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THE ANTIOXIDANT ROLE OF LOW MOLECULAR METABOLITES AND POLYPHENOL OXIDASE OF BRYOPHYTES IN POST-TECHNOGENIC TERRITORIES

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Background. The antioxidant system (AOS), which includes both high-molecular and low-molecular antioxidants, plays an important role in protecting plants from oxidative stress. Previous studies have shown that exposure to high temperature and insolation caused an increase in the activity and thermal stability of antioxidant enzymes, which can be considered a mechanism of adaptation of the protein-synthesizing system to the effects of high temperatures. The enzymatic antioxidant system does not provide 100 % protection to plant cells under stress. Antioxidant metabolites play an important role in plant adaptation to hyperthermia and other abiotic stress factors that lead to the generation of excessive amounts of ROS. The protective role of low-molecular-weight antioxidants in the development of stress tolerance to abnormally high temperatures and insolation, as well as changes in the activity of polyphenol oxidase (PPO) and phenolic content in bryophytes, has not been sufficiently studied. Therefore, the aim of the work was to study seasonal changes in the quantitative content of phenolic compounds (PhC), anthocyanin and carotenoid pigment complexes in mosses in post-anthropogenic areas, as well as changes in PPO activity as indicators of abiotic stress.

Materials and Methods. The objects of research were: the dominant moss species *Ptychostomum imbricatulum* (Müll. Hal.) Holyoak & N. Pedersen in the experimental areas of the sulfur mining dump of the Novoyavoriv State Mining and Chemical Enterprise "Sirka" (Lviv region), as well as the new species for the flora of Ukraine *Campylopus introflexus* (Hedw.) Brid., which was first discovered on the technogenic substrates the "Nadiya" mine dump in the Chervonohrad mining and industrial district, and later on former peat quarries in the vicinity of Lopatyn and Olesko in Lviv region. The research was conducted in the summer and autumn of 2023. The intensity of lighting in the experimental areas was measured with a GM1030C luxmeter (Benetech, China).

The determination of the total content of PhC in the gametophyte of mosses was carried out spectrophotometrically using the Folin-Denis reagent. Polyphenol oxidase activity was determined spectrophotometrically by the rate of oxidation of paraphenylenediamine. The quantitative content of carotenoids was determined using the method of D. Arnon. The content of anthocyanins was determined using a hydrochloric acid. The determination of flavonoids was carried out according to the method of A. Pekal. The results were statistically analyzed, determining the mean value, median, standard deviation (SD), and the first and the third quartiles for each characteristic in all the variants of the experiment.

Results and Discussion. The highest PhC content in *C. introflexus* plants from the studied areas was observed in July, August, and November, which is apparently due to the accumulation of these compounds under stressful conditions. The highest PPO activity in the gametophyte of the *C. introflexus* moss was observed in the summer in the northern section of the top of the "Nadiya" mine dump; in November, a higher PhC content and decreased PPO activity were detected. In summer, at high temperatures, light intensity, and pronounced moisture deficiency, an increase in the content of phenols, anthocyanins, carotenoids, and flavonoids was observed in samples of *P. imbricatulum* moss from the base to the top of the sulfur mining dump. In the autumn period, in response to decreasing temperature and light intensity, less intensive biosynthesis of antioxidant metabolites occurred on all research transects, although the trend of increasing of their content from the base to the top persisted.

Conclusion. The survival strategy of bryophytes under the intense influence of abiotic stress factors in the studied post-anthropogenic territories consists in increasing of the pool of low-molecular metabolites and an enhanced activity of polyphenol oxidase, which contributes to their stress resistance. Based on the research results, it can be assumed that the existence of *P. imbricatulum* and *C. introflexus* in post-technogenic territories initiates adaptogenesis and leads to the formation of mechanisms of moss resistance to the action of stress factors, which are based on nonspecific protective reactions that ensure the adaptation of the plant organism to changing environmental conditions.

Keywords: mosses, *Ptychostomum imbricatulum*, *Campylopus introflexus,* phenolic compounds, anthocyanins, carotenoids, flavonoids, polyphenol oxidase

INTRODUCTION

Hyperthermia is one of the powerful abiotic environmental factors that affect a plant organism leading to significant changes in cell metabolism. It is accompanied by an increase in the formation of reactive oxygen species (ROS) and, as a result, damage to their cellular structures (Wadavkar *et al.*, 2017; Zhang *et al.*, 2017). An important role in protecting plants from oxidative stress is played by the antioxidant system (AOS), which includes both high-molecular and low-molecular antioxidants. Studies using the example of the dominant species of moss *Ptychostomum imbricatulum* (Müll. Hal.) Holyoak & N. Pedersen (according to the old classification – *Bryum caespiticium* Hedw.) showed that the influence of high temperature and insolation caused an increase in the activity and thermal stability of antioxidant enzymes, which can be regarded as the mechanism of adaptation of the protein-synthesizing system to high temperatures (Baik *et al.*, 2021). It is believed that low-molecular antioxidants are one of the mechanisms of constitutive resistance of plants to hyperthermia. Under the influence of stressors on plants, rapid inactivation of the constitutive pool of antioxidant enzymes is observed. Therefore, in many cases, low-molecular-weight antioxidants are able to protect metabolism from ROS (Blokhina *et al.*, 2003; Arbona *et al.*, 2008) more effectively. Low-molecular antioxidants are compounds with different structure and chemical properties that are able to interact with oxygen and organic radicals and inhibit the course of free radical processes in cells (Zhang *et al.*, 2017).

Phenolic compounds (PhC) are considered the most important endogenous low-molecular organic antioxidants, which are active metabolites of cellular metabolism. The protective effect of plant phenols in vascular plants has been proven in cases of extreme high and low temperatures, high insolation, salinity, etc. (Rivero *et al.*, 2001; Edreva *et al.*, 2008; Li *et al.*, 2020; Peck & Mittler, 2020).

Along with the synthesis of phenolic compounds, polyphenol oxidase (PPO) is activated in bryophytes under stressful conditions, which catalyzes the oxidation of monophenols and/or o-diphenols to o-quinones with the reduction of oxygen to water, leading to the formation of brown melanin pigments (Vaughn *et al.*, 1988, Tilley *et al.*, 2023). PPO is found in most vascular plants and may have a role in either acclimation or short-term response to stress, indicated by circumstantial evidence such as enzyme localization. It is postulated that the occurrence of this enzyme is correlated with the emergence of land plants suggesting a role in adaption to abiotic stress (Boeckx *et al.*, 2015). It has been established that PPO contributes to the adaptation of plants to changing environmental conditions (Araji *et al.*, 2014; Smolińska-Kondla *et al.*, 2022). In recent years, the study of the participation of the enzyme in the metabolism of mosses is associated with the assessment of seasonal changes in the activity of PPO according to various physical and chemical characteristics of the substrates of localities (Richter *et al.*, 2005; Tahvanainen & Haraguchi, 2013; Thakur & Kapila, 2017).

Mosses are pioneer plants that settle in post-technogenic areas and contribute to their revitalization (Lobachevska *et al.*, 2022). Therefore, it is important to study the mechanisms of resistance of bryophytes to the influence of stress factors, which are based on non-specific protective reactions that ensure the adaptation of the plant organism to changing conditions of existence. The protective role of low-molecular-weight antioxidants in the development of stress tolerance to abnormally high temperatures and insolation, as well as changes in PPO activity and phenolic content in bryophytes, has not been sufficiently studied. Therefore, the aim of the work was to study seasonal changes in the quantitative content of PhC, anthocyanin and carotenoid pigment complexes in mosses in post-technogenic areas, as well as changes in PPO activity as indicators of abiotic stress.

MATERIAL AND METHODS

The objects of the research were: the dominant moss species *Ptychostomum imbricatulum* (Müll. Hal.) Holyoak & N. Pedersen in the experimental areas of the sulfur mining dump of the Novoyavoriv State Mining and Chemical Enterprise "Sirka" (Lviv region), as well as a new species for the flora of Ukraine, *Campylopus introflexus* (Hedw.) Brid., which was first discovered on technogenic substrates of the "Nadiya" mine dump in the Chervonohrad mining and industrial district, and later as well on former peat quarries in the vicinity of Lopatyn and Olesko in the Lviv region (**Fig. 1**). At present, no other locations of its distribution in Ukraine are known. Therefore, the areas of former peat quarries can be considered conditionally as control sites, as they are less devastated compared to the technogenic substrates of mine dumps.

Fig. 1. Species of mosses used for research: ***A*** − *Ptychostomum imbricatulum* (Müll. Hal.) Holyoak & N. Pedersen, ***B*** − *Campylopus introflexus* (Hedw.) Brid. Photos of the authors

The research was carried out in the summer (July, 2023) at temperatures t = +35.0 °C – +38.0 °C and lighting intensity of 100–110 thousand lux and in autumn (October, 2023) at temperatures t = +10 °C – +15 °C, insolation ≈ 80 thousand lux. The light intensity in the experimental areas was measured with a GM1030C luxmeter (Benetech, China).

Freshly collected samples of plant material were immediately analyzed in laboratory conditions using appropriate methods, and were also dried to an air-dry state to determine plant moisture content and convert the content of the studied compounds to the mass of dry matter.

Determination of the total content of phenolic compounds (PhC) in the gametophyte of mosses was carried out using a Specord 210 Plus spectrophotometer (manufacturer – AnalytikJena) with the Folin-Denis reagent (Anahita *et al.*, 2015). A portion of plant material (100 mg) was homogenized in 3 mL of 96% ethyl alcohol, centrifuged at 4000 g; 1 mL of supernatant was added to the test tube, 2.5 mL of distillate and 0.5 mL of Folin–Denis reagent were added. The samples were mixed and 1 mL of saturated Na_2CO_3 solution was added. The optical density of the extract was measured at a wavelength of 725 nm. The total content of phenolic compounds was calculated using a calibration curve based on chlorogenic acid and expressed in mg/g of dry weight.

The activity of polyphenol oxidase was determined spectrophotometrically by the rate of oxidation of paraphenylenediamine (Voitsekhivska *et al.*, 2010). A portion of plant material (50 mg) was homogenized in 5 mL of phosphate buffer, centrifuged at 4000 g. The enzyme activity was investigated by changing the p-phenylenediamine solution on a spectrophotometer at a wavelength of 420 nm.

Actual acidity (pH) was measured potentiometrically in an aqueous substrate-distillate extract (1:5) (Nikolaychuk, 2000).

To determine the amount of carotenoids, a portion of plant material (100–200 mg) was homogenized in 80 % acetone solution according to the method of D. Arnon (Arnon, 1949). The resulting extract, which contained the sum of green and yellow pigments, was centrifuged (10 min, 4000 g). The optical density of the extract was measured at a wavelength of 470 nm. The control and experimental suspensions were compared in terms of chlorophyll content.

The content of anthocyanins was determined using a hydrochloric acid (Kotova & Kotov, 2014; Khattab *et al.*, 2016; Burlaka *et al.*, 2021). A portion of plant material (50 mg) was homogenized in 10 mL of a 1% solution of hydrochloric acid in ethyl alcohol and kept in a water bath at a temperature of 40–45 °C for 20 min. The resulting homogenate was filtered, and the optical density of the filtrate was measured at a wavelength of 539 nm. The content of anthocyanins was expressed in mg/g of dry weight.

To determine flavonoids, 50 mg of shredded plant material was extracted with the addition of 2 mL of methanol during 24 hours. The homogenate was centrifuged at 5000 rpm for 10 min. Then, 1 mL of supernatant was taken, 0.5 mL of 2% $AlCl_3$ solution, 0.5 mL of 1M sodium acetate and 0.5 mL of distilled water were added. The mixture was kept for 10 min at room temperature and analyzed spectrophotometrically on a Specord 210 Plus spectrophotometer at a wavelength of 425 nm. The content of flavonoids was determined using a calibration curve based on quercetin and expressed in mg/g of dry weight (Pękal & Pyrzynska, 2014).

The results were statistically analyzed, determining the mean value, median, standard deviation (SD), and the first and the third quartiles for each characteristic in all the variants of the experiment. The selections were compared using two-way analysis of variance (two-way ANOVA) with Bonferroni post-hoc test, considering differences between the selections reliable at the level of $P < 0.05$, 0.01, and 0.001. All calculations and developments of diagrams were made using Statistica 8.0 software (StatSoft, USA, 2012).

RESULTS AND DISCUSSION

The highest content of phenolic compounds (PhC) in *C. introflexus* plants from the studied areas was noted in July, August and November (**Table 1**), which is obviously caused by the accumulation of these compounds under stressful conditions (increased temperature in summer and its decrease during autumn months).

The highest activity of polyphenol oxidase (PPO) in the gametophyte of the moss was noted in the summer in the northern part of the top of the "Nadiya" mine dump (**Table 2**). It was established that the content of phenols and the activity of polyphenol oxidase in the gametophyte *C. introflexus* were higher in the northern and northwestern areas, compared to the plants in the eastern area. In November, in the gametophyte of *C. introflexus* from the mine dump, a higher content of PhC and a decrease in the activity of PPO were determined, which may be due to a decrease in the temperature of the air (to 14 °C).

In plants of *C. introflexus* from the former peat quarries of Lopatyn and Olesko towns, the activity of polyphenol oxidase was higher than in plants from different exposures of the top of the "Nadiya" mine dump, which is caused by a higher pH of the substrates of the peat quarries (5.8–6.2), compared to dump substrates (5.2–5.6), because it is known from the literature that the activity of PPO depends on the actual acidity of the environment (Tahvanainen, Haraguchi, 2013).

It was shown that during the summer, when the air temperature range was 30–36 °C, the activity of PPO in the gametophyte *C introflexus* from all the studied territories was

Table 1. **The content of phenolic compounds in the gametophyte of the moss *Campylopus introflexus* (Hedw.) Brid. depending on local conditions, mg/g of dry weight**

Locality \ Month		July	August	October	November
Mine dump "Nadiya"	Eastern section	0.04±0.01	0.78±0.02 aaa, ccc	0.33±0.04 aaa, bbb, ddd	0.84±0.04 aaa, ccc
	North-western section	0.38±0.02***, @@, %%%, &&, bbb, c, ddd	0.97±0.03**, @, %%%, &&, aaa, ccc, dd	0.52±0.03**, @@@, %, &&, a, bbb, ddd	1.31±0.05**, @, %%, &&&, aaa, bb, ccc
	Northern section	0.72±0.03***, $$, %%%, cc, dd	0.85±0.02 $, %%%, &&&, c, d	1.09±0.03***, $$$, %%, &&&, aa, b	1.04±0.01**, $, %, &&, aa, b
Peat quarries	near Olesko village	1.28±0.02***, $$$, &&&, b, cc, d	1.49±0.01***, $$$, @@@, &, a,cc, dd	0.70±0.09*, $, @@, aa, bb	0.88±0.06 $$, @, a, bb
	near Lopatyn village	0.70±0.03***, $$, %%%, bbb, d	1.20±0.02***, $$, @@, %, aaa, ccc, ddd	0.76±0.03***, $$, @@@	0.81±0.01 $$$, @@, a, bbb

Locality: * – P <0.05; ** – P <0.01; *** – P <0.001 – statistically significant vs eastern section (control)
[$] – P <0.05; [$$] – P <0.01; [$$$] – P <0.001 – statistically significant vs north-western section
[@] – P <0.05; [@@] – P <0.01; [@@@] – P <0.001 – statistically significant vs northern section
[%] – P <0.05; [%%] – P <0.01; [%%%] – P <0.001 – statistically significant vs near Olesko village
[&] – P <0.05; [&&] – P <0.01; [&&&] – P <0.001 – statistically significant vs near Lopatyn village

Month: [a] – P <0.05; [aa] – P <0.01; [aaa] – P <0.001 – statistically significant vs July
[b] – P <0.05; [bb] – P <0.01; [bbb] – P <0.001 – statistically significant vs August
[c] – P <0.05; [cc] – P <0.01; [ccc] – P <0.001 – statistically significant vs October
[d] – P <0.05; [dd] – P <0.01; [ddd] – P <0.001 – statistically significant vs November

Table 2. **Seasonal changes in the activity of polyphenol oxidase in the gametophyte of the moss *Campylopus introflexus* (Hedw.) Brid., rel. unit/g of dry weight**

Locality \ Month		July	August	October	November
Mine dump "Nadiya"	Eastern section	56.5±7.2 bb	41.2±2.1 bb	95.2±4.1 aa, bbb, d	63.8±7.0 c
	North-western section	97.4±4.3*, @@, %%, &, b, cc, ddd	144.9±7.2***, %%, a, ccc, ddd	53.9±3.5**, @@@,%%%,&&&, aa, bbb	41.3±5.1 %%%, &, aaa, bbb
	Northern section	139.4±3.9***, $$, ddd	169.8±8.4***, %%, ddd	125.4±5.0***, $$$, %%%, &, ddd	50.4±4.4 %%%, &, aaa, bbb, ccc
Peat quarries	near Olesko village	137.1±4.9***, $$, bb, ccc, d	75.6±2.8**, $$, @@, &&, aa, ccc, ddd	334.4±8.1***, $$$, @@@, &&&, aaa, bbb, ddd	169.5±5.3***, $$$, @@@, &, a, bbb, ccc
	near Lopatyn village	143.2±9.7**, $$, d	148.5±3.2***, %%, d	152.0±4.9***, $$$, @, %%%, d	96.1±4.1*, %%%, a, b, c

Locality: * – P <0.05; ** – P <0.01; *** – P <0.001 – statistically significant vs eastern section (control)
[$] – P <0.05; [$$] – P <0.01; [$$$] – P <0.001 – statistically significant vs north-western section
[@] – P <0.05; [@@] – P <0.01; [@@@] – P <0.001 – statistically significant vs northern section
[%] – P <0.05; [%%] – P <0.01; [%%%] – P <0.001 – statistically significant vs near Olesko village
[&] – P <0.05; [&&] – P <0.01; [&&&] – P <0.001 – statistically significant vs near Lopatyn village

Month: [a] – P <0.05; [aa] – P <0.01; [aaa] – P <0.001 – statistically significant vs July
[b] – P <0.05; [bb] – P <0.01; [bbb] – P <0.001 – statistically significant vs August
[c] – P <0.05; [cc] – P <0.01; [ccc] – P <0.001 – statistically significant vs October
[d] – P <0.05; [dd] – P <0.01; [ddd] – P <0.001 – statistically significant vs November

higher, compared to the autumn months (the range of temperature variability was 15–24 °C). For most of the selected samples, an inverse relationship between the total content of PhC and activity of PPO was determined, which is obviously due to the oxidation of phenols, as well as the functioning of phenol oxidases (Tilley *et al.*, 2023).

One of the features of the formation of plant resistance to abiotic factors is the ability to synthesize secondary metabolites, which include PhC. It is known that PhC counteract with oxidative stress: they neutralize ROS, maintain the internal environment of cells in a restored state and have a positive effect on the activity of antioxidant enzymes (Baik *et al.*, 2021; Hasanuzzaman *et al.*, 2020; Durand, 2019; Ren *et al.*, 2021; Wani *et al.*, 2021). The activity of PPO, along with other redox enzymes of respiration, plays an important role in the processes of phytoimmunity to the action of abiotic and biotic factors, which is important for plant colonization of new, particularly, disturbed areas. (Lavid *et al.*, 2001). It is known about the induction of PhC synthesis in the gametophyte of mosses in response to exposure to UV radiation, high light intensity, temperature stress, and salinity (Bogdanović *et al.*, 2011). Unlike vascular plants, bryophytes do not have lignin in their epidermis, therefore they have evolved a molecular mechanism for their survival strategy, namely the biochemical ability to accumulate increased amounts of PhC. The red-brown color of the cell walls of rhizoids, caulonema stolons of protonema, macro- and micronema of shoots and brood bodies of bryophytes is mostly caused by PhC, which are of decisive importance for the formation of plant resistance (Smolińska-Kondla *et al.*, 2022).

The detected increase in PhC content indicates that under the action of extreme environmental factors, oxidative stress obviously develops, during which the physiological and biochemical adaptation systems of moss metabolism, aimed at restoring homeostasis, are activated (Lobachevska *et al.*, 2022). In response to temperature stress, nonspecific adaptive mechanisms are activated in plant cells. It is possible that the induction of these mechanisms is associated with an increase in the synthesis of PhC, which play the role of components of the plant antioxidant system (Smolińska-Kondla *et al.*, 2022). Along with the biosynthesis of phenols, there are reactions of their enzymatic oxidation by phenoloxidases, which include polyphenoloxidase (PPO), the activity of which is associated with adaptation processes of plants to changes in living conditions (Boeckx *et al.*, 2015). Products of oxidation and polymerization of phenols play a significant role in the protective reactions of plants to the influence of anthropogenic factors (Ortega-Garcha & Peragon, 2009; Mehr *et al.*, 2012). Therefore, their content can serve as one of the indicators of plant resistance to adverse environmental conditions.

The content of phenolic compounds in the dominant species of moss *P. imbricatulum* on the territory of the sulfur mining dump of the Novoyavorivsk State Mining and Chemical Enterprise "Sulfur" (Lviv region) largely depends on the microclimatic conditions on the experimental transects: the most optimal conditions, even in summer, are at the base of the dump, and the most extreme – at the top of the dump. It was found that in the summer period, when the temperature reached 35–38 °C, and the light intensity was 100–110 thousand lux, there was an increase in the content of phenols in *P. imbricatulum* moss samples from the base to the top of the dump (**Fig. 2**). In autumn, at lower temperatures (10–15 °C) and light intensity (70–80 thousand lux), the content of phenols decreased almost twice on all experimental transects: by 45 % at the base of the dump, by 44 % on the northern slope, by 55 % on the plateau and by 43 % at the top.

Thus, it was established that under stressful conditions, a large amount of phenols accumulates in *P. imbricatulum* moss, which contributes to its increased resistance to adverse microclimatic conditions.

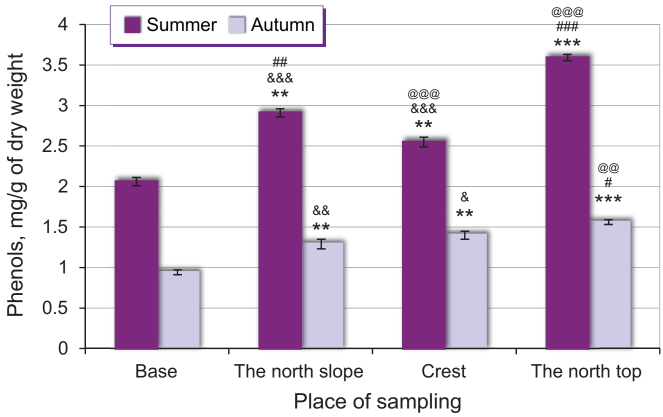

Fig. 2. The content of phenols (mg/g of dry weight) in the shoots of the moss *Ptychostomum imbricatulum* from different transects of the northern exposure of sulfur mining dump No. 1 (x±SD; n = 4); ** − difference between experimental sample and control (base) is statistically reliable at P < 0.01; *** – at P <0.001; @@ − P <0.01, @@@ - P <0.001 − statistically significant vs the north slope; # − P <0.05, ## − P <0.01, ### − P <0.001 - statistically significant vs the Crest; & − P <0.05, && − P <0.01, &&& − P <0.001 − statistically significant vs the north top

There are literary data (Bobo-Garcia *et al.*, 2015; Sharma *et al.*, 2019; Hurry, 2021) about changes in the content of certain PhC classes, as well as the adaptive role of anthocyanins under the influence of extreme abiotic factors. The basis for this assumption are the strong antioxidant properties of anthocyanins and the ability to control the level of hydrogen peroxide, which rises (Eghbaliferiz & Iranshahi, 2016). Anthocyanins are antioxidants that neutralize reactive oxygen species formed under stressful conditions. The content of anthocyanins is the dominant component of the phenolic complex. It is believed that anthocyanins participate in the protection of thylakoid membranes under stress (Neill *et al.*, 2002; Naing & Kim, 2021). We found a tendency for anthocyanins to accumulate under conditions of high temperatures and light intensity in the summer, especially at the top of the dump (by 40 %) and the northern slope of the dump (by 32 %), compared to the base (as control). In autumn, the content of anthocyanins slightly decreased on all experimental transects, although the trend of their content increasing from the base to the top persisted (**Fig. 3**).

The high content of anthocyanins in response to temperature stress and insolation indicates their important role in overcoming oxidative stress and moss adaptation. Obviously, an increase in the level of anthocyanins is the reaction of moss plants to adverse environmental factors aimed at neutralizing oxidative damage caused by the action of stress factors. It is known that there is a close relationship between the accumulation, qualitative and quantitative characteristics of anthocyanins in plants and environmental factors, in particular, temperature and light intensity (Lobachevska *et al.*, 2022).

Reactive oxygen species (ROS), especially singlet oxygen generated in chloroplasts under stress conditions, can oxidize carotenoids, leading to a variety of oxidized products, including aldehydes, ketones, endoperoxides, and lactones (Hasanuzzaman *et al.*, 2020). Some of these carotenoid derivatives are biologically active and can induce changes in gene expression leading to stress resistance (Havaux, 2013; Kulshrestha *et al.*, 2022). Carotenoids are components of the pigment complex and low-molecular terpenoid antioxidants. They absorb ROS formed during photo-oxidative stress and reduce the effects of extreme temperatures (Uarrota *et al.*, 2018).

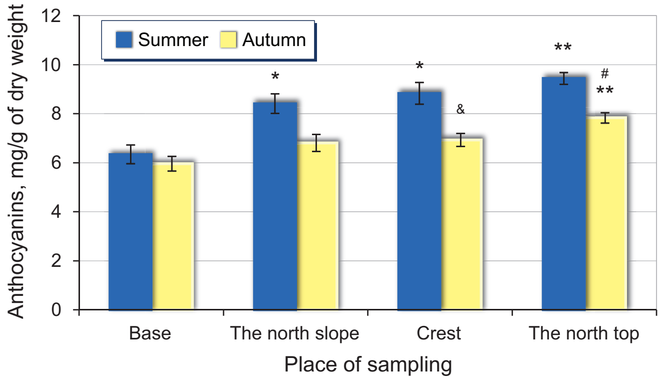

Fig. 3. Anthocyanin content (mg/g of dry weight) in shoots of the moss *Ptychostomum imbricatulum* from different transects of the northern exposure of sulfur mining dump No. 1 (x±SD; n = 4); * – difference between experimental sample and control (base) is statistically reliable at P <0.05; ** – at P <0.01; # – P <0.05 – statistically significant vs the Crest; & – P <0.05 – statistically significant vs the north top

The study has shown seasonal variability of the content of carotenoids in *P. imbricatulum* moss from experimental transects. An increase in the content of carotenoids was established on transects with high light intensity and temperature (northern peak and dump plateau) in summer. Thus, the content of carotenoids at the top of the dump was the highest in summer and amounted to 0.73±0.02 mg/g of dry weight, on the crest – 0.55±0.03 mg/g of dry weight. The lowest content of carotenoids was found in moss from the base of the dump – 0.41±0.05 mg/g of dry weight. Such an increase in the content of carotenoids indicates the development of protective reactions that contribute to the dissipation of excess light energy and neutralize ROS. In autumn, this trend of decreasing carotenoid content in samples from the top to the base of the dump persisted. Under optimal climatic conditions in autumn, the carotenoid content at the top of the dump decreased by 16 %, and at the base of the dump – by 7 % (**Fig. 4**).

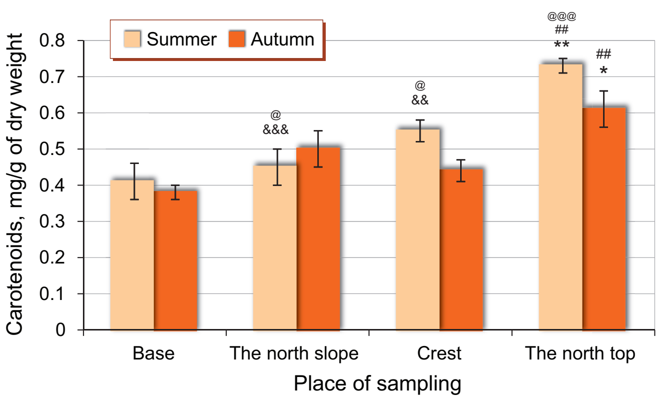

Fig. 4. The content of carotenoids (mg/g of dry weight) in the shoots of the moss *Ptychostomum imbricatulum* from different transects of the northern exposure of sulfur mining dump No. 1 (x±SD; n = 4); * – difference between experimental sample and control (base) is statistically reliable at P <0.05; ** – at P <0.01; @ – P <0.05, @@@ – P <0.001 – statistically significant vs the north slope; ## – P <0.01 – statistically significant vs the Crest; && – P <0.01 – statistically significant vs the north top

Taking into account the antioxidant properties of carotenoids, it is possible to assume their participation in the formation of adaptation to extreme environmental factors in post-technological territories. Obviously, the increased synthesis of carotenoids in *P. imbricatulum* is a necessary, genetically detenmined condition for survival under stress.

Thus, it was established that high light intensity and extreme temperatures in the summer, especially at the top of the rock dump, cause an increase in the pool of soluble phenolic compounds, anthocyanins and carotenoids, which contributes to the adaptation of *P. imbricatulum* moss to adverse abiotic factors and can serve as an indicator of physiological and biochemical state of the plant organism.

It is known that flavonoids are accumulated during stress, increasing the resistance of plants and are indicators of their physiological state (Han *et al.*, 2012; Kim *et al.*, 2021). The protective role of flavonoids is manifested in the increase of their biosynthesis as a response to the action of stress factors. It was found that in the summer, under high temperatures and light intensity, the content of flavonoids increased from the base to the top of the dump. Thus, at the top of the dump, the content of flavonoids was 19.56±0.21 mg/g of dry weight, on the northern slope – 17.61±0.21 mg/g of dry weight, on the crest – 18.21±0.30 mg/g of dry weight and in the basis – 14.51±0.21 mg/g of dry weight. In the autumn period, in response to decreasing temperatures, flavonoid biosynthesis was less intense by approximately 15 % on all transects (**Fig. 5**).

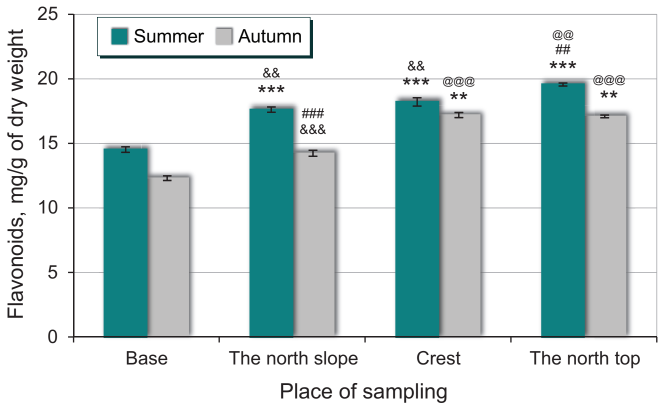

Fig. 5. The content of flavonoids (mg/g of dry weight) in shoots of the moss *Ptychostomum imbricatulum* from different transects of the northern exposure of sulfur mining dump No. 1 (x±SD; n = 4); ** − difference between experimental sample and control (base) is statistically reliable at P < 0.01; *** – at P <0.001; @@ – P <0.01; @@@ – P <0.001 − statistically significant vs the north slope; ## – P <0.01; ### – P <0.001 − statistically significant vs the Crest; && – P <0.01; &&& – P <0.001 − statistically significant vs the north top

Obviously, the increase in the content of flavonoids in autumn was accompanied by increasing resistance of moss cells to a decreased temperature, since the carbohydrate residues of flavonoids, similar to starch, delay the crystallization of water, and their hydroxyl groups can form hydrogen bonds with water molecules (Møller *et al.*, 2015). In addition, in the cousre of plant adaptation to stress factors, there is an increased expression of genes responsible for the synthesis of enzymes of flavone metabolism (Winkel-Shirley, 2001). Based on the obtained data, the seasonal dynamics of flavonoid accumulation and the relationship between the content of flavonoids and the negative impact of extreme stress factors in the moss *P. imbricatulum* were established. Therefore, in response to high insolation in summer and a decreasing of temperature in the autumn period, a change in the content of flavonoids is observed, indicating the adaptation of moss plants.

CONCLUSION

It has been established that in summer, at high temperatures and light intensity, the mosses *Ptychostomum imbricatulum* and *Campylopus introflexus* accumulate a large amount of phenols, which promotes an increase in resistance to adverse microclimatic conditions in post-technogenic territories. In autumn, when temperature and insolation indicators decrease, the content of phenols decreases.

Seasonal changes in the content of phenolic compounds and polyphenol oxidase activity in the gametophyte of the adventive/ adventitious moss *C. introflexus* are driven by both shifts in microclimatic conditions and the regulation of phenol biosynthesis and oxidation activation. These changes indicate the moss's tolerance to post-technogenic environmental conditions, enabling the species to expand its range.

The survival strategy of bryophytes under the intense influence of abiotic stress factors in the studied post-technogenic territories consists in increasing the pool of low-molecular metabolites (flavonoids, carotenoids, and anthocyanins) and increasing the activity of polyphenol oxidase, which contributes to their stress resistance.

Thus, based on the research results, it can be assumed that the existence of *Ptychostomum imbricatulum* and *Campylopus introflexus* in post-technogenic territories initiates adaptogenesis and leads to the formation of mechanisms of moss resistance to the action of stress factors. Mechanisms of moss resistance are based on nonspecific protective reactions that ensure the adaptation of the plant organism to changing environmental conditions.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: this article does not contain any studies with animal subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [O.B.; R.S.; V.H.]; methodology, [O.B.; R.S.]; validation, [O.B.; R.S.; V.H.]; formal analysis, [O.B.; R.S.; V.H.].; investigation, [O.B.; R.S.]; resources, [O.B.; R.S.; V.H.]; data curation, [O.B.; R.S.; V.H.]; writing – original draft preparation, [O.B.; R.S.]; writing – review and editing, [O.B.; R.S.]; visualization, [O.B.; R.S.]; supervision, [O.B.; R.S.; V.H.] ;project administration, [O.B.; R.S.; V.H.].

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АНТИОКСИДАНТНА РОЛЬ НИЗЬКОМОЛЕКУЛЯРНИХ МЕТАБОЛІТІВ І ПОЛІФЕНОЛОКСИДАЗИ БРІОФІТІВ НА ПОСТТЕХНОГЕННИХ ТЕРИТОРІЯХ

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Обґрунтування. Важливу роль у захисті рослин від окиснювального стресу відіграє антиоксидантна система (АОС), що включає як високомолекулярні, так і низькомолекулярні антиоксиданти. Попередніми дослідженнями з'ясовано, що вплив високої температури та інсоляції спричинював зростання активності й термостабільності ферментів-антиоксидантів, а це можна розглядати як механізм адаптації білоксинтезуючої системи до дії високих температур. Ензиматична антиоксидантна система не забезпечує 100 % захисту клітин рослин під час стресу. Антиоксидантні метаболіти відіграють важливу роль в адаптації рослин до гіпертермії та інших абіотичних стресових чинників, які призводять до генерації надлишкової кількості активних форм кисню (АФК). Захисна роль низькомолекулярних антиоксидантів у розвитку стрес-толерантності до дії аномально високих температур та інсоляції, а також зміна активності поліфенолоксидази (ПФО) та вмісту фенолів у бріофітів досліджені недостатньо. Тому метою роботи було вивчити сезонні зміни кількісного вмісту фенольних сполук (ФС), антоціанового та каротиноїдного пігментного комплексів у мохів на посттехногенних територіях, а також зміни активності ПФО як індикаторів абіотичного стресу.

Матеріали та методи. Об'єктами досліджень були: домінантний вид моху *Ptychostomum imbricatulum* (Müll. Hal.) Holyoak & N. Pedersen на дослідних ділянках відвалу видобутку сірки Новояворівського державного гірничо-хімічного підприємства “Сірка” (Львівська обл.), а також новий для флори України вид *Campylopus*

introflexus (Hedw.) Brid., який вперше виявлено на техногенних субстратах відвалу шахти "Надія" Червоноградського гірничопромислового району, а пізніше і на колишніх торфокар'єрах в околицях смт Лопатин і смт Олесько у Львівській області. Дослідження проводили влітку та восени 2023 року. Інтенсивність освітлення на дослідних ділянках вимірювали люксметром GM1030C (Benetech, Китай).

Визначення загального вмісту ФС у гаметофіті мохів проводили спектрофотометрично з використанням реактиву Фоліна–Деніса. Активність ПФО визначали спектрофотометрично за швидкістю окиснення парафенілендіаміну. Кількісний вміст каротиноїдів визначали за методом Д. Арнона. Вміст антоціанів визначали за допомогою соляної кислоти. Визначення флавоноїдів проводили за методом А. Пєкаль. Результати обробляли статистично, визначаючи середнє значення, медіану, стандартне відхилення, перший і третій квартилі для кожної ознаки в усіх варіантах експерименту.

Результати й обговорення. Найбільший вміст ФС у рослинах *C. introflexus* із досліджуваних територій відзначено у липні, серпні та листопаді, що, очевидно, зумовлено нагромадженням цих сполук за стресових умов. Найбільшу активність ПФО у гаметофіті моху *C. introflexus* відзначено влітку на північній ділянці вершини відвалу шахти "Надія", а у листопаді визначено більший вміст ФС і зменшення активності ПФО. Встановлено, що влітку за високих температур, інтенсивності освітлення та вираженого дефіциту вологи у зразках моху *P. imbricatulum* відбувалося зростання вмісту фенолів, антоціанів, каротиноїдів і флавоноїдів від основи до вершини відвалу сірчаного видобутку. В осінній період у відповідь на зниження температур та інтенсивності освітлення відбувався менш інтенсивний біосинтез низькомолекулярних антиоксидантів на всіх дослідних трансектах, хоча тенденція зростання їхнього вмісту від основи до вершини зберігалась.

Висновок. Стратегія виживання мохових рослин за інтенсивного впливу абіотичних стресових чинників на досліджуваних посттехногенних територіях полягає у зростанні пулу низькомолекулярних метаболітів і у збільшенні активності поліфенолоксидази, що сприяє їхній стресостійкості. Результати досліджень дають підстави вважати, що існування *P. imbricatulum* та *C. introflexus* на посттехногенних територіях ініціює адаптогенез і приводить до формування механізмів стійкості мохів до дії стресових чинників, в основі яких лежать неспецифічні захисні реакції, які забезпечують адаптацію рослинного організму до мінливих умов середовища.

Ключові слова: мохи, *Ptychostomum imbricatulum, Campylopus introflexus*, фенольні сполуки, антоціани, каротиноїди, флавоноїди, поліфенолоксидаза

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