

## Photosynthetic apparatus variability of the rigid hornwort (*Ceratophyllum demersum* L.) from the Saksagan River influenced by the mine activity

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Aquatic ecosystems are the final absorbers of various pollutants, so require complex monitoring to control their condition. Macrophytes and other aquatic plants serve as reliable indicators of the hydro ecosystems ecological state, as they provide an integral assessment of the aquatic environment in time and space. Changes in chlorophyll a and chlorophyll b content and ratio in the rigid hornwort (*Ceratophyllum demersum* L.) leaves depending on the water hydro chemical parameters of Saksagan River in the zones of influence of "Ternivska" and "Oktyabrsk" mines (Kryvyi Rih city) were studied. The purpose of the work was to find out the features of *C. demersum* photosynthetic apparatus reorganization as plants adaptive reaction to the technogenic transformation of river ecosystem. The water mineralization changes in the range of 1.2–2.9 g/L and fluctuations in the nitrates and nitrites content in the Saksagan River different sections were established. Both an increase and a decrease in the content of chlorophyll both forms in *C. demersum* leaves were detected, but more significant changes were related to chlorophyll b content. The photosynthetic pigments total content in plant leaves was the highest in the most mineralized areas (points of return water discharge and 500 m below of "Ternivska" mine), and it was the lowest due to sharp water mineralization increasing (500 m below the return water discharge of "Oktyabrsk" mine). Significant variability in the photosynthetic pigments content and ratio indicates flexible regulation of their synthesis in *C. demersum* leaves to maintain the efficiency of the photosynthetic apparatus under conditions of water pollution. The revealed regularities indicate the active protection of the photosynthetic process in the *C. demersum* leaves, which is an important prerequisite for the plant's survival in adverse conditions and the preservation of the structure and productivity of the water ecosystem of the Saksagan River.

**Keywords:** macrophytes; mining activity influence; water mineralization; bio indication; photosynthetic pigments; chlorophyll forms ratio

### Introduction

Mining activity is accompanied by the transformation of the ecosystems natural state, qualitative and quantitative changes in their components, and environmental pollution (Lykholat et al., 2021). Aquatic ecosystems are often the final collectors of various pollutants, so comprehensive contamination monitoring is necessary to control and prevent irreversible damage to these environments (Polechońska & Klink, 2022). Indispensable components of aquatic ecosystems are macrophytes, which together with algae form an autotrophic block of hydrobiocenoses. Aquatic plants are the primary producers of organic substances and play an important role in enriching the aquatic environment with oxygen. Macrophytes, like most other biological objects, show selective sensitivity to pollutants, so changes in their functioning can

characterize the dynamics of the water ecosystem conditions. That is why higher aquatic vegetation is often used as an indicator of the ecological state of hydro ecosystems during monitoring studies (Pasichna, 2013; Baldantoni & Alfani, 2016). Bioindication is the most effective direction in the environmental monitoring system. Aquatic plants serve as reliable indicators for assessing the state of reservoirs, as they provide an integral assessment of the aquatic environment in time and space (Klymenko et al., 2020).

The ubiquitous submerged macrophyte rigid hornwort (*Ceratophyllum demersum* L.) often serves as a test object for bioindication, in particular in the case of water body contamination with trace elements (Pasichna & Arsan, 2002; Rudecki et al., 2018). The species rigid hornwort is undemanding to lighting, and can grow both in the sun and in the shade, but does not like bright light; in the natural

conditions, it often displaces other species from water bodies. Underwater thickets of *C. demersum* play an important role in the water bodies' life, because they purify the water, saturate it with oxygen, and serve as a shelter for young fish and invertebrates; some fish and mollusks feed on them (Olkhovych et al., 2017). Widespread aquatic plant *C. demersum* is a cosmopolitan species in the reservoirs of the northern hemisphere and a typical representative of submerged macrophytes in the reservoirs of Ukraine, including the Saksagan River (Alexeyeva & Marenkov, 2021).

Various test parameters are used for bioindication and assessment of the pollutant's toxicity, including growth indicators, the photosynthesis intensity and the photosynthetic apparatus state, morphological characteristics, cytogenetic indicators, as well as various specific reactions of aquatic plants (Korobkova, 2017). Among the most informative indicators for assessing the plants physiological state, is the content of chlorophyll in the assimilation organs of plants. The content of plastid pigments and their condition determine the stability, viability and productivity of aquatic plants (Kiyak, 2018). The purpose of this work was to identify the patterns of the influence of mining activity on the content and ratio of chlorophyll different forms in the leaves of *C. demersum* from different sections of the Saksagan River.

## Materials and methods

Sampling of plant material of rigid hornwort (*Ceratophyllum demersum* L.) and water samples was carried out in May 2022 at six sections of the Saksagan River within the city of Kryvyi Rih near the "Ternivska" and "Oktyabrsk" mines. Both enterprises have permits for the release of return (rain and melt) water, which enters the Saksagan River after cleaning on local treatment facilities. For both mines, water and plant material sampling points were located at the place of return water discharge (48°03'55"N 33°31'29.9"E for "Ternivska" and 47°59'25.7" N 33°25'50"E for "Oktyabrsk" mines), and 500 meters above and below the return water discharge.

**Table 1**

Hydrochemical indicators of water samples from different sections of the Saksagan River in the zone of mining activity influence

| Sampling point                  | Mineralization, mg/L | NH <sub>4</sub> <sup>+</sup> content, mg/L | NO <sub>3</sub> <sup>-</sup> content, mg/L | NO <sub>2</sub> <sup>-</sup> content, mg/L |
|---------------------------------|----------------------|--------------------------------------------|--------------------------------------------|--------------------------------------------|
| "Oktyabrsk" main                |                      |                                            |                                            |                                            |
| Above return water discharge    | 1200.0 ± 15.1        | 0.48 ± 0.02                                | 2.83 ± 0.08                                | 0.06 ± 0.00                                |
| Place of return water discharge | 1210.1 ± 22.6        | 0.37 ± 0.01                                | 2.67 ± 0.06                                | 0.05 ± 0.00                                |
| Below return water discharge    | 1320.2 ± 24.8        | 0.60 ± 0.02                                | 2.61 ± 0.07                                | 0.08 ± 0.01                                |
| "Ternivska" main                |                      |                                            |                                            |                                            |
| Above return water discharge    | 2800.2 ± 28.6        | 0.62 ± 0.02                                | 3.33 ± 0.08                                | 0.01 ± 0.00                                |
| Place of return water discharge | 2885.0 ± 24.5        | 0.63 ± 0.02                                | 3.57 ± 0.09                                | 0.01 ± 0.00                                |
| Below return water discharge    | 2940.3 ± 29.8        | 0.47 ± 0.02                                | 2.91 ± 0.07                                | 0.01 ± 0.00                                |

In the area affected by the "Oktyabrsk" mine, the water mineralization levels of the Saksagan River differed insignificantly at the places of return water discharge and at 500 m above. At the same time, water mineralization was 110% higher ( $P \leq 0.05$ ) at the point below discharge of return water. Changes in the content of ammonia, nitrates, and nitrites depending on the water-sampling place had a different character, but the common feature was a decrease in indicators at the point of return water discharge compared to the point 500 m above.

In the area affected by the activity of the "Ternivska mine", the lowest water mineralization, detected at a point 500 m above the return water discharge, slightly increased at the other two sampling points. For the content of ammonia and nitrates, the highest indicators were found at the point of return water discharge, and the lowest at the point 500 m below.

The content of chlorophyll (Chl a, Chl b) and the amount of green pigments (Chl a + Chl b) in the *C. demersum* leaves were determined according to the method of Wintermans & De Mots (1965) with the help of a spectrophotometer "KFK-3" at wavelengths of 649 nm and 665 nm. The chlorophyll forms concentration (in mg/L) was determined according to equations (1–3):

$$C_{\text{Chl a}} = 13.70D_{665} - 5.76D_{649} \quad (1),$$

$$C_{\text{Chl b}} = 25.80D_{649} - 7.60D_{665} \quad (2),$$

$$C_{\text{Chl(a+b)}} = 6.10D_{665} + 20.04D_{649} \quad (3),$$

where:  $C_{\text{Chl a}}$  – concentration of chlorophyll a;  $C_{\text{Chl b}}$  – concentration of chlorophyll b;  $D_{665}$  – optical density of the extract at  $\lambda = 665$  nm;  $D_{649}$  – optical density of the extract at  $\lambda = 649$  nm.

The results of the calculations of the chlorophyll content in *C. demersum* leaves were expressed in mg/g of raw weight of plant material.

Hydrochemical studies were carried out according to generally accepted methods (Aršan et al., 2006). Analysis of water quality and determination of indicators was performed using the hydrochemical device EZODO AZ-86031 (Oximeter/pH-meter/conductometer/salt meter) and a set of express tests for rapid determination of water quality TESTLAB (JBL, Germany).

All determinations were carried out in triplicate; the results were statistically processed and presented as mean ± standard deviation ( $M \pm SD$ ). The research results were processed by the method of variation statistics using the Statistica 7.0 software package. The significance of the differences between the obtained data was assessed using the Student's t-test with a confidence level of  $P \leq 0.05$ .

## Results and discussion

In the water samples from different points on the Saksagan River, which receives return water from the territory of the "Ternivska" and "Oktyabrsk" mines, significant differences in the level of mineralization and the content of some ions were found (Table 1).

The study revealed noticeable photosynthetic pigments variability in the leaves of *C. demersum* plants from different sections of the Saksagan River. In the area affected by the "Oktyabrsk" mine, a significant increase in the content of chlorophyll both forms and chlorophyll total content was observed at the point of return water discharge compared to the point 500 m above (Fig. 1).

At the same time, the content of both photosynthetic pigments in *C. demersum* leaves significantly decreased at a point below the water discharge. The highest total chlorophyll content (Chl a + Chl b) was found in plants at the place of return water release. The ratio of Chl a/Chl b in plant leaves at the points of return water discharge and 500 m above did not differ much (on average 2.46 and 2.42, respectively). However, in plants from the point below the return water discharge, the Chl a/Chl b ratio increased to 4.55 due to a sharp decrease in the content of chlorophyll b.

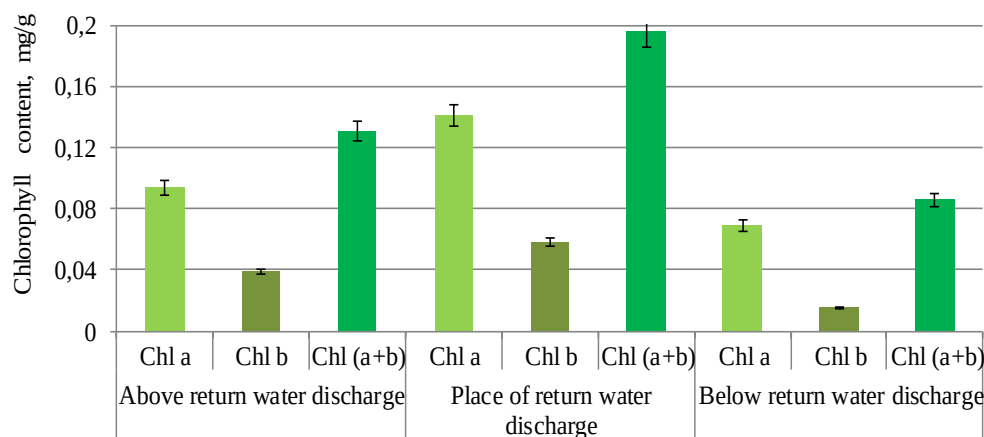


**Fig. 1.** Content of the photosynthetic pigments in leaves of *Ceratophyllum demersum* at different plots of Saksagan River influenced by "Oktyabrskaya" main working (Kryvyi Rih)

In area of «Ternivska» mine influence, chlorophyll a and chlorophyll b content in *C. demersum* from the place of return water discharge and 500 m below exceeded (by 1.4 – 1.5 times and 1.6 – 1.8 times, respectively) the indicators at the point above the return water discharge (Fig. 2).

The total pigments content (Chl a + Chl b) was the highest in *C. demersum* from the area 500 m below the return water discharge. The ratio of Chl a/Chl b in plant leaves was the largest (on average 2.12) at the plot 500 m above the return water discharge and noticeable lower at the other two sampling points (1.95 and 1.78, respectively) due to greater content of chlorophyll b.

Therefore, in the area of "Ternivska" mine activity, the photosynthetic pigments content in *C. demersum* leaves and almost all water hydrochemical indicators significantly

( $P \leq 0.05$ ) exceeded the levels in zone of "Oktyabrskaya" mine emissions, with the exception of nitrite content. In general, the water mineralization in the Saksagan River in the zone of both mines influence was much higher than that reported by Olkhovich et al. (2017) the optimal water mineralization index (237 mg/L), at which the ratio of Chl a/Chl b in *C. demersum* leaves was equal to 2.19.

Correlation analysis revealed different relationship between content of the photosynthetic pigments in *C. demersum* leaves and the mineralization level and some ions content in water in different sections of the Saksagan River (Table 2).

A high positive correlation of Chl a and Chl b content with the water mineralization level was found in the influence zone of "Ternivska" mine, while the correlation was negative in the samples from "Oktyabrskaya" mine influence zone. The effect of

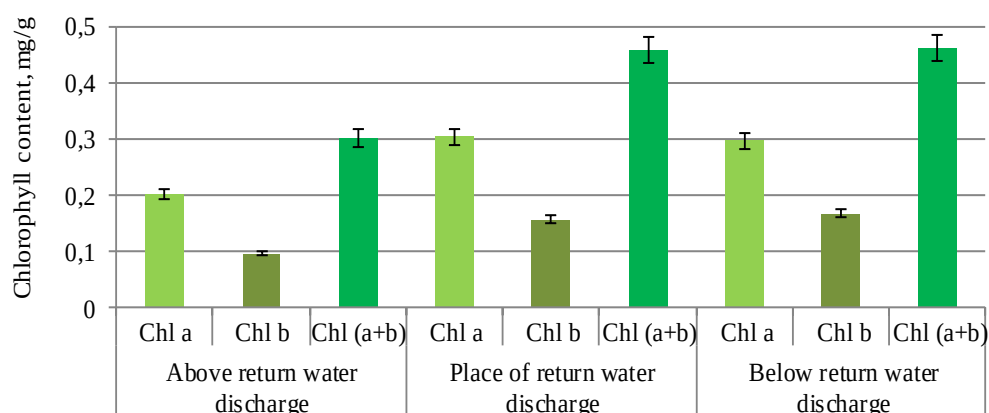


**Fig. 2.** Content of the photosynthetic pigments in leaves of *Ceratophyllum demersum* at different plots of Saksagan River influenced by "Ternivska" main working (Kryvyi Rih)

$\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$  on the content of photosynthetic pigments in *C. demersum* leaves also depended on their level in the Saksagan River water. A high level of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  negatively affects photosynthesis, since the process of nitrite conversion is localized in plastids, and nitrate reduction is indirectly related to photosynthesis through the opening of stomata or the provision of ion transport (Kolysnyk & Musienko, 2009).

The quantitative and qualitative composition of the pigment apparatus is the primary link in the process of photosynthesis, determines its intensity and can be a diagnostic sign of photosynthetic organisms' adaptability (Kiyak, 2018). The variability degree of photosynthetic apparatus depends on the plant's genetic characteristics, the pigments original composition and content, as well as the regulatory mechanisms of pigment synthesis (Los et al., 2008; Zhang et al., 2016). Submerged plants depend more on the chemical composition

and pH of water and gas content than plants with above-water leaves (Olkhovich et al., 2017).

It is obvious that high mineralization level, nitrates and nitrites in water create salt stress conditions for *C. demersum* plants and initiate the protective processes. Plants adaptation to an environment with a high salts level includes the photosynthetic apparatus rearrangements, which have species characteristics and concern both green pigments. It is known that chlorophyll a predominates in the photosystem I, while chlorophyll b makes up a significant share in light-harvesting complexes of photosystem II. For example, the pigments composition in bryophyte cells under the salt stress changed with a decrease in chlorophyll a proportion and an increase for chlorophyll b, which was a compensatory reaction of pigment apparatus to stress (Kiyak, 2018). The most noticeable changes in the photosynthetic process in halophyte plant occurred after a certain threshold value of salt stress and included a

**Table 2**

Correlation coefficients (r) between the content of photosynthetic pigments of *C. demersum* and hydrochemical parameters of the Saksagan River water in the zone of mining activity

| Photosynthetic pigment content, mg/g | Mineralization, mg/L | NH <sub>4</sub> <sup>+</sup> content, mg/L | NO <sub>3</sub> <sup>-</sup> content, mg/L | NO <sub>2</sub> <sup>-</sup> content, mg/L |
|--------------------------------------|----------------------|--------------------------------------------|--------------------------------------------|--------------------------------------------|
| “Oktyabrsk” main                     |                      |                                            |                                            |                                            |
| Chl a                                | r = - 0.71           | r = - 0.98                                 | r = 0.09                                   | r = - 0.93                                 |
| Chl b                                | r = - 0.86           | r = - 0.99                                 | r = 0.33                                   | r = - 0.99                                 |
| “Ternivska” main                     |                      |                                            |                                            |                                            |
| Chl a                                | r = 0.90             | r = - 0.40                                 | r = 0.00                                   | r = - 0.11                                 |
| Chl b                                | r = 0.97             | r = - 0.58                                 | r = 0.00                                   | r = - 0.30                                 |

photosynthesis rate decrease (Luo et al, 2017). A dose dependence of photosynthetic process in *C. demersum* leaves from exposure to manganese ions consisted in the toxic effects absence when the ion content in water was low, but a significant decrease in photosynthesis when the manganese content increased to 200 µg/L (Pasichna & Arsan, 2002). The toxicity of heavy metals is due to their ability to accumulate in chloroplasts and inhibit the synthesis of photosynthetic enzymes.

It is obvious that the adaptation of *C. demersum* photosynthetic apparatus to significantly different water mineralization levels in the Saksagan River demonstrates the plant ability to regulate the biosynthesis of chlorophyll both forms and, accordingly, their ratio. With less water mineralization, the increase in salinity, ammonia, and nitrites content caused a stressful effect on plants, because of which the chlorophyll b content sharply decreased, and chlorophyll a slightly increased. Under conditions of higher salinity, increased mineralization and nitrate content led to a simultaneous increase in the chlorophyll both forms content with the predominance of chlorophyll b. Therefore, regulated reduction/increase of the photosynthetic pigments content is an important component of the photosynthetic apparatus of *C. demersum* plants adaptation to functioning in conditions of both low and high levels of water mineralization of the Saksagan River.

## Conclusion

In the leaves of *C. demersum* plants from different sections of the Saksagan River, a dependence of the photosynthetic apparatus composition on the water hydro chemical parameters changes due to mine activity was found. In area with lower mineralization near “Oktyabrsk” mine, *C. demersum* plants experienced a sharp decrease in the content of Chl b and the total pigments content in case of increasing salinity. Under the water high mineralization in the area influenced by “Ternivska” mine, the content of both pigments increased (Chl b more than Chl a) in *C. demersum* leaves, which can be a compensatory reaction to maintain the optimal photosynthesis functioning. Probably, the photosynthetic apparatus of *C. demersum* is characterized by a flexible regulation of the response to a certain threshold value of water pollution. Such a protective and adaptive reaction corrects the biosynthesis of chlorophyll both forms, determines their content and ratio variability, and ensures the photosynthesis maintenance under the different water pollution levels. The findings indicate the active photosynthetic process protection in *C. demersum* leaves, which is an important prerequisite for the plant’s survival in adverse conditions and the preservation of the water ecosystem of the Saksagan River.

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