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## The strategies of nutrient removal compounds from wastewater by using aquatic plants in the Green deal implementation

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#### ABSTRACT

The aim of the study was to determine the efficiency of wastewater treatment from nitrogen compounds - ammonium, nitrite, nitrate, and phosphate - using *Lemna minor* and *Spirogyra sp.* in the context of the strategies toward Green Deal Implementation. Meanwhile, the various biological treatment methods of nutrients according to the value-added and application prospects were also discussed as a future solution in the Circular Economy Model. The complementary effect of *Lemna minor* and other higher aquatic plants, in particular *Spirogyra sp.* was established. The efficiency of nitrate removal for 7 days was 86.4 %, and phosphate removal was 78 %. The concentration of NO<sub>3</sub> <sup>-</sup> ions after treatment was 6.8 mg/dm<sup>3</sup>, and phosphate (P<sub>2</sub>O<sub>5</sub>) 2.2 mg/dm<sup>3</sup>. It was noted that the simultaneous use of an increased dose of duckweed *Lemna minor* and the algae *Spirogyra sp.* significantly increases the efficiency of nitrate removal from the experimental samples, and the efficiency of phosphate removal did not change compared to the experiment without the use of the algae Spirogyra sp. and with the use of a double dose of duckweed. Under these conditions, the nitrate removal efficiency for a duration of 7 days was 892 % and the phosphate (P<sub>2</sub>O<sub>5</sub>) 2 mg/dm<sup>3</sup>.

#### 1. Introduction

Recently, the method of wastewater treatment from various contaminants: metal ions, nitrogen and phosphorus compounds, etc., using higher aquatic plants, in particular duckweed - *Lemna minor*, has become widely used. The method is implemented in wetland-type facilities [1–5], where all biocenosis organisms are involved in wastewater treatment processes [6,7].

The ability of duckweed to remove up to 98 % of chromium, 30 % of lead, iron, cadmium, and copper from industrial wastewater has been established [8–12]. Duckweed is very active in physiological and biochemical aspects in relation to pollutants - it converts toxic compounds into less toxic ones [13,14]. They are capable of assimilating and transforming various substances, promote the precipitation of suspended solids, saturate water with oxygen, and intensify treatment processes.

The mechanism of nitrate and phosphate removal by the plant is that these substances are biogenic and are accumulated by the plant for their further involvement in biochemical transformations. Duckweed can remove up to 95 % of nitrates and up to 82 % of ammonia. Duckweed is undemanding to environmental conditions and can be easily cultivated under artificial conditions. Under favourable environmental conditions, duckweed can remove pollutant nitrogen and phosphate compounds several times faster than using biological wastewater treatment technologies in aeration tanks, even at high concentrations of pollutants [15]. Duckweed uses nitrogen compounds (ammonium, nitrates) as biogenic substances for the construction of cellular substances: proteins, amines, membranes, etc., in the processes of growth, reproduction growth of the biomass of aquatic plants.

*Lemna minor* is a species of free-floating higher aquatic plants that are among the smallest flowering plants in the world. The body of the plant consists of individual small rounded or egg-shaped plates that are connected and have a single root process. The diameter of the plates varies between 2 and 5 mm. It grows in groups, grows rapidly and forms a continuous green cover. The growth of duckweed biomass increases under conditions of stagnant water and high concentrations of biogenic

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nitrogen and phosphorus compounds. It is widespread in freshwater bodies with a slow flow: ponds, swamps, lakes, streams. These plants occupy the surface of the reservoir and are classified as phytoplankton. They reproduce through vegetative division with the help of buds, and thus each plant can form many new ones during its lifetime [16,17].

Lemna minor plants are warm and light-loving. Changes in optimal environmental conditions, such as a significant decrease in temperature and a change in the biorhythm associated with a reduction in daylight hours in autumn and winter, lead to a slowdown in the assimilation of essential trace elements, which include biogenic nitrogen and phosphorus compounds [18–21]. The death of the plants under study is also observed. That is why it is imperative to establish an optimal environment in artificial conditions. The growth of plant biomass directly depends on the assimilation of the main trace elements, which include nitrogen and phosphorus [22,23].

Therefore, the aim of the study was to determine the efficiency of wastewater treatment from nitrogen compounds - ammonium, nitrite, nitrate, and phosphate - using Lemna minor and Spirogyra sp. in the context of the strategies toward Green Deal Implementation. Meanwhile, the various biological treatment methods of nutrients according to the value-added and application prospects were also discussed as a future solution in the Circular Economy Model.

#### 2. Material and methods

#### 2.1. Experimental selection of plant material and cultivation

Cultivation of duckweed in laboratory conditions was carried out in a cultivator with dimensions of  $40 \times 30$  cm and a depth of 20 cm. To grow watercress, we used Steinberg's medium, prepared by dissolving a mixture of salts in distilled water (Table 1). Lighting was provided by a fluorescent lamp with a power of 15 W and a luminous flux of 3000 lux. The duration of daylight was 12 hours a day. The temperature was maintained by a heater at the level of 23-25°C.

To carry out research on the wastewater treatment process in the experimental bioreactor, watercress was taken from the cultivator in an amount of no more than 50 % of the surface of the water mirror and no more than once a week. Once a week, half of the volume of nutrient medium in the bioreactor was replaced with fresh.

During the selection of plant material, the appearance of the biomass of higher aquatic plants that were previously cultivated in the phytoreactor was carefully examined to detect any deviations from normally developed plants. An  $11 \times 9$  cm aquarium net made of nylon material was used to collect plants from the phytoreactor. Lemna minor was collected from the surface of the water in the phytoreactor by accumulating duckweed biomass in an aquarium net and allowing the excess water to drain for 1-2 minutes. After that, the net with duckweed was placed on a paper towel and allowed to absorb the excess moisture for another 1 min. After that, the duckweed was weighed in the required quantities on a Scout Pro laboratory balance and subsequently used in the experiments. Also, for a series of experiments, higher aquatic plants

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Table 1	
Concentrations of chemical compounds in a solution for cultivating w	vatercress.

Chemical compounds	Concentration in solution, mg/dm <sup>3</sup>
KNO <sub>3</sub>	350
$Ca(NO_3)_2 \cdot 4H_2O$	295
KH <sub>2</sub> PO <sub>4</sub>	90
K <sub>2</sub> HPO <sub>4</sub>	12,6
MgSO <sub>4</sub> ·7H <sub>2</sub> O	100
H <sub>3</sub> BO <sub>3</sub>	0,12
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0,18
Na2MoO4·2H2O	0,04
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0,18
FeCl <sub>3</sub> ·6H <sub>2</sub> O	0,76
Trylon B	1,5

Spirogyra sp. were used. These algae were collected by catching them with a net from the water column and allowing the excess water to drain for 1–2 minutes. After that, the net with the plants was placed on a paper towel and allowed to absorb the excess moisture for another 1 minute. After that, the algae were weighed in the required quantities on a Scout Pro laboratory balance and further used in the experiments.

Duckweed can be cultivated both in separate phytocultivators and directly in biological ponds, where the plant is used for biological wastewater treatment. The optimal density of the mother crop for the most efficient biomass growth is 500–700 g/m<sup>2</sup>. Increasing the planting density does not always slow down the biomass growth, as even under such conditions, sunlight is sufficient for the plants due to the stratification. When the crop density exceeds 4000  $g/m^2$ , the intensity with which biomass is accumulated decreases significantly, and a decrease in the crop density to 100–300  $g/m^2$  leads to a massive increase in the amount of blue-green, green and diatoms. The intensity of biomass accumulation also depends on the frequency of harvesting its growth. Too frequent harvesting of duckweed leads to mechanical damage to the plants and negatively affects the growth, while too rare harvesting of the growth leads to an excess of the optimal crop density, which results in a decrease in the accumulation rate.

#### 2.2. Preparation of model solutions and determination of nitrogen ion and phosphate content

To determine the efficiency of ammonium nitrogen removal by Lemna minor, a model solution was prepared using NH<sub>4</sub>Cl salt with concentration of ammonium nitrogen of 5 mg/dm<sup>3</sup>. To determine the concentration of NH<sub>4</sub><sup>+</sup> ions in the experimental solutions, the ionometer I160 MI and the measuring electrode to it ELIS-121 NH<sub>4</sub><sup>+</sup> were used.

To determine the efficiency of nitrate removal by Lemna minor, a model solution was prepared using KNO3 salt with nitrate concentration of 50 mg/dm<sup>3</sup>. To determine the concentration of  $NO_3^{-1}$  ions in the test solutions, the ionometer I160 MI and the measuring electrode to it were used ELIS -121 NO3 -.

To determine the efficiency of phosphate removal by Lemna minor, a model solution was prepared using KH<sub>2</sub>PO<sub>4</sub> salt with phosphate concentration of 63 mg/dm<sup>3</sup>. Phosphates (P<sub>2</sub>O<sub>5</sub>) were determined by a spectrophotometric method based on measuring the optical density of coloured solutions formed by the interaction of orthophosphate ions with molybdate in an acidic environment. This process produces a yellow heteropolyacid, which is converted into an intensely coloured blue compound under the influence of reducing agents. The optical density was determined using a ULAB 102 spectrophotometer at a wavelength of  $\lambda = 690$  nm.

#### 2.3. Experimental setup

The experimental setup used for the study was in the form of model bioponds, the dimensions of which were 12x9x2 cm, 216 ml in volume, made of plastic. A total of 32 models of such bioponds were used in the experimental studies. To conduct the experiment, the model biopond was filled with the appropriate model solution, biological plant material was added and left for the required period of time in a place with direct access to sunlight.

#### 2.4. Methodology of the experiment

The experiment was conducted in several stages. All experiments were divided into two series: determining the efficiency of sample treatment from nitrates by higher aquatic plants and determining the efficiency of sample treatment from phosphates by higher aquatic plants.

1) Determination of the treatment efficiency of a model solution containing NO3 <sup>-</sup> ions with a concentration of 50 mg/dm3. In all

installations, the volume of the model solution was the same and amounted to 100 ml. All experiments were conducted under exactly the same conditions. The duration of the experiment was 7 days (144 hours). For this experiment, 16 model units were used. The biological material was the higher aquatic plants Lemna minor and Spirogyra sp. 3 types of higher aquatic plants were used for the experiments  $m_1 = 11$  g (Lemna minor);  $m_2 = 22$  g (Lemna minor);  $m_3 = 3$  g (Spirogyra sp.), which were prepared as described above. The model solution was poured into the model units and the biological material was distributed as follows: in units 1, 2, 3, 4, the higher aquatic plants Lemna minor were loaded with 11 g each; in units 5, 6, 7, 8, the higher aquatic plants Lemna minor were loaded with 22 g each; in units 9, 10, 11, 12, the higher aquatic plants Lemna minor and Spirogyra sp. were loaded with 11 g and 3 g, respectively; in units 13, 14, 15, 16, the higher aquatic plants Lemna minor and Spirogyra sp. were loaded with 22 g and 3 g, respectively. The readings were taken on the first, third, fifth and seventh days of the experiment. On the first day of the experiment, NO<sub>3</sub><sup>-</sup> ion concentration readings were taken from units 1, 5, 9, 13; on the third day from units 2, 6, 10, 14; on the fifth day of the experiment from units 3, 7, 11, 15; on the seventh day of the experiment from units 4, 8, 12, 16. The data obtained were processed using Microsoft Excel.

2) Determination of the treatment efficiency of the model solution containing phosphate ( $P_2O_5$ ) and a concentration of 10 mg/dm<sup>3</sup>. In all installations, the volume of the model solution was the same and amounted to 100 ml. All experiments were conducted under exactly the same conditions. The duration of the experiment was 7 days (144 hours). For this experiment, 16 model units were used. The biological material was the higher aquatic plants Lemna minor and Spirogyra sp. 3 types of higher aquatic plants were used for the experiments:  $m_1 = 11$  g (Lemna minor);  $m_2 = 22$  g (Lemna minor);  $m_3$ = 3 g (*Spirogyra sp.*), which were prepared as described above. Model solution was poured into the model units and the biological material was distributed as follows: in units 1, 2, 3, 4, the higher aquatic plants Lemna minor were loaded with 11 g each; in units 5, 6, 7, 8, the higher aquatic plants Lemna minor were loaded with 22 g each; in units 9, 10, 11, 12, the higher aquatic plants Lemna minor and Spirogyra sp. weighing 11 g and 3 g, respectively; in units 13, 14, 15, 16, higher aquatic plants Lemna minor and Spirogyra sp. weighing 22 g and 3 g, respectively, were loaded. The readings were taken on the first, third, fifth and seventh days of the experiment. On the first day of the experiment, the concentration of phosphate  $(P_2O_5)$  was taken from units 1, 5, 9, 13; on the third day from units 2, 6, 10, 14; on the fifth day of the experiment from units 3, 7, 11, 15; on the seventh day of the experiment from units 4, 8, 12, 16. The concentration of phosphate (P2O5) was determined as described above. The data obtained were processed using Microsoft Excel and MATLAB.

#### 3. Results and discussion

### 3.1. Research to establish the optimal conditions required for Lemna minor

Since there are no data in the literature on the optimal conditions required for *Lemna minor*, a series of experiments were conducted to establish them. Fig. 1 shows a phytobioreactor for the cultivation of *Lemna minor* with artificial lighting and heating.

For the experiment, a model solution based on water from a natural reservoir with an initial concentration of nitrate ions of 50 mg/dm<sup>3</sup>, ammonium nitrogen of 1.1 mg/dm<sup>3</sup>, and phosphate of 2 mg/dm<sup>3</sup> was used. In the autumn and winter period, there is a slowdown in biomass accumulation and the onset of yellowing and death of some plants. When a heater was installed in the phytobioreactor at 23–26°C and artificial fluorescent lighting was applied for 8–9 hours, biomass accumulation resumed and massive plant death stopped. After 24 hours, the concentration of nitrate ions was 21.4 mg/dm<sup>3</sup>, ammonium nitrogen

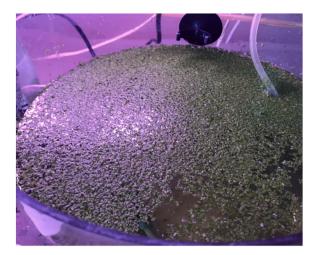


Fig. 1. Phytobioreactor with higher aquatic plants Lemna minor.

1 mg/dm<sup>3</sup>, and phosphate  $1.8 \text{ mg/dm}^3$ . After 48 hours, the concentration of nitrate ions was 13 mg/dm<sup>3</sup>, ammonium nitrogen  $0.8 \text{ mg/dm}^3$ , and phosphate  $1.6 \text{ mg/dm}^3$ .

A decrease in the concentration of nutrients indicates normal, uninhibited metabolic processes of these higher aquatic plants.

To determine the difference in wastewater treatment efficiency under conditions typical for the autumn-winter period (reduced daylight hours, low water and air temperatures) and under conditions favourable for plant growth, the following series of experiments was conducted.

A model solution of settled tap water with a volume of  $0.5 \text{ dm}^3$  and an initial concentration of ammonium nitrogen of 5 mg/dm<sup>3</sup> was used for the experiment. *Lemna minor* duckweed biomass weighing 10 g was used. The experiment involved a three-stage water treatment for 6 days, with the replacement of duckweed every 2 days with a fresh portion of biomass of the same amount. After 48, 96, 144 hours from the start of the experiment, the concentration of ammonium nitrogen was 3.18 mg/ dm<sup>3</sup>, 2.04 mg/dm<sup>3</sup> and 0.98 mg/dm<sup>3</sup>, respectively, with a treatment effect of 80.4 %.

In parallel, a similar experiment was conducted, but with optimal environmental conditions: a water heater at  $23^{\circ}$ C and artificial fluorescent lighting with a duration of 8 hours. A model solution of settled tap water with a volume of  $0.5 \text{ dm}^3$  and an initial concentration of ammonium nitrogen of 5 mg/dm<sup>3</sup> was used for the experiment. *Lemna minor* duckweed biomass weighing 10 g was used. The experiment involved a three-stage water treatment for 7 days, with the replacement of duckweed every 2 days with a fresh portion of biomass of the same amount. 48, 96, 144 h after the start of the experiment, the concentration of ammonium nitrogen was  $1.12 \text{ mg/dm}^3$ ,  $0.76 \text{ mg/dm}^3$  and  $0.49 \text{ mg/dm}^3$ , respectively, with a 90.2 % effect of such treatment.

A comparison of the effects of water treatment from ammonium nitrogen by the higher aquatic plant *Lemna minor* under different environmental conditions is shown in Fig. 2.

The influence of more favourable conditions can be noted even in the external condition of duckweed. In the sample where artificial lighting and heating were used, much fewer yellowed plants were observed (Fig. 3).

The study of biological treatment of biogenic phosphorus and nitrogenous compounds using the higher aquatic plant *Lemna minor* was carried out using model solutions prepared based on the concentrations of pollutants inherent in real wastewater.

After analysing the content and concentrations of real Ukrainian wastewater, a model solution was prepared with a nitrate concentration of 50 mg/dm<sup>3</sup> and a phosphate concentration of 10 mg/dm<sup>3</sup>. After treatment in accordance with the current Ukrainian standards, the concentrations of nitrates and phosphates in the treated water before discharge into a natural water body, which will not cause environmental

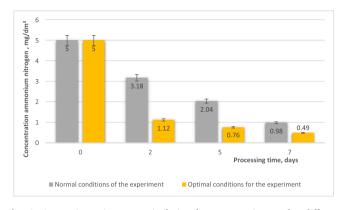
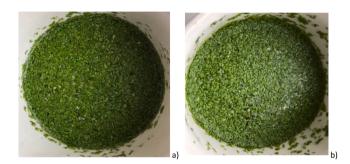


Fig. 2. Ammonium nitrogen assimilation by *Lemna minor* under different environmental conditions depending on the treatment duration.



**Fig. 3.** Photos of *Lemna minor* under different conditions of the experiment: a) normal conditions; b) with heating and artificial lighting.

damage to the water body itself and the environment, should not exceed 10 mg/dm $^3$  and 2 mg/dm $^3$ , respectively.

The model solutions were prepared in accordance with the methodology described above, and the studies were carried out on the model installation in accordance with the methodology described above.

The study was divided into several stages and conducted on modern laboratory equipment in the laboratory in compliance with all safety requirements.

### 3.2. Investigation of the dependence of the effect of nitrate removal by Lemna minor on the duration and weight of nitrate removal

The research consisted of two parallel experiments (experiment 1 and experiment 2), which were conducted under the same conditions for 7 days. For experiment 1, a duckweed weight of  $m_1$ = 11 g was used. For experiment 2, a duckweed sample weighing  $m_2$ = 22 g was used. The results were measured on the 2nd, 5th and 7th day after the start of the experiment. The same plastic containers were used for the experiment 1 and 4 pieces for experiment 2. The biological pond models were placed in one place with direct access to sunlight. Fig. 4 show the change in the measured values of nitrate concentration depending on the duration (in days) from the beginning of the experiment.

From the results of the solution concentrations obtained, the efficiency of treatment of the model solution from nitrates was calculated depending on the duration of the experiment. Fig. 4 show the change in the calculated nitrate removal efficiency as a function of the duration from the beginning of the experiment.

Thus, after analysing the data obtained, it can be stated that the treatment of polluted water from nitrogen compounds is different depending on the weight of duckweed used. The reduction of nitrate concentration in the model solutions is better in Experiment 2, where a larger weight of *Lemna minor* was used. This is also evidenced by the

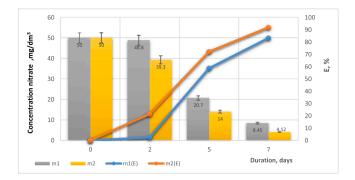


Fig. 4. Changes in nitrate concentration and removal efficiency in the model solution with the duration of the experiment.

calculated treatment efficiency, which was 83.1% in experiment 1 against 91.8% in experiment 2. The decrease in nitrate concentration occurs due to the increase in biomass over time, which leads to an increase in the need for nitrogen to build cellular matter.

## 3.3. Investigation of the dependence of the effect of phosphate removal by Lemna minor on the duration and weight of the plant

The research consisted of two parallel experiments (experiment 3 and experiment 4), which were conducted under the same conditions for 7 days. For experiment 3, a duckweed weight of  $m_1$ = 11 g was used. For experiment 4, a duckweed sample weighing  $m_2$  = 22 g was used. The results were measured on the 2nd, 5th and 7th day after the start of the experiment. The same plastic containers were used for the experiment 4. The biological pond models were placed in one place with direct access to sunlight. Fig. 5 show the change in the measured phosphate concentration values as a function of time since the start of the experiment.

From the results of the solution concentrations obtained, the efficiency of treatment of the model solution from phosphates was calculated depending on the duration of the experiment. Fig. 5 show the change in the calculated phosphate removal efficiency as a function of the duration from the beginning of the experiment.

Thus, after analysing the data obtained, it can be stated that the treatment of contaminated water from phosphorus compounds is different depending on the weight of duckweed used. The reduction of phosphate concentration in the model solutions is better in Experiment 4, where a larger weight of *Lemna minor* was used. This is also evidenced by the calculated treatment efficiency, where the treatment effect in experiment 3 was 73 % compared to 81 % in experiment 4.

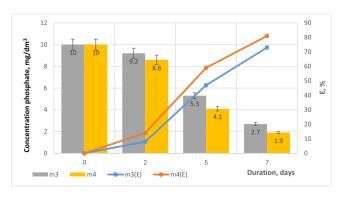


Fig. 5. Changes in phosphate concentration and removal efficiency in the model solution with the duration of the experiment.

3.4. Investigation of the dependence of the effect of nitrate removal by the higher aquatic plants Lemna minor and Spirogyra sp. on the duration and their weight

The research consisted of two parallel experiments (experiment 5 and experiment 6), which were conducted under the same conditions for 7 days. For experiment 5, a duckweed sample of  $m_1 = 11$  g and an algae sample of  $m_3 = 3$  g were used. For experiment 6, a duckweed sample weighing  $m_2 = 22$  g and an algae sample  $m_3 = 3$  g were used. The results were measured on the 2nd, 5th and 7th day after the start of the experiment. The same plastic containers were used for the experiment in the amount of 4 pieces for experiment 5 and 4 pieces for experiment 6. The biological pond models were placed in one place with direct access to sunlight. Fig. 6 show the change in the measured nitrate concentration values as a function of time since the start of the experiment.

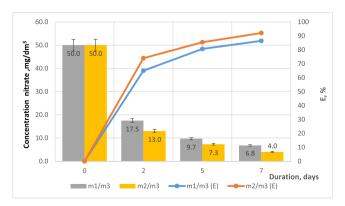
From the results of the solution concentrations obtained, the efficiency of treatment of the model solution from nitrates was calculated depending on the duration of the experiment. Fig. 6 show the change in the calculated nitrate removal efficiency as a function of the duration from the beginning of the experiment.

Thus, after analysing the data obtained, it can be stated that the treatment of polluted water from nitrogen compounds is different depending on the masses of duckweed and algae used. The reduction of nitrate concentration in the model solutions is better in Experiment 6, where a larger weight of *Lemna minor* was used. This is also evidenced by the calculated treatment efficiency, where the treatment effect in experiment 5 was 86.4 % compared to 92.0 % in experiment 6.

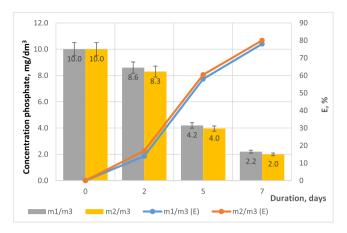
# 3.5. Investigation of the dependence of the effect of phosphate removal by the higher aquatic plants Lemna minor and Spirogyra sp. on the duration and their weight

The research consisted of two parallel experiments (experiment 7 and experiment 8), which were conducted under the same conditions for 7 days. For experiment 7, a duckweed sample of  $m_1 = 11$  g and an algae sample of  $m_3 = 3$  g were used. For experiment 8, a duckweed sample of  $m_2 = 22$  g and an algae sample of  $m_3 = 3$  g were used. The results were measured on the 2nd, 5th and 7th day after the start of the experiment. The same plastic containers were used for the experiment and 4 pieces for experiment 8. The biological pond models were placed in one place with direct access to sunlight. Fig. 7 show the change in the measured phosphate concentration values as a function of time since the start of the experiment.

From the results of the solution concentrations obtained, the efficiency of treatment of the model solution from phosphates was calculated depending on the duration of the experiment. Fig. 7 show the change in the efficiency of phosphate removal depending on the duration from the beginning of the experiment.



**Fig. 6.** Changes in nitrate concentration and removal efficiency in the model solution with the duration of the experiment.



**Fig. 7.** Changes in the concentration and removal efficiency of phosphate in the model solution with the duration of the experiment.

Thus, after analysing the data obtained, it can be stated that the treatment of contaminated water from phosphorus compounds is different depending on the masses of duckweed and algae used. The reduction of phosphate concentration in the model solutions is better in experiment 8, where a larger weight of duckweed *Lemna minor* was used. This is also evidenced by the calculated treatment efficiency, where the treatment effect in experiment 7 was 78 % compared to 80 % in experiment 8.

#### 4. Conclusions

Based on the research, it was found that with an increase in the amount of Lemna minor biomass, the efficiency of treatment of model solutions from nitrogen and phosphorus compounds does not decrease, but rather increases. Even with an increased density, the plants have enough light for photosynthesis and no duckweed death in the lower layer is observed. The duckweed planted in a single layer shows good results, and the water treated by this method meets the standards for discharge into the reservoir in terms of nitrate content and requires minor additional treatment in terms of phosphate content. The efficiency of such treatment for nitrogen over a period of 7 days is 83 % and for phosphorus 73 %, and the concentrations of  $NO_3$  <sup>-</sup> and phosphate (P<sub>2</sub>O<sub>5</sub>) after treatment are 8.45 mg/dm<sup>3</sup> and 2.7 mg/dm<sup>3</sup>, respectively. With the use of a double duckweed Lemna minor, the efficiency of treatment of model solutions from both phosphorus and nitrogen compounds for a duration of 7 days increases and amounts to 81 % and 91.8 %, respectively, and the concentration of NO3<sup>-</sup> ions after treatment under such conditions is  $4.12 \text{ mg/dm}^3$  and phosphorus  $1.9 \text{ mg/dm}^3$ . These concentrations do not exceed the current standards for discharging treated water into natural water bodies.

The complementary effect of Lemna minor and other higher aquatic plants, in particular Spirogyra sp. was established. The efficiency of nitrate removal for 7 days was 86.4 %, and phosphate removal was 78 %. The concentration of NO<sub>3</sub>  $^{-}$  ions after treatment was 6.8 mg/dm<sup>3</sup>, and phosphate  $(P_2O_5)$  2.2 mg/dm<sup>3</sup>. It was noted that the simultaneous use of an increased dose of duckweed Lemna minor and the algae Spirogyra sp. significantly increases the efficiency of nitrate removal from the experimental samples, and the efficiency of phosphate removal did not change compared to the experiment without the use of the algae Spirogyra sp. and with the use of a double dose of duckweed. Under these conditions, the nitrate removal efficiency for a duration of 7 days was 92 % and the phosphate removal efficiency was 80 %. The concentration of NO $_3$  <sup>-</sup> ions after treatment was 4 mg/dm $^3$ , and phosphate  $(P_2O_5)$  2 mg/dm<sup>3</sup>. A decrease in the concentration of known elements such as nitrogen and phosphorus occurs due to an increase in biomass over time, which leads to an increase in the need for nitrogen to build

the cellular substance of duckweed itself.

Thus, the biological method of water and wastewater purification from nitrogen and phosphorus compounds using *Lemna minor* alone or in combination with Spirogyra sp. have shown high efficiency in removing such compounds and can be implemented in water treatment plants for deep water purification with low economic costs. These results are contained in the proposals for the renewal of the Urban Wastewater Treatment Directive, which has been in force since 1991 and must be updated, adapted to new challenges and realities, according to the decision of the European Commission as part of the European Green Deal in 2022. Such as setting stricter standards for nutrient removal and aligning the directive with the ambitions of the Green Deal, with a commitment to energy neutrality for treatment plants.

The used and removed higher aquatic plants can be used as fertilizer or as feedstock for biogas production in a circular economy model. It is also known that duckweed has a significant amount of protein and is food for waterfowl, as well as (mixed with bran and flour) for pigs, geese and chickens. Therefore, a large-scale and efficient nutrient recovery system using *Lemna minor* alone or in combination with *Spirogyra sp.* should be studied and proposed. from ecological and economic points of view in the context of implementing the assumptions of the circular economy.

#### CRediT authorship contribution statement

**Zhukova Veronika:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Data curation. **Jakub Drewnowski:** Writing – review & editing, Supervision, Resources, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sablii Larysa:** Writing – original draft, Validation, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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