

SEASONAL CHANGES IN THE CONTENT OF PHOTOSYNTHETIC PIGMENTS OF DOMINANT MACROPHYTES IN THE BARDAČA FISHPOND AREA

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Abstract

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In this paper, changes in the content of photosynthetic pigments during the vegetation season in the leaves of *Phragmites communis* Trin., *Salvinia natans* (L.) All., *Trapa natans* L. and *Utricularia vulgaris* L. in the area of Bardača fishpond (Sinjak pond) were monitored. Physical and chemical characteristics of water are defined in order to determine their association with the content of photosynthetic pigments. The obtained results indicate the specificity in the content of photosynthetic pigments between aquatic macrophytes and significant variations during the vegetation season. The maximum content of pigments was determined in the leaves of the emerged species, while the minimum content was measured in the submerged and floating leaves. The investigated macrophytic species showed a similar trend in the total chlorophyll content during the vegetation period; with two maximums recorded – May and September, while the minimum values were recorded with the senescence process (October). Total chlorophyll and carotenoids were in a negative correlation with temperature, pH, saturation, water transparency and orthophosphate content in water. On the other hand, they were in a positive correlation with the content of nitrates in water. The correlation analysis of the physical and chemical parameters of water quality and the concentration of macrophytes photosynthetic pigments showed a significant influence of certain abiotic factors on the photosynthetic pigments' content in the leaves of *Phragmites communis* Trin., *Salvinia natans* (L.) All., *Trapa natans* L. and *Utricularia vulgaris* L.

Key words: photosynthetic pigments, seasonal dynamics, aquatic macrophytes, physical and chemical parameters of water.

Introduction

Aquatic macrophytes beside algae represent the most important primary producers of freshwater ecosystems and play a significant role in the structure and functioning thereof (Lukina, Smirnova, 1988; Ronzhina et al., 2004; Chambers et al., 2008; Nikolić et al., 2009; Dar et al., 2013; Horvatić et al., 2013; Atapaththu et al., 2017), while contributing to the natural process of self-purification of

water (Prasad et al., 2003, 2006; Dar et al., 2012, 2013). It is known that the photosynthetic activity of most plant species, and also of the investigated macrophytic species, depends mostly on the light intensity and quality, structural and morphological properties, the total chlorophyll content per unit of leaf surface, as well as on the abiotic factor of the environment (temperature, water mineral nutrition content) (Ronzhina et al., 2001; Nekrasova et al., 2003; Ronzhina et al., 2004; Nikolić et al., 2009; Rocha et al., 2009; Dar et al., 2012, 2013; Horvatić et al., 2013). Light intensity and temperature together affect growth, morphology, photosynthesis, growth rate and chlorophyll content (Beck, Redman, 1940; Ivanković et al., 2011). It is natural that chlorophyll levels change over the course of the season (Hreeb, 2017), and that changes in the chlorophyll content can provide valuable information on the physiological state of the plant, indicate the changes in plant growth, photosynthetic capacity, or the impact of various stress factors (Beck, Redman, 1940). In aquatic basins, a full spectrum of light is adopted by emerged and floating water plants, while submerged plants living at higher depths absorb a smaller amount of visible radiation, and also of variable quality, so that the variation in the content of photosynthetic pigments as well as their representation is one of the ways of adaptation to different light conditions in the aqueous layer (Lukina, Smirnova, 1988; Stevanović, Janković, 2001; Ronzhina et al., 2004, Dar et al., 2013; Horvatić et al., 2013). Most studies (Nikolić et al., 2009; Horvatić et al., 2013; Hreeb, 2017) indicate that various physical and chemical conditions play a key role in the biosynthesis of photosynthetic pigments, and that these changes mostly reflect on submerged plants and that their photosynthetic apparatus differs significantly in relation to emerged and floating plants. The content of total chlorophyll, chlorophyll-*a* and chlorophyll-*b* also depends on the chemical environment such as oxygen level, pH, nitrate content, phosphate content and so on (Dar et al., 2013; Horvatić et al., 2013). Even Beck, Redman (1940) have shown in their research that the spectral compositions of light and light intensity are very important factors determining the seasonal variation of photosynthetic pigments in the plant leaves. Also, the same authors state that for each species, there is an optimum of the light intensity and temperature, before the optimum is reached, the production of pigments grows, and above the optimum, the reduction occurs. In aquatic ecosystems, the lack of mineral elements can limit photosynthesis, and on the other hand, excess nutrients can be very harmful and lead to eutrophication (Chambers et al., 2008; Nikolić et al., 2009). Dar et al. (2013), Al-Hamdani, Sirna (2008) in their research state that the chlorophyll content grows along with the increase of nutrients in water, with small amounts of nutrients remaining in water, which leads to the purification of water basins. In their paper, Kitajima, Hogan (2003) state that the chlorophyll *a/b* ratio increases with increasing light intensity and increasing N availability. On the other hand, the large production of biomass of aquatic plants can create a problem, especially at the moment of decomposition of their biomass because it can accelerate the eutrophication process (Nikolić et al., 2009; Dar et al., 2012, 2013) and quickly lead to a change in water quality (Taniguchi et al., 2004; Thomaz et al., 2004). With the senescence process as a consequence of chlorophyll degradation, there is a significant reduction in the content of photosynthetic pigments (Ronzhina et al., 2004). Based on previous research, the area of Bardača fishpond (Lolić et al., 2012, 2014) belongs to the category of eutrophic waters. As one of the possible solutions for eutrophication is the use of water macrophytes, which thanks to their hyperaccumulation ability, significantly reduce levels of mineral elements and heavy metals in water (Prasad et al., 2003, 2006).

In this paper, changes in the content of photosynthetic pigments during the vegetation season, as well as the differences in the content of pigments between emerged, submerged and floating plants, were monitored. Also, the correlation between the content of photosynthetic pigments and the physical and chemical parameters of water (water temperature, transparency, electrical conductivity, oxygen, saturation, pH) was made, as well as in relation to the concentrations of nutrients in water (ammonium, nitrate, nitrite nitrogen and orthophosphates) in order to determine the degree of their interconnectedness.

Material and methods

Research area

The area of Bardača represents a unique wetland-pond ecosystem located on the far north-east of Lijevo Polje, bordered by the Sava River from the north, the Brzaja and Vrbas rivers from the east, the Matura from the west, while on the south side there is a perimeter canal Osorna-Borna-Ljevanica (O-B-LJ) in the immediate vicinity. The geographical location of the Bardača area is at the center of the moderate belt (45° 08' N and 17° 25' E), at about 100 m above sea level, at the foot of Mount Motajica. Since 2007, Bardača was named Ramsar Area, which confirmed its international importance as an "Important Bird Area". Spatially, Bardača covers 2810 ha (810 ha of aquatic and 2000 ha of land ecosystem with a total of 11 ponds (Đurić et al., 2004). The research was carried out at the site Sinjak, which was an active fishpond during the sampling period (Đurić et al., 2004).

Sample analysis

For the sampling of plant material and its preparation for analysis, the standard methods applied to aquatic plants (APHA, 1995) were used. Samples of aquatic macrophytes were collected in the field and delivered to the laboratory in containers with liquid nitrogen. Water samples were sampled alongside for the purpose of analyzing physical and chemical characteristics. The temperature of water and air was determined by the mercury thermometer in the morning hours. Water transparency was determined using the Secchi disk. The oxygen concentration was determined using the Hach HQ30 flexi oximeter, for the pH value and electrical conductivity, the Eutech Cyberscan PC 10 pH-meter was used, and turbidimeter Eutech TN-100 for the determination of turbidity. The determination of the concentration of ammonium ions (NH_4), nitrates (NO_3), nitrites (NO_2) and orthophosphates (o-PO_4) was performed using the HACH DR2800 spectrophotometer. Samples were taken at the same profile points, and the measurements were carried out in a layer of water 30 to 50 cm below the surface. The samples were then transported on ice at a temperature of +4 °C and analyzed within 24 hours. The content of photosynthetic pigments in the aqueous macrophyte leaves was determined spectrophotometrically in acetone extract using standard methods (APHA, 1995), and the reading was performed using UV-VIS Shimadzu UV-160 spectrophotometer, Kyoto, Japan.

Statistical analysis

All the data were statistically processed in SPSS (Statistical Package for the Social Sciences) program. The analyses were done in three independent repetitions, and the analyzed parameters were analyzed using the analysis of variance method (ANOVA) of factorial assay, descriptive statistics and comparison of the obtained data. To compare the results and examine the intergroup differences from ANOVA analysis within *Post hoc* tests, LSD test for the level of significance $p < 0.05$ was used. The influence of physical and chemical parameters of water quality on the concentration of photosynthetic pigments of macrophytes is expressed by the Spearman's rank correlation coefficient at the level of significance $p < 0.05$ and $p < 0.01$.

Results and discussion

In the period of research, the values of water temperature at the Sinjak were in accordance with the air temperature and varied from 15.5 °C in October to 31.1 °C as measured in July (Table 1).

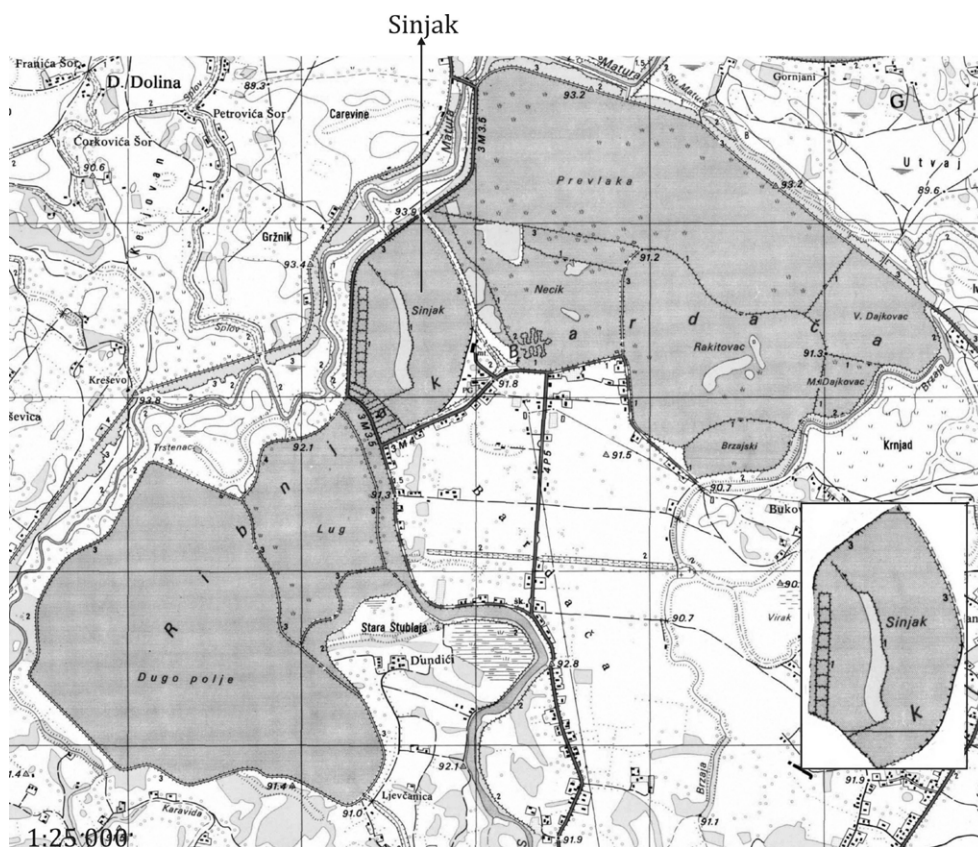


Fig. 1. Map of Bardača fishpond (a detail from topographic map 1:25000, Nova Gradiška (Razboj – Ljevnanski) 4-4, Vojno-geografski institut, 1977 (*Military Geographic Institute*)).

The pH value oscillated from 6.98 in September to 8.35 in May, and the water on average was slightly alkaline. The electric conductivity values ranged from a minimum of 364 $\mu\text{S}/\text{cm}$ in July to a maximum of 488 $\mu\text{S}/\text{cm}$ in May, indicating a moderate water load with dissolved ions.

During the spring, maximum development of phytoplankton (Lolić et al., 2014), in the period from May to July, the water was hyper saturated with dissolved oxygen. In May, due to the intensive process of photosynthesis, the oversaturation of water with oxygen was as high as 139.2%. In August and September, due to high water temperature and intense bacterial oxidation of organic matter, the concentration of dissolved oxygen in water dropped significantly, so the water saturation amounted to only 56.8%, which is in accordance with the findings of the research of Futog pond (Matavulj et al., 1990). The lack of oxygen in the water leads to a multiple negative effects on hydrobionts and on the general physical and chemical state of water. Organic matter is accumulated as their oxidation slows down, numerous saprophytic organisms

T a b l e 1. Physical and chemical parameters of water in the locality Sinjak.

Parameters	May	June	July	August	September	October
Air temperature (°C)	22.1	25.4	36.0	27.5	20.1	15.3
Water temperature (°C)	23.3	25.2	31.1	27.2	20.0	15.5
pH	8.35	8.16	7.94	7.74	6.98	8.01
Electrical conductivity µS/cm)	488	425	364	369	406	374
Turbidity (NTU)	22.00	18.16	10.71	28.10	22.60	33.20
Dissolved oxygen (mg/l)	11.68	9.56	8.00	5.27	5.07	8.30
Saturation (%)	139.2	117.8	109.5	67.6	56.8	83.1
Ammonium nitrogen (mg/l)	0.26	0.05	0.00	0.36	0.12	0.11
Nitrate nitrogen (mg/l)	0.3	0.2	0.2	0.3	0.5	0.5
Nitric nitrogen (mg/l)	0.009	0.002	0.000	0.015	0.013	0.013
Orthophosphates (mg/l)	1.69	0.18	0.01	0.40	0.06	0.06
Sulphates (mg/l)	23	14	12	10	23	15
Transparency (cm)	49	52	70	36	33	40
Depth (cm)	110	105	76	80	120	165

are developed, among which are many pathogens, the activity of fish is reduced, they make less use of available food and are less resistant to other adverse environmental factors (Bojčić et al., 1982). In June, a low concentration of ammonium nitrogen of 0.05 mg/l was measured, while in July we did not detect ammonium nitrogen, so that in August, it reached its maximum concentration of 0.36 mg/l. The same trend was obtained for nitrite nitrogen, which was not detected in July, and in August, the maximum concentration of 0.015 mg/l was measured. At the investigated site, the effect of summer phosphate consumption (0.01 mg/l) was visible due to its participation in biological processes during the active growth season. Levado (2001), monitoring changes in nitrate and orthophosphate concentrations in the eutrophic lake Rostherne Mere in the United Kingdom, came to the similar results. The highest concentration of nutrients in water was recorded at the end of the winter phase, and then their concentration rapidly declined in the spring when there was mass development of phytoplankton (Lolić et al., 2014). During the spring and summer, the concentration of nutrients did not significantly vary until the summer/autumn maximum of phytoplankton development, when the lowest annual concentrations of nutrients were measured. At the end of this phase, there was a renewed increase in the concentration of nutrients in water. Namely, inorganic forms of nitrogen and phosphorus represent the basic nutrients that limit the development of phytoplankton in water basins and due to the intense development of phytoplankton, their total consumption can happen. Subsequently, the phytoplankton dying out follows, and due to bacterial oxidation of dead organic matter, the concentration of nutrients in water increases, and hence, the consumption of dissolved oxygen (Dodds, 2006). During the entire investigated period, our results indicate low nitrate nitrogen concentrations in the Sinjak fishpond (Table 1). The water load by suspended substances of different origin, as well as the intensive development of phytoplankton, indicates high turbidity values and extremely low transparency throughout the vegetation period (Table 1).

The results of the research in the Sinjak fishpond area showed significant variations in the content of photosynthetic pigments between emerged, free-floating and submerged leaves, which changed seasonally and depending on the physical and chemical conditions of the environment (Fig. 2). Aqueous macrophytes are characterized by a lower concentration of photosynthetic pigments per fresh biomass (1–2 mg/g) compared to terrestrial plants (2–3 mg/g) (Ronzhina et al., 2004), which was shown by the results obtained in this paper. Uvalle Saucedo et al. (2008) indicate that the reduction in the content of photosynthetic pigments can be associated with the reduction of N content in leaves and changes in the activity of nitrate-reductase, changes during phenophases (blossoming and seed production). Dar et al. (2012) and Horvatić et al. (2013) state that the changes in the content of photosynthetic pigments in macrophytic species are the result of adapting to different ecological conditions, primarily different intensity of light and temperature, which can be associated with our research.

In this paper, the maximum content of pigments for *Phragmites communis* was recorded during the period of May and September, which is characterized by the highest nutrient content and optimal water temperature. Concentration of total chlorophyll on the Sinjak fishpond in leaves of *P. communis* varies depending on the sampling period (Fig. 2a). In relation to the analyzed species, the maximum content of photosynthetic pigments was determined in the leaves of *P. communis*, which, during the season, varied in the range 0.650–0.985 mg/g FW (Fig. 2a). The obtained values of the total chlorophyll in the leaves of *P. communis* were approximate to the values received by Ronzhina et al. (2004) for *P. communis* ($1.78 \text{ mg/gFW} \pm 0.08$), for *Typha latifolia* ($0.66 \text{ mg/gFW} \pm 0.02$) and *T. angustifolia* ($0.86 \text{ mg/gFW} \pm 0.02$). The content of photosynthetic pigments varied considerably during the season and phases of plant development, as recorded by other researchers (Mészáros et al., 2003). A significant reduction in the chlorophyll content of *Phragmites communis* was recorded during the period July–August, which is probably due to the stress caused by elevated temperature (Table 1) and high light intensity, which can be associated with other researches (Uvalle Saucedo et al., 2008; Temali, Ylli, 2017; Sheikh et al., 2017). The content of total chlorophyll during the season was higher than the content of carotenoids (0.095–0.206 mg/gFW) with the maximum during September. The a/b ratio also changed during the season (2.214–2.797 mg/gFW), it was significantly higher during July and October which is probably the result of changes of the different intensity of light and temperature, while the lowest a/b ratio was determined during August as a consequence of the reduced N content during that period, as recorded by other researchers as well (Lippert et al., 2001; Mészáros et al., 2003).

In the leaves of *Salvinia natans*, the total chlorophyll content was significantly lower in relation to the other investigated species. During the season, the values varied from 0.240–0.404 mg/gFW, whereby it is noticed that the highest values were recorded during July and August when this species reached maximum growth, and the minimum was recorded with aging (September). Also, the values of carotenoids showed the same seasonal dynamics. The measured values were similar to those obtained by other researchers in their paper (Ronzhina et al., 2004) for *Lemna gibba* (0.01–0.59 mg/gFW) and *Lemna minor* (0.04–0.77 mg/gFW) and for *Salvinia natans* (0.511 mg/gFW) (Horvatić et al., 2012). In this paper, the lower content of photosyn-

thetic pigments was observed in floating versus emerged, and higher in relation to submerged leaves, which was contrary to the research by other authors (Ronzhina et al., 2004; Dar et al., 2012).

In plants adapted to high radiation intensities such as floating, normal life activity is facilitated by a relatively lower chlorophyll content in leaves in comparison to the emerged and submerged species, whereby the damage of cells from photooxidation is reduced (Nekrasova et al., 2003), which is in accordance with our results. In the floating leaves of the investigated species, higher a/b ratio was recorded compared to the emerged and submerged leaves, which is a consequence of adaptation to environmental conditions.

Research of Al-Hamdani, Sirna (2008) showed that the concentrations of chlorophyll and carotenoids in leaves of *Salvinia minima* increase up to 100 times with increasing nutrients

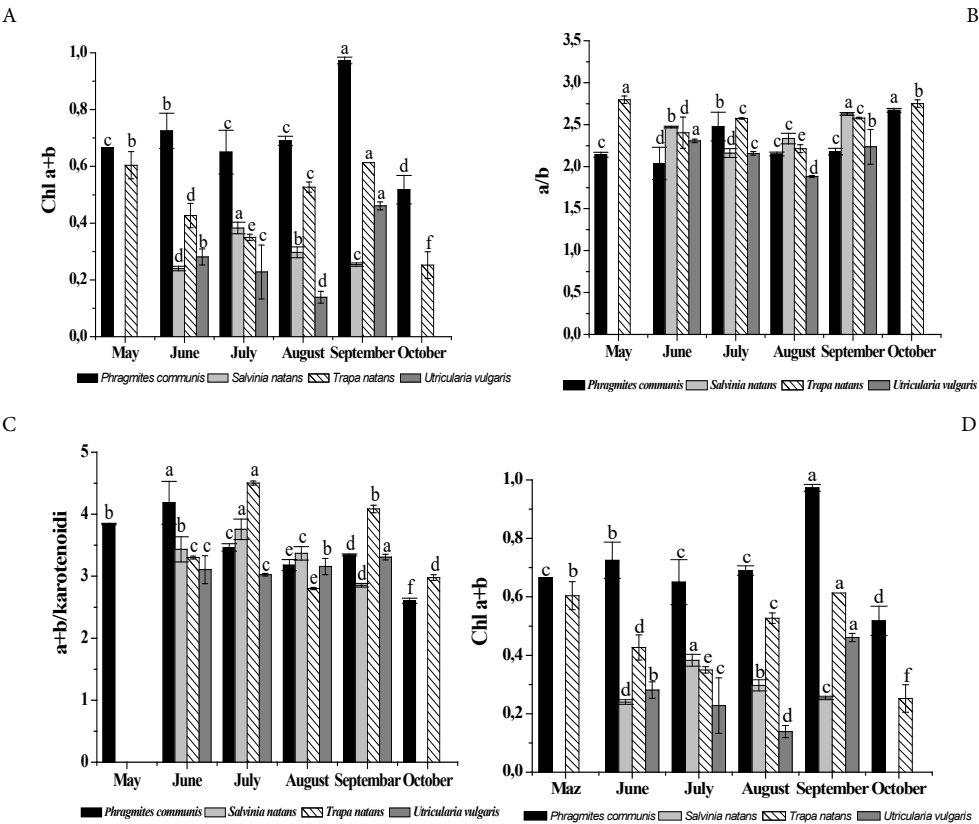


Fig. 2. Seasonal variations in the total chlorophyll content (a+b) (A), rates of chlorophyll-a and b (Chl-a/b) (B), rates of total chlorophyll content and carotenoids (a+b/carotenoids) (C), and carotenoids (D) (mg/gFW) in aquatic macrophytes in the Sinjak fishpond area.

in water. It is also known that many types of floating plants can efficiently remove all forms of nitrogen from aquatic ecosystems (Jampeetong, Brix, 2009; Saeed, Sun, 2012), which has a practical application in its removal from water, and which is one of the reasons for a significant reduction of nutrient content during the summer period in this paper.

In floating leaves of *Trapa natans*, the total chlorophyll content was higher compared to the submerged ones of *Utricularia vulgaris* and floating of *Salvinia natans*, while it was lower in compared to reed leaves. The lowest content of chlorophyll was measured at the end of the season (October) in the aging phase (0.252 mg/gFW) and in July (0.350 mg/gFW), while it was the highest in the spring during the active phase of growth and development (0.604 mg/g FW-May) and September (0.613 mg/gFW). Such seasonal trend in the total chlorophyll content can be attributed to the influence of various abiotic factors and the adaptability of the investigated species to the conditions of the environment. A similar seasonal trend was obtained for carotenoids, but on average, it was significantly lower. The intensified intensity of light and temperature during the summer period acted stressfully so that the floating species *Trapa natans* showed a trend of decrease in total chlorophyll during that period, which was in agreement with the research by Horvatić et al. (2013).

The chlorophyll a/b ratio also changed during the season (2.8–2.752), with a maximum in May and October. The reason for this may be the high negative correlation between the temperature and a/b. The different trend is recorded for the ratio a+b/ carotenoids, the highest values were obtained during June and August, while 2–3 times lower content was determined in October. The negative correlation was recorded between pH, orthophosphate and a + b/carotenoids.

In submerged leaves of *Utricularia vulgaris* total chlorophyll content ranged from 0.139–0.461 mg/gFW, which was lower compared to the results by Horvatić et al. (2013) with the same species. The maximum values of total chlorophyll were measured at the end of the season (September) and the minimum during August. During the summer period (August), due to the intensive development of floating vegetation, submerged species become shadowed, which significantly reduced the available light and, consequently, the concentration of photosynthetic pigments. Other researchers came to similar results (Ronzhina et al., 2004; Nikolić et al., 2009; Horvatić et al., 2013). The concentration of chloroplast pigments and photosynthetic activity depends on the degree of contact of the leaves with water, whereby the submerged plants have a much lower content of photosynthetic pigments since they are adapted to low illumination conditions, which can be compared with terrestrial sciophytes (Nekrasova et al., 2003; Ronzhina et al., 2004). Different studies conducted on water macrophytes (Ronzhina et al., 2004; Nikolić et al., 2009; Horvatić et al., 2013) attributed to the difference in concentration of pigments to their specific morphological-anatomical structure and abiotic factors of the environment (reduction in intensity and quality of light, reduced water level and an increase in turbidity degree), which can be associated with the results in this paper also. Also, the same authors state that the submerged leaves are characterized by the hydration similar to the succulent plants (up to 95%), which may be one of the reasons for the lower chlorophyll content compared to emerged plants (Nekrasova et al., 2003; Ronzhina et al., 2004).

It is known that total chlorophyll and a chlorophyll a/b ratio vary with shading, that is, that chlorophyll a/b increases with decreasing light intensity as one kind of adaptation to maximally use the available light, as shown in numerous papers (Dale, Causton, 1992), which is the rea-

son for increasing chlorophyll a/b ratio by the end of the vegetation period in all investigated species. Changes in chlorophyll a/b ratio can be associated with a selective increase in the light capacity so that the changes in the ratio should be proportional to the level of available light, indicating that the flotation leaves that are more exposed to solar radiation differ significantly from the submerged and emerged in terms of utilizing the available light.

The lower chlorophyll a/b ratio during the summer period (July-August) was determined in the floating and submerged leaves, probably as a consequence of adaptation to reduced light intensity due to the shadowing of macrophyte species by their intensive development (Dale, Causton, 1992). The chlorophyll a/b ratio in the leaves of *Utricularia vulgaris* also changed during the season in the range (1.8–2.307 mg/gFW), which was the highest in June and the lowest in August.

The increased chlorophyll a/b ratio in floating and submerged leaves at the beginning of the season (May-June) and at the end (September-October) can be explained by the availability of nutrients, that is, with increasing nutrient concentrations, the chlorophyll a/b ratio was higher, as other researchers (Wetzel, 2001; Kitajima, Hogan, 2003) showed. During the summer period, a lower chlorophyll a/b ratio was measured, which can be the consequence of photoinhibition due to an increase in the intensity of light and a reduction in the availability of nutrients or their complete lack. In their researches, Kitajima, Hogan (2003) found that the chlorophyll a/b ratio increases with the increase in N content in leaves exposed to higher light intensity compared to shadow leaves, which was contrary to the results obtained in this paper. Our results indicate that the chlorophyll a/b ratio was reduced with a decrease in the N content and the available light.

At the investigated site, a wide fluctuation of physical and chemical parameters was recorded during the research period, which can be considered as the main cause of variations in the content of photosynthetic pigments (Maksimović et al., 2019). Research (Shilla, Dativa, 2008; Dar et al., 2012, 2013) indicates that temperatures significantly determine the level of chlorophyll biosynthesis in most macrophytes and that the highest concentrations of chlorophyll are measured at a maximum atmospheric temperature. The same authors state that a reduction in total chlorophyll content during the season can occur due to a relatively lower temperature in the initial growth stage causing delay in blooming or due to early macrophyte decay caused by a sudden increase in temperature during peak growth. We came to similar results in this paper.

Table 2 presents correlations between the total chlorophyll and carotenoid content with water parameters in the Sinjak fishpond for all four investigated plant species. On the basis of the obtained results, we can say that a similar trend of correlation occurs with *Phragmites communis* and *Trapa natans*, and on the other hand, for the *Salvinia natans* and *Utricularia vulgaris*.

Total chlorophyll and carotenoid content in *Phragmites communis*, *Trapa natans* and *Utricularia vulgaris* was in negative correlation with temperature. The reason for this can be explained by the changes that occur during the season, whereby due to the increase in the rate of degradation of organic matter, the oxygen content is very rapidly decreasing (Taniguchi et al., 2004; Thomaz et al., 2004; Townsend, 2006). This process quickly leads to a change in water quality, due to decomposition, the release of ions from detritus and reduction of dissolved oxygen takes place as a result of increased microbial breathing (Carvalho et al., 2003; Bianchini et al. 2008). In addition, oxygen deficiency stimulates the release of ions from the sediment (e.g.,

T a b l e 2. Correlation coefficients between physical and chemical parameters of water quality and content of pigments in leaves of *Phragmites communis*, *Trapa natans*, *Salvinia natans* and *Utricularia vulgaris* at Sinjak pond.

Parameters	Water temperature (°C)	pH	Electric conductivity (µS/cm)	Saturation %	Nitrate nitrogen (mg/l)	Orthophosphates (mg/l)	Transparency (cm)
Water temperature (°C)	1.000						
pH	-0.086	1.000					
Electric conductivity (µS/cm)	0.029	-0.600	1.000				
Saturation %	0.086	0.143	0.486	1.000			
Nitrate nitrogen (mg/l)	-0.493	-0.522	0.348	-0.551	1.000		
Orthophosphates (mg/l)	-0.429	-0.143	-0.029	0.371	-0.203	1.000	
Transparency (cm)	0.174	0.261	0.377	0.986**	-0.662	0.290	1.000
<i>Phragmites communis</i>	Total chlorophyll	-0.081	0.270	-0.154	0.795*	-0.840*	-0.290
	Carotenoids	-0.392	-0.285	-0.693	0.904*	-0.690	-0.522
<i>Trapa natans</i>	Total chlorophyll	-0.086	0.714	-0.200	0.812*	-0.486	-0.290
	Carotenoids	-0.657	0.143	-0.429	0.841*	-0.086	-0.522
<i>Salvinia natans</i>	Total chlorophyll	0.800	-0.400	0.800	-0.949	-0.400	1.000**
	Carotenoids	0.211	0.316	0.949	-0.889	-0.211	0.738
<i>Utricularia vulgaris</i>	Total chlorophyll	-0.600	0.900*	0.700	0.000	-0.100	0.100
	Carotenoids	-0.600	0.900*	0.700	0.000	-0.100	0.100

reactive phosphorus), increasing electrical conductivity. A strong negative correlation between the content of total chlorophyll and carotenoids and saturation is in the summer period in *Phragmites communis* and *Trapa natans*, which could be explained by the state of stress during the summer, when a decrease in oxygen concentration happens due to decomposition of organic matter, especially in shallow ponds. The content of carotenoids increases with increasing light intensity because it protects chlorophyll from photooxidations induced by enhanced UV radiation (Stevenson et al., 1996), which can be linked to the results obtained in this paper. However, in *Salvinia natans* and *Utricularia vulgaris* a positive correlation was shown between total chlorophyll and carotenoid content and saturation.

In the investigated area, a great variation of the mineral forms of nitrogen and orthophosphate was observed, which are incorporated in the production processes, and are released from the biomass of aquatic organisms in degradation processes. For all four plant species, a negative correlation was found between total chlorophyll and carotenoids and the orthophosphate content in water.

During the summer period, a drastic reduction in all forms of nitrogen is observed in order to increase their value later in the degradation process. A positive relationship between the nitrate and the concentration of chlorophyll and carotenoids was demonstrated in *Phragmites communis* and *Trapa natans*, which can be explained by the dependence of the photosynthesis process on the supply of nitrates (Wetzel, 2001), which we have also noted in this paper. By contrast, for *Salvinia natans*, a negative correlation was obtained, and no correlation was established for *Utricularia vulgaris*.

The correlation between transparency and total chlorophyll and carotenoid content at the investigated site was negative for *Phragmites communis* and *Trapa natans*. In limnology of shallow lakes, commonly under the strong influence of wind, re-suspension of the sediment occurs, which contributes to turbidity and transparency reduction. Similar results were recorded by Ivanković et al. (2011) for Blidinje. On the other hand, a positive correlation between transparency and the content of chlorophyll and carotenoids was detected in *Salvinia natans*.

Conclusion

By analyzing the content of photosynthetic pigments in aquatic macrophytes in the Sinjak fishpond, different seasonal dynamics and specificities between the investigated species were determined. The lowest content of total chlorophyll and carotenoids was found in the floating leaves of *Salvinia natans*, followed by *Utricularia vulgaris* and *Trapa natans*, while the highest content of photosynthetic pigments was found in the emerged leaves of *Phragmites communis*. Spearman's rank correlation coefficient indicated that the physical and chemical conditions of the environment have far-reaching influence on the content of photosynthetic pigments of aquatic plants and that certain abiotic factors significantly determine the biosynthesis of photosynthetic pigments of aquatic macrophytes (primarily temperature, nutrients and saturation). The results obtained in this paper provide valuable information on the concentration of photosynthetic pigments in aquatic plants, as well as changes in the content thereof, which were created as a result of the light limitation, the increase in temperature and other abiotic factors of the environment.

References

- Al-Hamdani, S. & Sirna C.B. (2008). Physiological responses of *Salvinia minima* to different phosphorus and nitrogen concentrations. *Am. Fern J.*, 98(2), 71–82. DOI: 10.1640/0002-8444(2008)98[71:PROSMT]2.0.CO;2.
- American Public Health Association (APHA) (1995). *Standard methods for the examinations of water and wastewater*. Washington.
- Atapaththu, K.S.S., Asaeda, T., Yamamuro, M. & Kamiya H. (2017). Effects of water turbulence on plant, sediment and water quality in reed (*Phragmites australis*) community. *Ekológia (Bratislava)*, 36(1), 1–9. DOI: 10.1515/eko-2017-0001.
- Beck, W.A. & Redman R. (1940). Seasonal variations in the production of plant pigments. *Plant Physiol.*, 15(1), 81–94. DOI: 10.1104/pp.15.1.81.
- Bianchini Jr., I., Cunha-Santino, M.B. & Peret A.M. (2008). Oxygen demand during mineralization of aquatic macrophytes from an oxbow lake. *Revista Brasileira de Biologia*, 68(1), 61–67. DOI: 10.1590/S1519-69842008000100009.
- Bojčić, C., Debeljak, Lj., Vuković, T., Jovanović-Kršljanin, B., Apostolski, K., Ržaničanin, B., Turk, M., Volk, S., Drecun, Đ., Habeković, D., Hristić, Đ., Fijan, N., Pažur, K., Bunjevac, I. & Marošević Đ. (1982). *Slatkovodno ribarstvo*. Zagreb: Jugoslavenska Medicinska Naklada.
- Carvalho, P., Thomaz, S.M. & Bini L.M. (2003). Effects of water level, abiotic and biotic factors on bacterioplankton abundance in lagoons of a tropical floodplain (Paraná River, Brazil). *Hydrobiologia*, 510(1–3), 67–74. DOI: 10.1023/B:HYDR.0000008532.71152.38.
- Chambers, P.A., Lacoul, P., Murphy, K.J. & Thomaz S.M. (2008). Global diversity of aquatic macrophytes in freshwater. *Hydrobiologia*, 595, 9–26. DOI: 10.1007/s10750-007-9154-6.
- Dale, M.P. & Causton D.R. (1992). Use of chlorophyll a/b ratio as a bioassay for the light environment of a plant. *Funct. Ecol.*, 6, 190–196. DOI: 10.2307/2389754.
- Dar, N.A., Hamid, A., Ganai, B.A., Bhat, S.U. & Pandit A.K. (2012). Primary production dynamics of two dominant macrophytes in Wular lake, a Ramsar site in Kashmir Himalaya. *Ecologia Balkanica*, 4(2), 77–83.
- Dar, N.A., Pandit, A.K. & Ganai B.A. (2013). Seasonal variation in the pigment content of dominant macrophytes from Wular lake, Kashmir Himalaya, India. *Biochemistry and Pharmacology: Open Access*, 2(4), 1–6. DOI: 10.4172/2167-0501.1000124.
- Dodds, W.K. (2006). Eutrophication and trophic state in rivers and streams. *Limnol. Oceanogr.*, 51(1), 671–680. DOI: 10.4319/lo.2006.51.1_part_2.0671.
- Đurić, D., Sopić, D., Trifković, A. & Jandrić B. (2004). Hidrotehnički radovi u području močvare Bardača. In Ž. Šarić, M. Stanković & D. Butler (Eds.), *Život u močvari* (pp. 17–27). Banja Luka: Urbanistički Zavod Republike Srpske.
- Horvatić, J., Kočić, A., Bučanac, D., Peršić, V. & Varga M. (2013). Preliminarna istraživanja sezonskih promjena slobodno-plivajućih i submerznih makrofita u kanalu Barbara (Baranja). *Hrvatske Vode*, 21(85), 215–224.
- Hreeb, K.K. (2017). Effect of different water temperatures on growth of aquatic plants *Salvinia natans* and *Ceratophyllum demersum*. *Journal of Coastal Life Medicine*, 5(1), 13–15. DOI: 10.12980/jclm.5.20176-213.
- Ivanković, A., Velagić Habul, E. & Knezović Z. (2011). Oceanological and hydrobiological studies. *Oceanological and Hydrobiological Studies*, 40(3), 19–27. DOI: 10.2478/s13545-011-0025-4.
- Jampeetong, A. & Brix H. (2009). Nitrogen nutrition of *Salvinia natans*: Effects of inorganic nitrogen form on growth, morphology, nitrate reductase activity and uptake kinetics of ammonium and nitrate. *Aquat. Bot.*, 90, 67–73. DOI: 10.1016/j.aquabot.2008.06.005.
- Kitajima, K. & Hogan K.P. (2003). Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant, Cell Environ.*, 26, 857–865. DOI: 10.1046/j.1365-3040.2003.01017.x.
- Levado, E. (2001). *Studies on phytoplankton diversity within the water column of two freshwater lakes – Rostherne Mere (UK) and Lake Glubokoe (Russia)*. PhD Thesis, University of Manchester.
- Lippert, I., Rolletschek, H. & Kohl J.-G. (2001). Photosynthetic pigments and efficiencies of two *Phragmites australis* stands in different nitrogen availabilities. *Aquat. Bot.*, 69, 359–365. DOI: 10.1016/S0304-3770(01)00148-6.
- Lolić, S., Matavulj, M. & Maksimović T. (2012). *Komparativna analiza kvaliteta vode rijeke Mature i bazena Prevlaka na Bardači* (pp.121–128). Banja Luka.
- Lolić, S., Matavulj, M., Dekić, R. & Maksimović T. (2014). *Kvalitativni i kvantitativni sastav fitoplanktona u vodi bazena Sinjak (ribnjak Bardača)* (pp. 39–47). Banja Luka.
- Lukina, L.F. & Smirnova N.N. (1988). *Fiziologija viših vodnih rastenii*. Kiev: Naukova Dumka.
- Matavulj, M., Gajin, S. & Bokorov M. (1990). Sezonska dinamika fosfomineralizatora u vodi ribnjaka i njihov odnos sa drugim članovima mikrobiocenoze, ribnjak Futog II. In *Zbornik radova II Jugoslovenskog simpozija mikrobne ekologije* (pp. 219–227). Zagreb.

- Maksimović, T., Rončević, S. & Kukavica B. (2019). *Utricularia vulgaris* L. and *Salvinia natans* (L.) All. heavy metal (Fe, Mn, Cu, Zn and Pb) bioaccumulation specificity in the area of Bardača fishpond. *Ekológia (Bratislava)*, 38(3), 300–313. DOI: 10.2478/eko-2019-0016.
- Mészáros, I., Veres, S., Dinka, M. & Lakatos G. (2003). Variations in leaf pigments content and photosynthetic activity of *Phragmites australis* in healthy and die-back reed stands of lake Fertő/Neusiedlersee. *Hydrobiologia*, 506, 681–686. DOI: 10.1007/BF03335350.
- Nekrasova, G.F., Ronzhina, D.A., Maleva, M.G. & P'yankov V.I. (2003). Photosynthetic metabolism and activity of carboxylating enzymes in emergent, floating, and submerged leaves of hydrophytes. *Russian Journal of Plant Physiology*, 50, 57–67. DOI: 10.1023/A:1021936418052.
- Nikolić, Lj., Pajević, S. & Ljevnaić B. (2009). Primary production dynamics of dominant hydrophytes in Lake Provala (Serbia). *Central European Journal of Biology*, 4(2), 250–257. DOI: 10.2478/s11535-009-0013-5.
- Prasad, M.N.V. & Freitas H.M.D. (2003). Metal hyperaccumulation in plants - Biodiversity prospecting for phytoremediation technology. *Electronic Journal of Biotechnology*, 6(3), 285–321. DOI: 10.2225/vol6-issue3-fulltext-6.
- Prasad, M.N.V., Greger, M. & Aravind P. (2006). Biogeochemical cycling of trace elements by aquatic and wetland plants: relevance to phytoremediation. In M.N.V. Prasad, K.S. Sajvan & R. Naidu (Eds.), *Trace elements in the environment, biogeochemistry, biotechnology, and bioremediation* (pp. 483–507). Boca Raton: CRC. DOI: 10.1201/9781420032048.
- Rocha, R.R.A., Thomaz, S.M., Carvalho, P. & Gomes L.C. (2009). Modeling chlorophyll-a and dissolved oxygen concentration in tropical floodplain lakes (Paraná, River, Brazil). *Braz. J. Biol.*, 69(Suppl. 2), 491–500. DOI: 10.1590/S1519-69842009000300005.
- Ronzhina, D.A. & P'yankov V.I. (2001). Structure of the photosynthetic apparatus in leaves of freshwater hydrophytes: 2. Quantitative characterization of leaf mesophyll and the functional activity of leaves with different degrees of submersion. *Russian Journal of Plant Physiology*, 48, 723–732. DOI: 10.1023/A:1012544105453.
- Ronzhina, D.A., Nekrasova, G.F. & P'yankov V.I. (2004). Comparative characterization of the pigment complex in emergent, floating, and submerged leaves of hydrophytes. *Russian Journal of Plant Physiology*, 51, 21–27. DOI: 10.1023/B:RUPP.0000011299.93961.8f.
- Saeed, T. & Sun G. (2012). A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: dependency on environmental parameters, operating conditions and supporting media. *J. Environ. Manag.*, 112, 429–448. DOI: 10.1016/j.jenvman.2012.08.011.
- Sheikh, A.Q., Pandit, A.K. & Ganai B.A. (2017). Seasonal variation in chlorophyll content of some selected plant species of Yousmarg grassland ecosystem. *Asian Journal of Plant Science and Research*, 7(2), 33–36.
- Shilla, D. & Dativa J. (2008). Biomass dynamics of charophyte-dominated submerged macrophyte communities in Myall Lake, NSW, Australia. *Chemistry and Ecology*, 24, 367–377. DOI: 10.1080/02757540802351185.
- Stevanović, M. & Janković M.M. (2001). *Ekologija biljaka sa osnovama fiziološke ekologije biljaka*. Beograd: NNK.
- Stevenson, R., Bothwell, M., Lowe, R. & Thorp J.H. (1996). *Algal ecology: Freshwater benthic ecosystems*. Academic Press.
- Taniguchi, G.M., Bicudo, D.C. & Senna P.A.C. (2004). Abiotic variables in littoral-limnetic gradient of an Oxbow lake of Mogi-Guaçu River floodplain, southeastern, Brazil. *Braz. Arch. Biol. Technol.*, 47(6), 961–971. DOI: 10.1590/S1516-89132004000600016.
- Temali, A. & Ylli A. (2017). Variations of photosynthetic pigments of the specie *Najas marina* related to the water quality in the opposite shore of Skadar lake. *International Educational Scientific Research Journal*, 3(1), 5–12.
- Thomaz, S.M., Pagioro, T.A., Bini, L.M., Roberto, M.C., & Rocha R.R.A. (2004). Limnology of the Upper Paraná Floodplain habitats: patterns of spatio-temporal variations and influence of the water levels. In S.M. Thomaz, A.A. Agostinho & N.S. Hahn (Eds.), *The Upper Paraná River and its floodplain: physical aspects, ecology and conservation* (pp. 76–102). Leiden: Backhuys Publishers.
- Townsend, A.S. (2006). Hydraulic phases, persistent stratification, and phytoplankton in a tropical floodplain lake (Mary River, northern Australia). *Hydrobiologia*, 556(1), 163–179. DOI: 10.1007/s10750-005-0885-y.
- Uvalle Saucedo, J.I., Gonzalez Rodriguez, H., Ramirez Lozano, R.G., Cantu Silva, I. & Gomez Meza M.V. (2008). Seasonal trends of chlorophylls a and b and carotenoids in native trees and shrubs of Northeastern Mexico. *J. Biol. Sci.*, 8, 258–267. DOI: 10.3923/jbs.2008.258.267.
- Wetzel, R.G. (2001). *Limnology: lake and river ecosystems*. San Diego: Academic Press.