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Foliar Content of Phenolic Compounds in *Platanthera bifolia* from Natural and Transformed Ecosystems at Different Stages of Orchid Development

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Abstract. The representatives of the family Orchidaceae Juss. are often used as a source of natural antioxidants, including phenolic compounds, which play an important role in plant resistance under stressful conditions. This study investigates the content of lipid peroxidation products and soluble phenolic compounds in flowering plants of *Platanthera bifolia* (L.) Rich. growing in natural (forest park) and transformed (fly ash dumps of Thermal Power Stations) ecosystems of the Middle Urals, Russia, as well as the content of flavonoids at different stages of orchid development. Research has shown that in disturbed habitats, *P. bifolia* is capable of forming abundant populations containing a significant portion of the flowering plants. Additionally, flowering orchids from fly ash dumps contained an average 20 % more lipid peroxidation products, which indicated a shift in the redox balance towards oxidative processes. An increase by 2.4 times on average in the content of phenolic compounds, particularly flavonoids, was observed at all developmental stages of the plants growing in the transformed ecosystems. Regardless of the growing conditions, the non-flowering mature individuals were characterized by a minimum content of flavonoids, probably due to pre-generative metabolic restructuring. Yet, in the period of orchid blooming, the flavonoid content in their leaves increased again in all study sites. At the same time, the flavonoid proportion of the total soluble phenolic compounds was 42 % in the natural habitat, increasing to 66 % on average in the transformed ecosystems. Thus, flavonoids are involved

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in the protective adaptive responses of *P. bifolia*, not only ensuring the survival of this orchid but also contributing to the implementation of its ontogenetic program.

Keywords: Orchidáceae, fly ash substrates, oxidative stress, redox balance, antioxidants, flavonoids, age structure of population.

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Содержание фенольных соединений в листьях *Platanthera bifolia* из естественной и трансформированных экосистем на разных стадиях развития орхидеи

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Аннотация. Представители семейства Orchidaceae Juss. нередко являются источником природных антиоксидантов, к числу которых относятся фенольные соединения, играющие важную роль в формировании устойчивости растений к стрессовым факторам. Изучено содержание продуктов перекисного окисления липидов (ПОЛ) и растворимых фенольных соединений у генеративных особей *Platanthera bifolia* (L.) Rich., произрастающих в естественной (лесопарк) и трансформированных (золоотвалы ГРЭС) экосистемах Среднего Урала, а также содержание флавоноидов в листьях орхидеи на разных стадиях ее развития. Обнаружено, что в нарушенных местообитаниях *P. bifolia* способна формировать ценопопуляции с высокой численностью и значительным вкладом в возрастной спектр генеративных особей. При этом цветущие орхидеи с золоотвалов содержали в среднем на 20 % больше продуктов ПОЛ, что свидетельствует о сдвиге редокс-баланса в сторону окислительных процессов. Кроме того, у растений, произрастающих в трансформированных экосистемах, наблюдалось увеличение содержания фенольных соединений, в частности флавоноидов (в среднем в 2,4 раза), на всех изученных стадиях. Независимо от условий произрастания виргинильные особи характеризовались минимальным содержанием флавоноидов, вероятно, из-за метаболических перестроек в период закладки генеративных органов. В период цветения количество флавоноидов в листьях орхидеи снова увеличивалось на всех участках. При этом доля флавоноидов от общего содержания фенольных соединений возрастала от 42 % в естественном фитоценозе до 66 % в среднем в трансформированных экосистемах. Сделано заключение, что флавоноиды участвуют в защитно-приспособительных реакциях *P. bifolia*, не только обеспечивая выживание этой орхидеи, но и способствуя реализации ее онтогенетической программы.

Ключевые слова: Orchidáceae, зольные субстраты, окислительный стресс, редокс-баланс, антиоксиданты, флавоноиды, возрастная структура популяции.

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Introduction

In recent decades, the search for promising sources of plant raw materials for preparing effective formulations with a high content of biologically active compounds has become increasingly important (Gutierrez, 2010). Phenolic compounds are unique substances of secondary metabolism with various properties and play an essential role in plants. This is a very diverse group of compounds, which differ in their chemical structure, varying from simple molecules such as phenolic acids to highly polymerized compounds such as condensed tannins. It is well known that the categorization of a compound is based on the number of constituent carbon atoms in combination with the structure of the main phenolic skeleton (Michalak, 2006; Fraga et al., 2009). Moreover, the diversity of the chemical structures of phenolic compounds determines the wide range of properties and functions not only in the cells, but also in the entire plant organisms. For example, lignin plays an essential role in the structural organization of plant cell walls (Landolino, Cook, 2009). Tannins present in vacuoles take part in plant defense against pathogens. Other phenolic compounds, such as phenylpropanoids, protect cell structures against the chain reactions of free radicals due to excessive light; thus, they act as antioxidants (Babenko et al., 2019). Simple phenols are also widely distributed phenolic compounds, which play an important biological role. Some of them (salicylic acid) act as signaling molecules; others (for example, *n*-hydroxybenzoic acid) are precursors of compounds such as ubiquinones and plastoquinones (Marchiosi et al., 2020).

Flavonoids are the most abundant group of plant polyphenols. These substances play a significant role in the processes of cell signaling, can serve as messengers of chemical signals, and participate in the processes of plant reproduction (Takahama, Oniki, 2000; Pourcel et al., 2007). They are also involved in protecting plants against various adverse environmental factors. One of the most notable functions of these secondary metabolites is plant protection against oxidative stress due to their pronounced antioxidant activity (Takahama, Oniki, 2000; Pourcel et al., 2007; Tarakhovsky et al., 2013).

Different species of the Orchidaceae family are known as the sources of natural antioxidants (Gutierrez, 2010). Many of them have long been used in traditional medicine. However, the chemical composition of orchids as potential sources of biologically active substances has been insufficiently studied. In addition, there is practically no information on the protective antioxidant responses of orchids growing in industrially disturbed territories.

Platanthera bifolia (L.) Rich., commonly known as the lesser butterfly-orchid, is one of the orchid plants with pronounced medicinal properties (Shreter, 1975). It is a protected orchid species listed as «rare» (category III) in many regional Red Data Books of Russia. In the Red Book of the Sverdlovsk Region, though, this species is in category V as «rehabilitating» (Red Book…, 2018). At the same time, in recent years, populations of *P. bifolia* have been found in industrially disturbed areas of the Middle Urals and other regions (Filimonova et al., 2014; Mishagina, 2018; Romanova, Mongush, 2019). Our study focuses on the numerous population of *P. bifolia* found in forest communities formed on the fly ash dumps of Thermal Power Stations (TPS) of the Sverdlovsk Region (Filimonova et al., 2014).

The aim of the study was 1) to estimate the content of lipid peroxidation products and the total soluble phenolic compounds in leaves of *P. bifolia* from natural (forest park) and transformed (fly ash dumps of the TPS) ecosystems of the Middle Urals, Russia; and 2) to compare the foliar flavonoid content at different stages of orchid development.

Materials and methods

P. bifolia is an herbaceous perennial orchid plant with a thickened fusiform stem-root tuberoid; it is a mesophyte, a European–West Asian boreal-nemoral species. It exhibits broad ecological tolerance, but is not strictly confined to a certain plant community (Vakhrameeva et al., 2008; Mishagina, 2018).

In this work, local populations of *P. bifolia* from three different forest communities of the Sverdlovsk Region, Russia (subzone of southern taiga), were studied. Site 1 was located in the natural plant community of the Southwestern Forest Park in the city of Ekaterinburg (56º46'25''N; 60º32'32''E). Site 2 was located in the transformed territory with a forest community (age 45–50 years) shaped like a strip along the inner dam of the fly ash dump of the Sredneural'skaya Thermal Power Station near the town of Sredneural'sk (SUTPS, 57°0'37''N; 60°27'58''E). Site 3 was represented by a forest community (age 35–40 years) naturally colonizing fly ash dump of the Verkhnetagil'skaya Thermal Power Station (VTTPS), the town of Verkhniy Tagil (57°20'46''N; 59°56'45''E) (Fig. 1).

Geobotanical characteristics such as crown density, total shrub projective cover, total

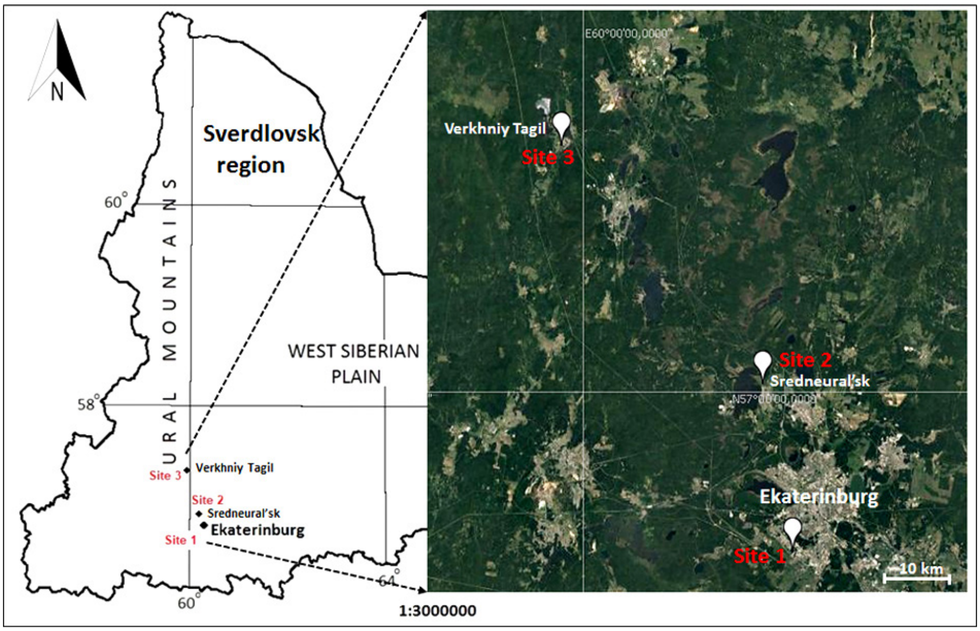

Fig. 1. The schematic map of the Sverdlovsk Region and sampling points of *P. bifolia*

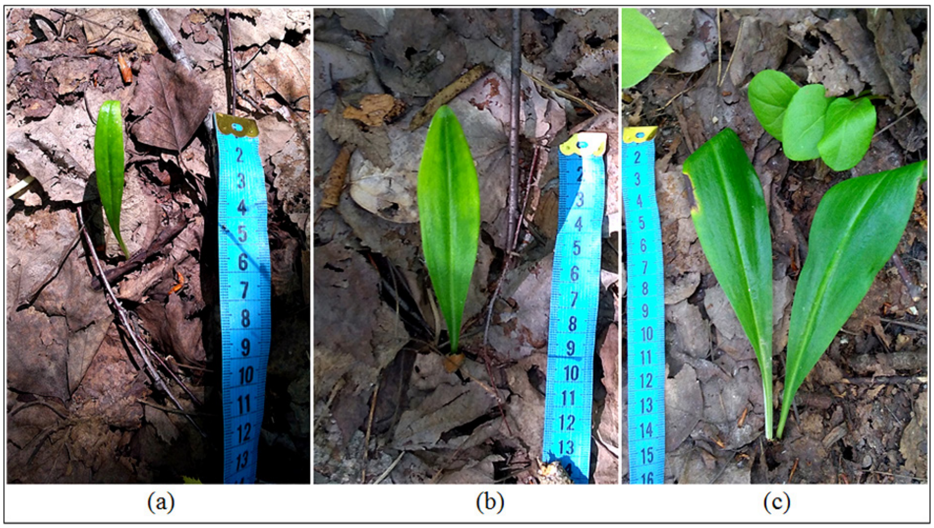

Fig. 2. Age groups of *P. bifolia* individuals: (a) juvenile, (b) immature, and (c) non-flowering mature

herbaceous projective cover, and total projective cover of the moss-lichen layer were studied using standard methods.

In each *P. bifolia* population, the juvenile, immature, non-flowering mature (Fig. 2), and flowering (Fig. 3) developmental stages of individuals were studied.

A juvenile plant had at least one narrow-lanceolate leaf up to 5–6 cm long and about 1 cm wide, with 2–4 veins (Fig. 2a). An immature plant was represented by a small shoot with one lanceolate-elliptical leaf up to 8–10 cm long and up to 2.5 cm wide, with 6–8 veins (Fig. 2b). A non-flowering mature plant had two almost opposite

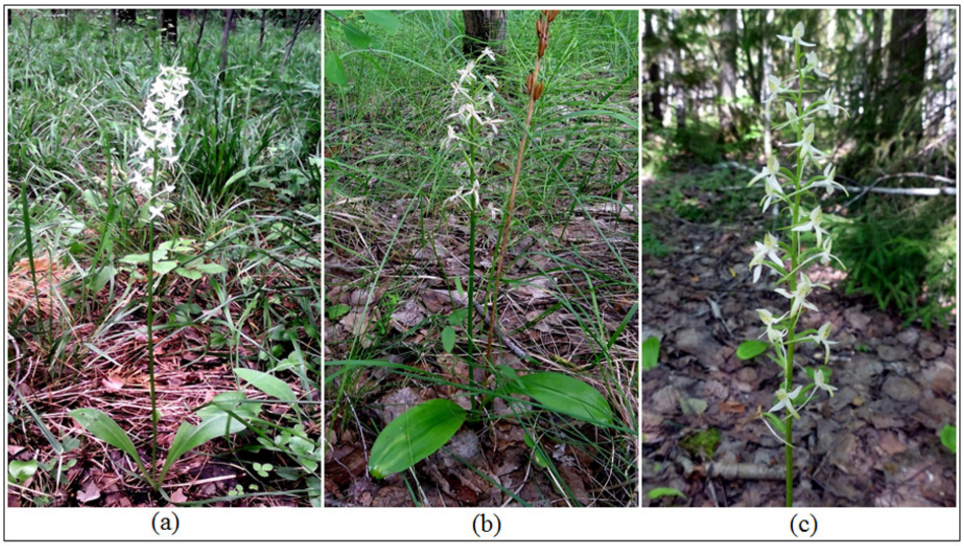

Fig. 3. The individuals of *P. bifolia* at the flowering stage: (a) in natural forest community, (b) on fly ash dump of SUTPS, and (c) on fly ash dump of VTTPS

leaves (rarely one leaf) – elliptical or oblong-elliptical leaves up to 16 cm long and 3.5–4.0 cm wide, with 8–10 veins (Fig. 2c). A flowering plant was represented by a generative shoot with two (less often one) elliptical or oblong-elliptical leaves, up to 20 cm long and 4.5–6.0 cm wide, with 10–12 veins, and inflorescence with 12–20 (occasionally up to 30–35) flowers (Fig. 3).

Leaf samples were collected in mid-July, during the blooming period of orchids. For determination of lipid peroxidation products (malondialdehyde, MDA) and soluble phenolic compounds, the leaves of flowering *P. bifolia* plants were collected from four plants from each study site. For the measurement of flavonoids, the leaves were collected at different stages of development.

Lipid peroxidation was estimated according to Heath and Packer (1968) by measuring the total thiobarbituric acid reactive products at 532 and 600 nm and calculated as MDA content.

The soluble phenolic compounds were determined using the Folin-Ciocalteu assay (Singleton et al., 1999). Briefly, 0.1 mL of crude 96 %-ethanol extract was mixed thoroughly with 0.5 mL of the Folin-Ciocalteu reagent for 3 min, followed by the addition of 0.4 mL of 7.5 % (*w/v*) sodium carbonate. The mixture was allowed to stand for 60 min in the dark, and absorbance was measured at 760 nm. Gallic acid was used as a reference standard.

For determination of flavonoids, 0.5 g of fresh leaf sample was crushed, extracted with 96 % ethanol solution containing 1 % Triton X-100 (*v/v*), and finally filtered through a paper filter. The flavonoid content was measured spectrophotometrically at 420 nm after reaction with citroborat reagent (a mixture of 20 % citric acid and 5 % boric acid at a ratio of 1:1) as described by Rogozhin (2006). A standard curve was plotted using rutin as a standard.

All parameters were measured using fresh plant material in four biological and three analytical replicates and then calculated as per g of dry weight (DW). In order to estimate the DW, the specific amount of fresh leaves was dried at 75 °C for 24 h. The significant difference between the study sites was determined by one-way

Table 1. General geobotanical characteristics of the study sites

Characteristics	Site 1	Site 2	Site 3
Crown density	0.6–0.7	0.4–0.5	0.5–0.6
Total shrub projective cover, %	15–20	15–35	20–30
Total herbaceous projective cover, %	65–80	40–60	10–25
Total projective cover of the moss-lichen layer, %	10–15	5–10	5–8
Number of orchid individuals per 1 m^2	0.4	1.3	2.7

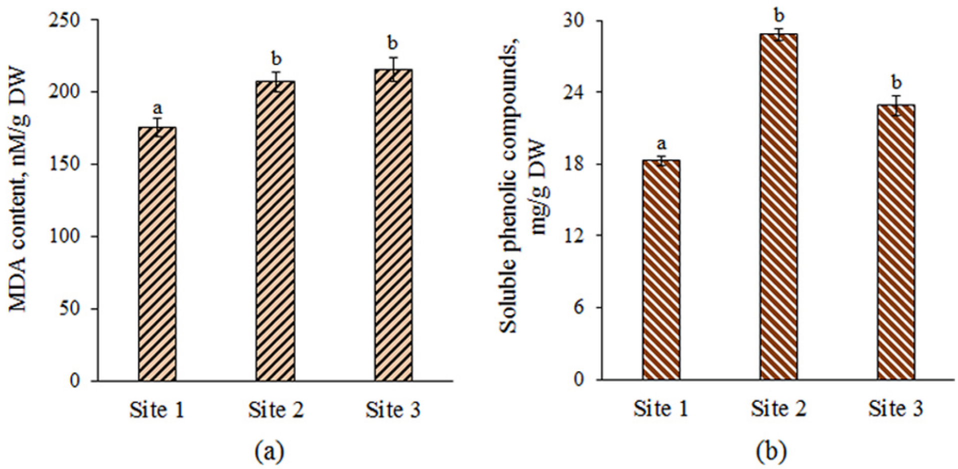

Fig. 4. The foliar content of (a) malondialdehyde (MDA) and (b) soluble phenolic compounds in flowering individuals of *P. bifolia* from natural (Site 1) and transformed (Sites 2 and 3) ecosystems. Data represent means ± SE (n = 12). Different letters indicate significant differences between the study sites at $p < 0.05$

ANOVA followed by Tukey's test ($p < 0.05$). The range values of some geobotanical characteristics of the study sites are listed in Table 1. Data shown in Fig. 4 and 5 are the means ± standard errors (SE).

Results and discussion

The natural recreation zone located far away from the industrial city activities was taken as a control habitat (Site 1) and represented by a 115–130-year-old forest. The soil of this site was sod-medium podzolic (Filimonova et al., 2020). Among the woody species, *Pinus sylvestris* L. dominated, and *Betula pendula* Roth and *Populus tremula* L. were co-dominants. The height of the first layer trees was 20–30 m and the height of the second layer trees was 10–15 m, with a tree crown density of 0.6–0.7 (Table 1). *Salix caprea* L. and *Sorbus aucuparia* L. were found in the underwood. Total shrub projective cover was 15–20 % and contained the dominant *Rubus idaeus* L. and *Rosa acicularis* Lindl. The total herbaceous projective cover was 65–80 %, dominated by *Vaccinium myrtilis* L., *V. vitis-idaea* L., *Aegopodium podagraria* L., *Calamagrostis arundinaceae* (L.) Roth, *Orthilia secunda* (L.) House, *Vicia sylvatica* L., *Maianthemum bifolium* (L.) FW Schmidt, etc. The moss-lichen layer was not well developed whereas leaf litter was poorly developed or absent. *P. bifolia* plants were found both as single individuals and in scattered groups on an area of 400 m^2. Orchid individuals grew at

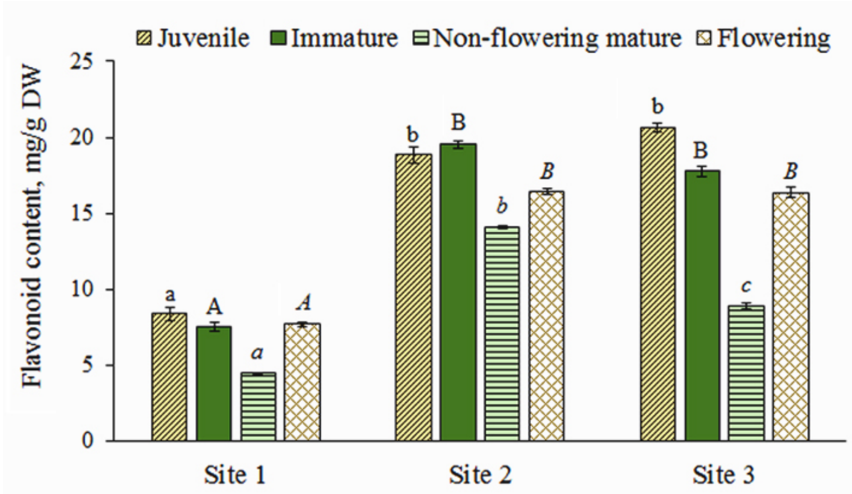

Fig. 5. The foliar flavonoid content at different developmental stages of *P. bifolia* from natural (Site 1) and transformed (Sites 2 and 3) ecosystems. Data represent means ± SE (n = 12). Different small and capital letters indicate significant differences between the study sites at $p < 0.05$

the edge, surrounded by woody plants, in shaded conditions. The total number of plants in control population (P1) was 163 (Table 1).

Site 2 was represented by sparse growth of trees, which naturally colonized the southeast part of the SUTPS fly ash dump. *P. sylvestris*, *B. pendula*, and *P. tremula* were dominant tree species in this forest. The tree crown density varied from 0.4 to 0.5 with the height of the trees between 18 and 20 m. The second layer was dominated by *P. tremula*, *S. caprea*, *S. aucuparia*, *Salix myrsinifolia* Salisb., *Padus avium* L., *Viburnum opulus* L., *R. acicularis* and *Caragana arborescens* Lam. Total shrub projective cover varied from 15 to 35 % whereas the total herbaceous projective cover was 40–60 % (in some places reaching 80–100 %) and was represented by *O. secunda*, *Pyrola rotundifolia* L., *Hieracium umbellatum* L., *P. bifolia*, *Trifolium pratense* L., *Amoria repens* (L.) C. Presl, *Vicia cracca* L., *Poa angustifolia* L., *P. pratensis* L., *Festuca rubra* L., and *Equisetum pratense* L. No significant development of moss-lichen layer was observed. The orchid population (P2) grew on sunny open glades (400 m^2 area) and contained about 518 individuals (Table 1).

Site 3 showed a rather dense orchid population (P3) in the forest naturally colonizing the VTTPS fly ash dump area (150 m^2). The forest was represented by small-leaved trees dominated by *B. pendula*, *P. tremula*, and *P. sylvestris* with the total crown density between 0.5–0.6. The undergrowth included *B. pubescens*, *P. obovata*, and *Larix sibirica* Ledeb. Total shrub projective cover was 20–30 %, while total herbaceous projective cover was 10–25 % and was represented by *Calamagrostis epigeios* (L.) Roth, *P. pratensis*, *A. repens*, *F. rubra*, *Deschampsia cespitosa* (L.) Beauv, *Fragaria vesca* L., *Chimaphila umbellata* (L.) W. Barton, *Pyrola rotundifolia* L., *O. secunda*, *Pyrola chlorantha* Sw., *Chimaphila umbellata* (L.) W. Barton, and *P. bifolia*. Mosses grew only near tree trunks. The population (P3) of *P. bifolia* grew in a sparse forest under diffused lighting conditions, with a total population of 411 individuals (Table 1).

Our study showed that all local orchid populations were normal, incomplete, and capable of self-maintenance (Table 2). *P. bifolia*

Table 2. Structure of *P. bifolia* populations from natural forest community (P1), fly ash dump of SUTPS (P2), and fly ash dump of VTTPS (P3)

Number of individuals at different stages	P1	P2	P3
Juvenile	21	77	62
Immature	33	73	138
Non-flowering mature	49	171	118
Flowering	60	197	93

plants grew in small groups, with the flowering individuals in the center (Fig. 3) surrounded by juvenile, immature, and non-flowering mature plants (Fig. 2). A similar distribution of *P. bifolia* was noted by other authors (Stetsuk, 2010). In the orchid populations in Site 1 (P1), the flowering individuals constituted 37 %, while the juveniles, immature, and non-flowering mature individuals made up 13, 20, and 30 %, respectively. The portion of flowering *P. bifolia* plants in transformed habitats was smaller (23 %) only on Site 3 (P3). A more significant contribution to this population was made by immature and non-flowering mature individuals (62 %).

As is known, the fly ash substrates have unfavorable composition, pH, and other physicochemical properties, a reduced number of microorganisms and fungi, insufficient supply of nutrients (especially nitrogen), and elevated concentrations of some heavy metal(oid)s such as As, Cd, Cr, Hg, Pb, etc. (Chibrik et al., 2016; Gajic et al., 2018). At the same time, the size of *P. bifolia* populations in the fly ash dumps was significantly greater than in the forest park (Table 1). Plants growing on such substrates often experience significant stress, which leads to increased oxidation processes due to overproduction of reactive oxygen species (ROS) in their cells. This is evidenced by the high level of lipid peroxidation, in particular the content of malondialdehyde – a product of membrane lipid degradation.

The level of lipid peroxidation in orchid leaves growing on fly ash substrates was found to be slightly higher (by 20 % on average, Fig. 4a). This indicates the negative effect of the conditions in these industrially disturbed habitats. According to Gajic et al. (2016), in the leaves of *Festuca rubra* grown on fly ash deposits, the content of MDA was high probably as a result of toxic concentrations of As and B and the low content of Cu, Zn, and Mn (Gajic et al., 2016).

Adaptive plant responses to industrially induced stress include changes in metabolism due to the activation of biosynthesis of non-enzymatic antioxidants such as natural phenols. They bind ions well and form complexes with them, preventing the formation of ROS. They are also able to give up a hydrogen atom from the OH-group of the aromatic ring to eliminate free radicals oxidizing lipids and other biomolecules (Michalak, 2006). Therefore, it is interesting to compare the total contents of phenolic compounds in plants from natural and disturbed habitats.

The content of soluble phenolic compounds in the leaves of flowering plants growing in fly ash dumps (Sites 2 and 3) was on average 1.4 times higher than in the natural plant community (Fig. 4b). Results of the present study were in good agreement with the data of other authors (Gajic et al., 2013, 2018), who reported a significant increase in the phenolic compound contents of the leaves and roots of different plant species growing on fly ash deposits.

As noted previously, such phenolic compounds as flavonoids perform a number of functions in the life cycle of plants. This fact is associated with the ability of flavonoids to inactivate ROS, thus preventing the development of oxidative stress and increasing the tolerance of plants to stressful conditions. Their protective role, as a rule, is expressed as an increase in the biosynthesis of these compounds in response to unfavorable factors (Takahama, Oniki, 2000; Khramova et al., 2006; Pourcel et al., 2007).

The results of determining the foliar flavonoid content at different stages of *P. bifolia* development in the natural forest community and disturbed habitats are shown in Fig. 5.

The ontogenetic state can be considered as a key point in the plant development. At the same time, there are very few works addressing the features of physiological processes in different age groups of plants under the anthropogenic stress (Polovnikova, Voskresenskaya, 2008). Therefore, the assessment of physiological parameters at different stages of plant development is of particular relevance.

In the present study, the foliar flavonoid content of *P. bifolia* plants from the fly ash dumps (Sites 2 and 3) was on an average 2.4 times higher compared to the plants from the natural habitat (Site 1). Flavonoids constituted 42 % of the total soluble phenolic compounds in orchid plants from the forest park but 66 % in the plants from the fly ash dumps.

The increased content of flavonoids in the plants from disturbed areas indicates a change in the metabolic processes and phytochemical composition of plants, which are forced to adapt to their environment. Other authors also noted an increase in the flavonoid content in the plants affected by industrial (Nemereshina, Gusev, 2004) and radioactive (Khramova et al., 2006) contamination. For example, under increasing anthropogenic stress, the content of flavonoids and other phenolic compounds in plants was negatively correlated with the soil concentrations of macronutrients (calcium, magnesium, manganese) but positively correlated with concentrations of several trace elements (aluminum and copper) (Artemkina, 2010).

Another possible reason for the increase in the flavonoid content of the orchids from disturbed habitats may be a higher level of light exposure in the fly ash dumps due to the lower total herbaceous projective cover (Table 1). As is known, light induces activation of a number of enzymes that take part in the biosynthesis of phenolic compounds in plants (Zaprometov, 1996).

The present study demonstrated that changes in the flavonoid content in *P. bifolia* leaves occurred in all sites and depended on the age of the plants (Fig. 5). The lowest level of accumulation of these antioxidants was noted in the non-flowering mature stage. An obvious explanation is the depletion of the flavonoid pool due to an imbalance between the processes of flavonoid synthesis and consumption during the period of generative organ formation, when the requirements of plants for active biomolecules are the highest.

Conclusions

In the transformed habitats, the orchid *P. bifolia* is capable of forming abundant populations containing a significant portion of the flowering plants. Regardless of the growing conditions, the non-flowering mature individuals were characterized by a minimal content of flavonoids, probably due to pre-generative metabolic restructuring.

Increased biosynthesis of soluble phenolic compounds, particularly flavonoids, in response to oxidative imbalance in leaves of the *P. bifolia* growing on fly ash substrate was observed at all stages of development studied here. This indicates high adaptive potential of the orchid *P. bifolia* to endure unfavorable conditions prevailing on fly ash deposits. Natural phenolic compounds are the important antioxidants in plants. A significant increase in their content in *P. bifolia* from fly ash dumps points to the conclusion that they take part in protective adaptive responses, which not only ensure the survival of *P. bifolia* but also contribute to the implementation of its ontogenetic program.

The possibilities of using these orchids in medicine as potential sources of antioxidants and other biologically active substances are limited because of the lack of raw material and the special protected status of orchids. Therefore, the introduction of the orchid into botanical and nursery gardens and the search for biotechnological approaches to their cultivation *in vitro* are of particular relevance.

References

Artemkina N.A. (2010) The content of phenolic compounds in *Vaccinium vitis-idaea* from pine forests of the Kola Peninsula. *Chemistry of Plant Raw Material* [Khimiya Rastitel'nogo Syr'ya], 3: 153–150 (in Russian)

Babenko L.M., Smirnov O.E., Romanenko K.O., Trunova O.K., Kosakivska I.V. (2019) Phenolic compounds in plants: biogenesis and functions. *Ukrainian Biochemical Journal*, 91(3): 5–18

Chibrik T.S., Lukina N.V., Filimonova E.I., Rakov E.A., Maleva M.G., Prasad M.N.V. (2016) Biological recultivation of mine industry deserts: facilitating the formation of phytocoenosis in the Middle Ural region, Russia. *Bioremediation and Bioeconomy*. Prasad M.N.V. (ed.) Amsterdam, Elsevier, p. 389–418

Filimonova E.I., Lukina N.V., Glazyrina M.A. (2014) Orchids in technogenic ecosystems of Ural. *Optimization and Protection of Ecosystems* [Ekosistemy, ikh optimizatsiya i okhrana], 11: 68–75 (in Russian)

Filimonova E.I., Lukina N.V., Glazyrina M.A. (2020) *Platanthera bifolia* (L.) Rich. on industrial dumps in the Middle Urals. *Problems of Botany of South Siberia and Mongolia* [Problemy botaniki Yuzhnoi Sibiri i Mongolii], 19–2: 215–220 (in Russian)

Fraga C.G., Celep G.S., Galleano M. (2009) Biochemical actions of plant phenolics compounds: thermodynamic and kinetic aspects. *Plant phenolics and human health: biochemistry, nutrition, and pharmacology.* Fraga C.G. (ed.) Hoboken, New Jersey, John Wiley & Sons, Inc., p. 91–106

Gajic G., Pavlovic P., Kostic O., Jaric S., Durdevic L., Pavlovic D., Mitrovic M. (2013) Ecophysiological and biochemical traits of three herbaceous plants growing of the disposed coal combustion fly ash of different weathering stage. *Archives of Biological Sciences*, 65(4): 1651–1667

Gajic G., Djurdjevic L., Kostic O., Jaric S., Mitrovic M., Stevanovic B., Pavlovic P. (2016) Assessment of the phytoremediation potential and an adaptive response of *Festuca rubra* L. sown on fly ash deposits: native grass has a pivotal role in ecorestoration management. *Ecological Engineering*, 93: 250–261

Gajic G., Djurdjevic L., Kostic O., Jaric S., Mitrovic M., Pavlovic P. (2018) Ecological potential of plants for phytoremediation and ecorestoration of fly ash deposits and mine wastes. *Frontiers in Environmental Science*, 6: 124

Gutierrez R.M.P. (2010) Orchids: a review of uses in traditional medicine, its phytochemistry and pharmacology. *Journal of Medicinal Plants Research*, 4(8): 592–638

Heath R.L., Packer L. (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125(1): 189–198

Khramova E. P., Tarasov O. V., Krylova E. I., Syyeva S. Y. (2006) Peculiarities of flavonoid accumulation in plants in conditions of radiation contamination. *Issues of Radiation Safety* [Voprosy radiatsionnoi bezopasnosti], 4: 13–21 (in Russian)

Landolino A. B., Cook D. R. (2009) Phenylpropanoid metabolism in plants: biochemistry, functional biology, and metabolic engineering. *Plant phenolics and human health: biochemistry, nutrition, and pharmacology.* Fraga C. G. (ed.) Hoboken, New Jersey, John Wiley & Sons, Inc., p. 489–563

Marchiosi R., dos Santos W. D., Constantin R. P., de Lima R. B., Soares A. R., Finger-Teixeira A., Mota T. R., de Oliveira D. M., Foletto-Felipe M.P., Abrahao J., Ferrarese-Filho O. (2020) Biosynthesis and metabolic actions of simple phenolic acids in plants. *Phytochemistry Reviews*, 19(4): 865–906

Michalak A. (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*, 15(4): 523–530

Mishagina D. A. (2018) Types of the technogenically-modified Orchidaceae family ecosystems in the Ivanovo region. *Advances in Current Natural Sciences* [Uspekhi sovremennogo estestvoznaniya], 6: 102–106 (in Russian)

Nemereshina O. N., Gusev N. F. (2004) Influence of industrial pollution on the content of flavonoids in plants of Scrophulariáceae family of the Steppe Urals. *Orenburg State University Bulletin* [Vestnik Orenburgskogo gosudarstvennogo universiteta], 10: 123–126 (in Russian)

Polovnikova M. G., Voskresenskaya O. L. (2008) Activities of antioxidant system components and polyphenol oxidase in ontogeny of lawn grasses under megapolis conditions. *Russian Journal of Plant Physiology*, 55(5): 699–705

Pourcel L., Routaboul J.-M., Cheynier V., Lepiniec L., Debeaujon I. (2007) Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends in Plant Science*, 12(1): 29–36

Red Book of the Sverdlovsk Region: animals, plants, mushrooms (2018) Ekaterinburg, Mir LLC, 450 p. (in Russian)

Rogozhin V. V. (2006) *Workshop on biological chemistry.* SPb, Publishing House «Lan», 256 p. (in Russian)

Romanova N. G., Mongush B. O. (2019) The coenopopulation state of *Platanthera bifolia* (L.) Rich. (Orchidaceae) on self-growing rock dumps. *Problems of Botany of South Siberia and Mongolia* [Problemy botaniki Yuzhnoi Sibiri i Mongolii], 18: 377–382 (in Russian)

Shreter A. I. (1975) *Medicinal flora of the Soviet Far East*. Moscow, 328 p. (in Russian)

Singleton V. L., Orthofer R., Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299: 152–178

Stetsuk N. P. (2010) Ecological features of *Platanthera bifolia* (L.) Rich. on the territory of the Southern Urals. *Orenburg State University Bulletin* [Vestnik Orenburgskogo gosudarstvennogo universiteta], 6: 34–37 (in Russian)

Takahama U., Oniki T. (2000) Flavonoids and some other phenolics as substrates of peroxidase: physiological significance of the redox reactions. *Journal of Plant Research*, 113(1111): 301–309

Tarakhovsky Yu.S., Kim Yu.A., Abdrasilov B. S., Muzafarov E. N. (2013) *Flavonoids: biochemistry, biophysics, medicine*. Pushchino, Synchrobook, 310 p. (in Russian)

Vakhrameeva M.G., Tatarenko I.V., Varlygina T.I., Torosyan G.K., Zagulskii M.N. (2008) *Orchids of Russia and adjacent countries (within the borders of the former USSR)*. Koenigstein, Germany, Koeltz Scientific Books, 690 p.

Zaprometov M.N. (1996) *Phenolic compounds and their role in plant life*. Moscow, Nauka, 45 p. (in Russian)