Phytoremediation efficiency of duckweed communities for mining enterprises wastewater treatment from nitrogen compounds

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Abstract. The main purpose of the work was to determine the methods of mining enterprises wastewater secondary treatment from nitrogenous compounds. The chemical analysis of wastewater was carried out, and substances with exceeding concentrations were established. The accumulating power of indigenous species of higher aquatic vegetation has been estimated. The absorption regularities of nitrates of duckweed (Lemna minor) in solutions with different concentrations were determined. The expediency of duckweed cultivating with the aim of phytoremediation has been proved.

1. Introduction
One of the most acute problems, that appears during the process of development of mineral deposits, is huge amount of wastewater polluted with different chemical compounds [1-4]. For enterprises using open-pit mining method the list of contaminating components includes such components as nitrogen compounds, mostly in nitrate form. They permeate in waters because of usage of nitrogenous explosives [5-6]. Hardware-based treatment with application of such methods as chlorination, ozonisation, ultraviolet irradiation, electrolysis, ion exchange and others is often ineffective and does not justify the high costs of equipment installation, reagents and operating costs. Due to significant quarry waters volume use of aerotanks for nitrogen compound extraction is not that effective as well.

Modification of mining technology decreases the efficiency index of existing wastewater treatment facilities. Moreover, designing and operating of new wastewater treatment facilities require significant capital and operating costs, what is usually impractical from an economic point of view. That’s why it is obvious that the most perspective methods are those that can be implemented in already existing technological tanks that are currently under operation.

Nowadays, plants growing on a substrate, in particular, sedge, are cultivated on the constructed wetlands. However, the use of fluctuant plants that do not need to fix the root system on the substrate would be an apt supplement to the method of natural biological treatment. In the climatic conditions under consideration, fluctuant plants such as bur-reed and duckweed are occurred.

Savage duckweed (Lemna minor) could be found in the clearing pools. This fact serves as an evidence that the water of the clearing pool is not toxic to the duckweed and contributes to its
development and reproduction. Furthermore, it is known that duckweed absorbs pollutants, including nitrogen-containing compounds in the form of nitrates, therefore, for the successful implementation of wastewater treatment the intensification of denitrification processes is not required [7-11].

2. Methods

In the present study, the clearing pool of an iron-mining enterprise is used as a matter of research. The clearing pool is formed on the site of the former quarry and reaches a length of 260 meters, and in the widest part - 100 meters. It is divided into two sections by means of a filtering protruding dam. Water enters a larger section, where the water is desilting, after which, passing through the filtering dam, it falls into a smaller section where biological treatment takes place. Purified water is discharged into the swamp through an overflow well and branch duct, reaching 54 m lengthwise. The potential output of the clearing pool is 3,590 thousand m³/year, i.e. 9 800 m³/day.

Current situation is - the concentration of nitrates in the water of sedimentation basin exceeds the permissible level several times even after treatment. This situation constitutes a great threat to the environment and requires the adoption of immediate actions for solving this problem. The cause of contamination is the use of explosives containing nitrates, which subsequently permeate into the water drained from the pit. On average, 5000-6000 kg of nitrates, 30-50 kg of nitrite, 60-80 kg of ammonium nitrogen enter the clearing pool monthly.

The bottom relief of the clearing pool is uneven and has a number of areas where its depth of is more than 2 meters. This peculiarity creates difficulties for the application of generally used biological wastewater treatment technologies by creating a protruding bed for planting higher aquatic vegetation. Therefore, constructed wetlands technology was introduced at the clearing pool of the mining and processing enterprise, which is designed to provide the wastewater purification from nitrates.

During the study, the following tasks were sequentially solved:

- current state assessment of the ecosystem of the clearing pool;
- effectiveness evaluation of current wastewater treatment methods from nitrogen compounds;
- development of technology for the wastewater secondary treatment from nitrogen-containing compounds using the method of natural biological treatment.

In order to carry out research on the water state, four samples were taken, three of which - directly from the clearing pool: at the entrance of the pool, after the filtering dam and at the exit from the treatment plant. The fourth water withdrawal was made from a stream flowing through the territory of the enterprise in the clearing pool immediate nearness. Vegetation samples were also taken: of sedge and willow growing in the shallow part of the clearing pool, and of the fluctuant plant - duckweed, for the nitrates content analysis.

Substance mass concentration determination in the water was carried out according to the current methods using photocolorimetry with a portable photocolorimeter «Ecotest 2020». Since the nitrates content in the 1st and 2nd samples was too high, these samples were diluted five times, and further processing of the results was carried out taking into account this dilution. In order to increase the analysis results reliability, the nitrates concentration in water samples was additionally determined on a liquid chromatograph. The metal content in the water samples was as well determined on a parallel-action optical emission spectrophotometer with inductively coupled plasma ICP-9000. The advantage of the ICP-9000 spectrometer is spectral overlays diagnostics, which allows to determine the number of several elements simultaneously and high sensitivity to concentrations up to a few µg/l.

Plant samples analysis is carried out in order to determine the nitrates which were absorbed by the plants from polluted water. This analysis can be performed by analyzing plant extracts on a DR 5000 spectrophotometer or on a liquid chromatograph. For the water extracts preparation, the plants were disintegrated in the mill to a fraction of less than 1 micron. After that a sample of milled plants weighing 1 g is placed in a measuring flask per 100 ml and 50 ml of a 1% potassium alum solution is poured. Further the suspension is stirred using a magnetic stirrer and then filtered to obtain solutions,
which are subjected to further analysis. Since the solutions are highly colored, they must be discolored for optical analysis. The discoloration was conducted using activated carbon adsorption. The discolored solutions were analyzed for nitrate content on a DR 5000 spectrophotometer.

In order to study the duckweed absorptivity an experiment was conducted in laboratory conditions, during which solutions with a nitrate content of 100 mg/l, 250 mg/l and 450 mg/l using natural reservoir water were prepared. Solutions of each concentration were placed in two identical vessels in a volume of 250 ml. In one of the two solutions of the same concentration 50 duckweed leaf blades were placed. The second solution, without duckweed, was used as a reference solution, since the concentration of nitrates may vary with time due to various natural factors. Also, duckweed in the amount of 50 leaf blades was placed in a vessel with water used to prepare solutions of a given concentration. To reduce the evaporation rate, the solutions were covered with a transparent polyethylene film. Seven prepared solutions (two solutions of each concentration and a test solution) were placed in a climate chamber with the maintained air temperature of 20 °C and a light mode changing the time of a day (day-night) every 12 hours. During the next 2 weeks the observations on the growth of duckweed and the nitrates concentration in solutions were made.

3. Results and discussion

The results of the quarry wastewater samples analysis at different treatment stages are presented in table 1.

| № of sample | Concentration of NO$_3^-$, mg/l |
|-------------|--------------------------------|
|             | Photocolorimetric analysis («Ecotest 2020») | Chromatographic method of analysis (LC-20 Prominence) |
| Sample 1   | 476,83 | 312,67 |
| Sample 2   | 443,50 | 287,47 |
| Sample 3   | 133,70 | 77,36  |
| Sample 4   | 113,37 | 63,15  |

As it can be seen from the study results, the content of nitrates in water significantly exceeds the permissible level (40 mg/l) even after purification [12]. Exceeding of the maximum permissible concentration is observed even in a nearby stream (sample 4), this allows making an assumption that water from the clearing pool is seeping through the soil, polluting adjacent surface water bodies. All these facts point on the necessity to improve water purification system from nitrates.

The spectrophotometer measurement results are presented in the table 2.

| Metal | Concentration of NO$_3^-$, mg/l | Spectrometric Analysis Method (ICPE-9000) |
|-------|--------------------------------|------------------------------------------|
|       | sample 1 | sample 2 | sample 3 | sample 4 | MPC       |
| Ba    | 0,551 ± 0,001 | 0,553 ± 0,002 | 0,516 ± 0,003 | 0,578 ± 0,002 | 0,1       |
| Ca    | 96,3 ± 0,4   | 93,1 ± 0,4   | 84,8 ± 0,2   | 95,3 ± 0,5   | 40        |
| Cd    | 0,0005 ± 0,0001 | < 0,0005   | 0,0002 ± 0,0002 | 0,0004 ± 0,00004 | 0,01  |
| Cu    | 0,2 ± 0,02   | 0,79 ± 0,1   | 0,04 ± 0,02  | 0,26 ± 0,025 | 1         |
| Fe    | 0,0083 ± 0,0004 | 0,008 ± 0,001 | 0,008 ± 0,00004 | 0,0114 ± 0,0002 | 0,5 |
| K     | 122,0 ± 0,4  | 119,0 ± 0,04 | 110,0 ± 1   | 121,0 ± 0,07 | 180       |
| Mg    | 14,7 ± 0,05  | 14,5 ± 0,1   | 13,2 ± 0,0005 | 14,7 ± 0,07 | 120       |
| Mn    | 0,037 ± 0,0001 | < 0,0002   | 0,0004 ± 0,0002 | 0,0003 ± 0,0001 | 0,1 |

Table 1. Nitrates content in water samples.

Table 2. Metal content in water samples.
Analysis of water samples revealed more than twice time exceeding of calcium concentration and more than five times of barium. This indicates the need for additional water treatment from these metals, but this problem was not considered in the framework of this work.

The results of the phytomass study of aquatic vegetation on the content of nitrates are given in table 3.

**Table 3.** The results of measuring the nitrates content in the samples of plants.

| Plant name          | Absorption signal | Concentration of NO$_3^-$, mg/l | Concentration of NO$_3^-$, mg/kg | Nitrogen content, mg/kg |
|---------------------|-------------------|--------------------------------|---------------------------------|------------------------|
| Sedge               | 0,305             | 25,95                          | 12973,91                        | 2929,59                |
| Goat willow         | 0,019             | 1,95                           | 973,91                          | 219,92                 |
| Duckweed            | 0,269             | 22,99                          | 11408,70                        | 2576,16                |

The results of the study show a high absorption capacity for nitrates in sedge and duckweed, which determines the choice of these particular plant species for phytoremediation.

An experiment of nitrates absorption efficiency by duckweed under climate chamber conditions made it possible to evaluate the plant's response to the initial pollutant concentration in the solution and the absorption efficiency of nitrates by the plant. The results are shown in figure 1.
excess of nitrates in water limit the development of the plant and reduce the effectiveness of bioremediation.

4. Conclusion
As a result of the study, it was determined that the wastewater of the enterprise contains high concentrations of nitrogen compounds. The currently used scheme of biological treatment with use of constructed wetlands does not reduce the concentration of nitrates to the maximum permissible level. The macrophytes growing in the water area of the clearing pool may be used as an additional method of treatment. In the existing range of nitrogenous compounds concentrations, the cultivation of duckweed (Lemna minor) can be an effective and reasonably priced method of secondary treatment. Phytomass excess can be used as a fertilizer or as a source of extra nutrition in livestock or fish farming [13], [14].

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