results

Figures 1-2 show the results of the accumulation of P and S in the vegetative mass of phytoremediant plants under the influence of technogenically polluted wastewater from mining enterprises by the end of the experiment.

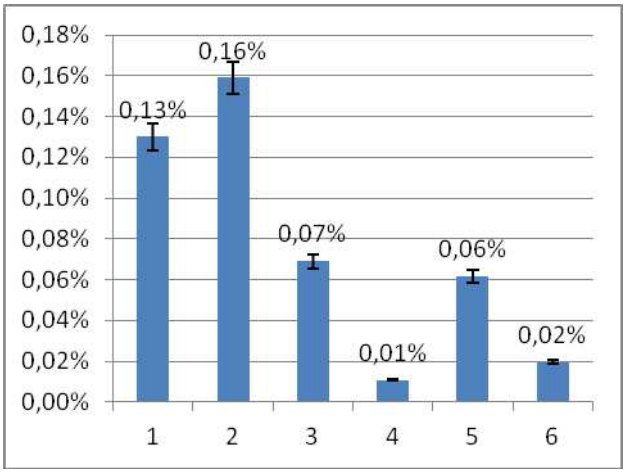

Fig. 1. The level of phosphorus accumulation in phytoremediant plants according to the experimental scheme.

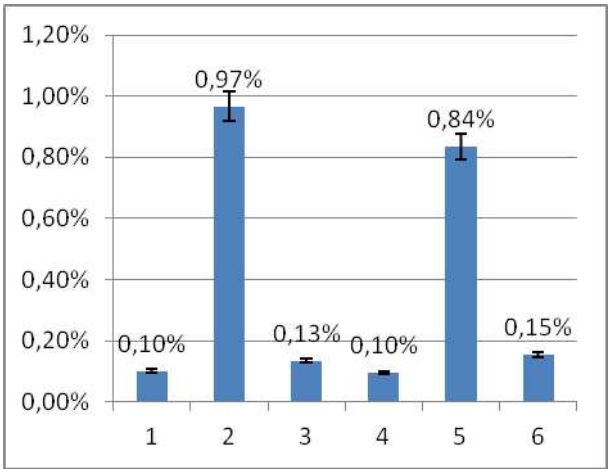

Fig. 2. The level of sulfur accumulation in phytoremediant plants according to the experimental scheme.

Figure 1 shows that the highest degree of phosphorus accumulation in the leaf mass occurs in Phragmites australis; in plants of the genus *Typha* in the control, the level of accumulation is two times lower. When exposed to technogenically polluted wastewater from mining enterprises, the phosphorus content increases only in *Phragmites australis*; in plants of the genus *Typha*, a significant decrease occurs. The absorption of sulfur by plants (Figure 2) in the control occurs as follows: the greatest accumulation is observed in *Typha latifolia*, 7 times more than in *Phragmites australis*. The response of plants to the impact of technogenically polluted wastewater from mining enterprises is similar to how phosphorus accumulated. *Phragmites* absorbs sulfur much more actively than phosphorus, while plants of the genus *Typha* reduce the concentration of this element.

Phosphorus is involved in the synthesis of carbohydrates, is part of nucleic acids and plays a significant role in the process of plant respiration; they are a key component of substances that are vital for the growth and development of cells, as is sulfur, which is part of proteins. In this regard, of particular interest was the assessment of the dynamics of the

morpho-physiological parameters of the development of plants that receive vital elements along with pollutants.

Digital biomass, as an indicator of plant condition, is calculated as the product of the height and area of a 3D leaf, provided that the plant has a shape whose volume can be calculated taking into account the height and length in cm^3 [23-24]. In Figures 3-5 show the dynamics of changes in this parameter during the experiment.

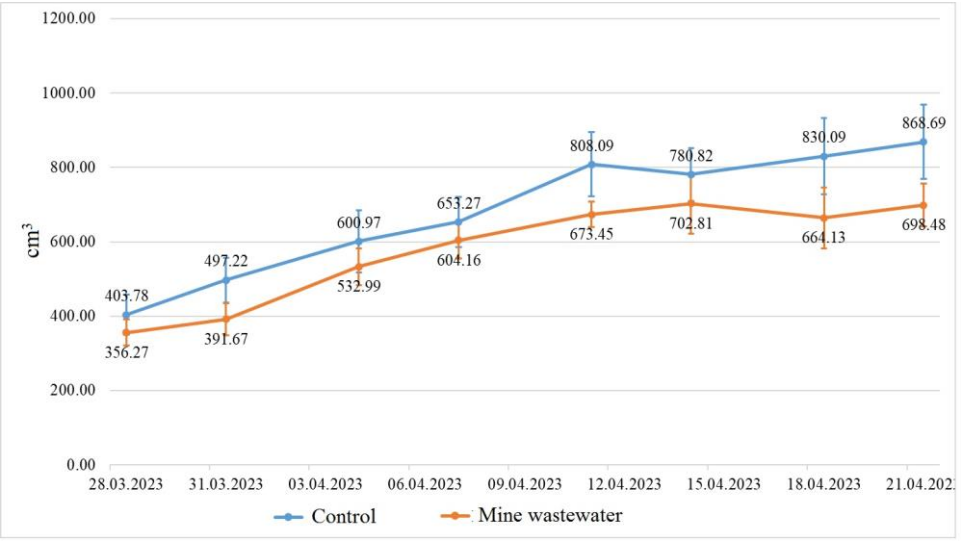

Fig. 3. Dynamics of changes in the digital biomass of *Phragmites australis*.

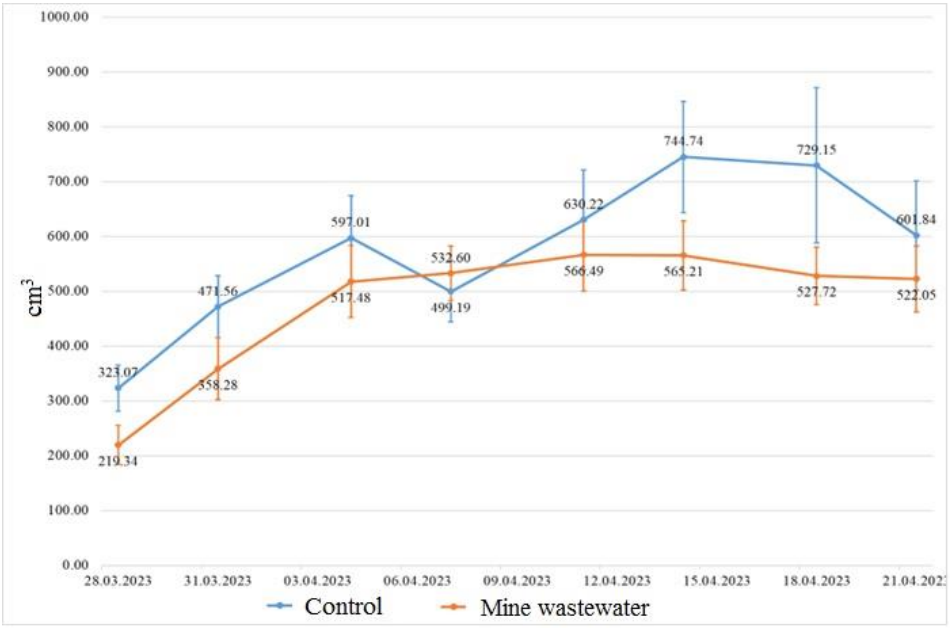

Fig. 4. Dynamics of changes in the digital biomass of *Typha angustifolia*.

Fig. 5. Dynamics of changes in the digital biomass of *Typha latifolia*.

In all three studied plant species, there is a natural increase in digital biomass, both in the control and under the influence of technogenically polluted wastewater from mining enterprises, while the growth rate of digital biomass at the end of the experiment in the control for *Phragmites australis* was 115.14%, and under the influence of mine wastewater 96.05%, which indicates inhibition of growth processes in plants. The growth rate of *Typha latifolia* in the control was 86.29%, and under the influence of mine wastewater 138.01%, so we can confidently say that mine wastewater acts as a catalyst for growth processes for this species. An interesting fact is that the closely related species *Typha angustifolia* practically did not react in any way to the influence of the anthropogenic factor: the growth rate in the control was 99.24%, under the influence of mine wastewater 95.76% - statistically unreliable.

The Normalized Chlorophyll Ratio Index (NPCRI) is an index measured at blue light wavelengths (UV rays) compared to red wavelengths. Chlorophyll fluorescence occurs at both wavelengths; the difference between these two wavelengths, calculated using the formula: (RED − BLUE)/(RED + BLUE), allows one to calculate values correlating with the chlorophyll content [25, 26]. Figures 6-8 display the dynamics of changes in the normalized chlorophyll index of phytoremediant plants under the influence of mine wastewater in the control and model experiments.

The presented graphs show that in *Phragmites australis* there is a trend towards a decrease in the index, so by the end of the experiment the growth rate in the control was -5.72%, and when using mine wastewater 190.64%, thus the composition of the elements contained in the water is acted as catalysts for increasing photosynthetic activity. At the same time, for *Typha latifolia* the growth rate of the index in the control by the end of the experiment was 13.99%, and under the influence of mine wastewater it was 1.97%, and for *Typha angustifolia* it was 4.69% and 31.78%, respectively.

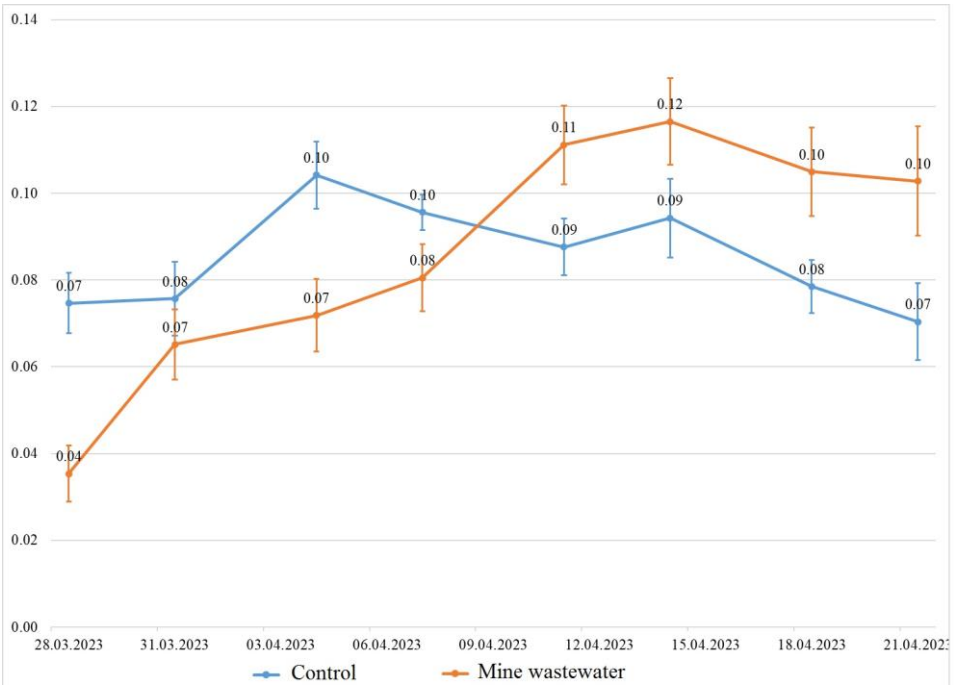

Fig. 6. Dynamics of changes in NPCRI Phragmites australis.

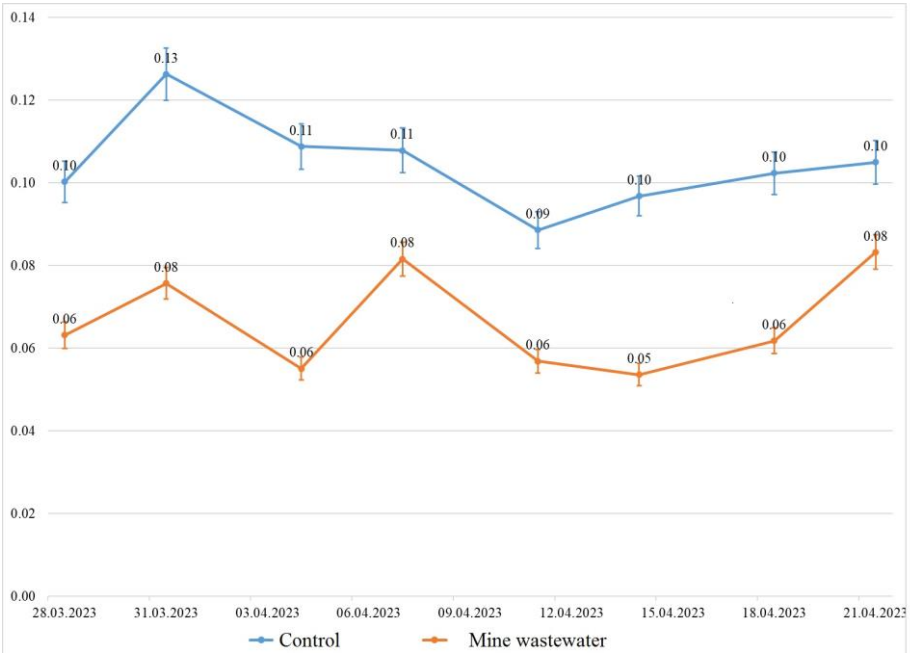

Fig. 7. Dynamics of changes in NPCRI Typha angustifolia.

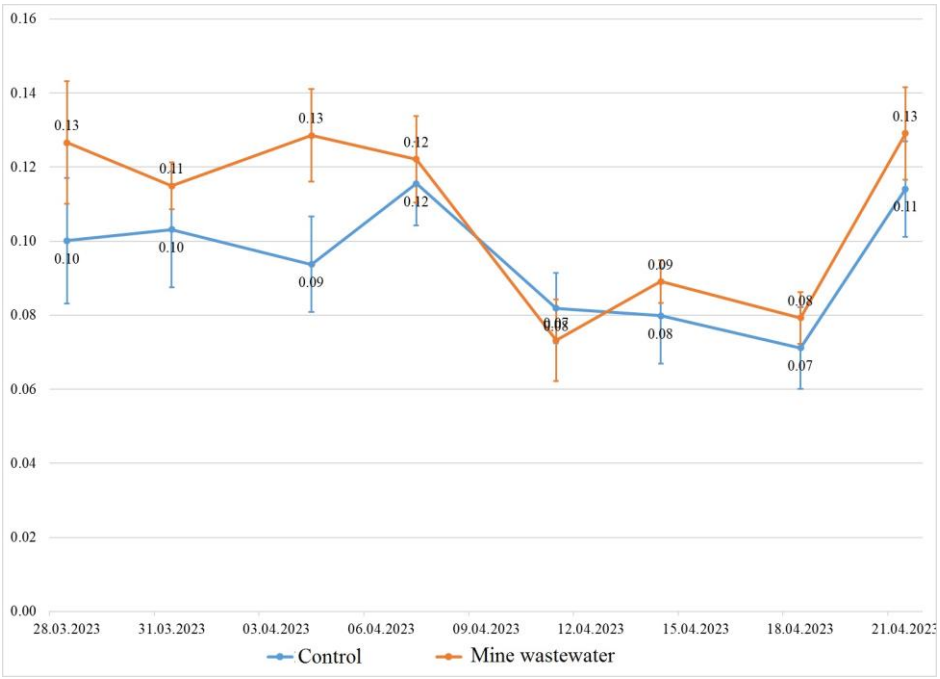

Fig. 8. Dynamics of changes in NPCRI *Typha latifolia*.

4 Discussion

Different degrees of accumulation of phosphorus and sulfur phytoremediants in the green mass of plants are due to differences in localization in vegetative organs. Plants of both genera are rhizomatous perennials, however, *Phragmites* contains a lot of sugar and protein substances in the green grass before the formation of panicles, and in plants of the genus *Typha*, accumulation primarily occurs in the rhizome. This is what most likely explains the decrease in phosphorus and sulfur under the influence of technogenically polluted wastewater from mining enterprises in *Typha latifolia* and *Typha angustifolia* in the experiment. The physiological state of plants, assessed through digital biomass and the NPCRI index, indicates an activation of growth processes in *Phragmites australis* and *Typha latifolia* compared to the control and an increase in photosynthetic activity in *Phragmites australis* and *Typha angustifolia*. This opposite effect of technogenically polluted wastewater from mining enterprises on two closely related species is due to the morphological characteristics of the leaf blades. Thus, *Typha angustifolia* stops growth processes associated with an increase in leaf area, but begins to actively accumulate photosynthetic pigments, and *Typha latifolia* activates growth processes in leaf blades, while there are not enough nutrients for the formation of photosynthetic pigments. It is necessary to conduct research related to the accumulation of phosphorus and sulfur in the rhizomes of the plants under study.

5 Conclusion

During the experiment it was established:

- Technogenically polluted wastewater from mining enterprises increases the content of phosphorus and sulfur in the vegetative green mass of *Phragmites australis* and

decreases it in *Typha angustifolia* and *Typha latifolia* according to the results obtained on an AVIO 220 Max optical emission spectrometer.

- Under the influence of technogenically polluted wastewater from mining enterprises, only in the species *Typha latifolia* there is an increase in the growth rate of digital biomass compared to the control, which is confirmed by the results obtained using the PlantEye F500 multispectral 3D installation when assessing the dynamics of plant development in the experiment.
- The normalized chlorophyll ratio index increases significantly in Phragmites australis and not significantly in *Typha angustifolia*, which indicates the activation of photosynthetic processes under the influence of mine wastewater.
- *Phragmites australis* can act as a source of sulfur and phosphorus for producing green fertilizers and at the same time form biomass in the same volume as under conditions of technogenic pollution. This makes this species the most promising for research in the direction related to the breeding of varieties for various types of pollution and opens up opportunities for a new round of so-called technogenic selection, which will consist in breeding plants with a high accumulating ability to absorb pollutants.

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