

CHLOROPLAST PIGMENTS IN POST-FIRE-GROWN CRYPTOPHYTES ON VIDLIČ MOUNTAIN (SOUTHEASTERN SERBIA)

MARIJA S. MARKOVIĆ^{1*}, DRAGANA V. PAVLOVIĆ², SVETLANA M. TOŠIĆ¹,
VESNA P. STANKOV-JOVANOVIĆ³, N. S. KRSTIĆ³, S. M. STAMENKOVIĆ¹,
TATJANA LJ. MITROVIĆ¹ and V. LJ. MARKOVIĆ⁴

¹Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, 18000 Niš, Serbia

²Institute of Biology and Ecology, Faculty of Science and Mathematics, University of Kragujevac, 34000 Kragujevac, Serbia

³Department of Chemistry, Faculty of Science and Mathematics, University of Niš, 18000 Niš, Serbia

⁴Department of Physics, Faculty of Science and Mathematics, University of Niš, 18000 Niš, Serbia

Abstract - In this study the content of chloroplast pigments (chlorophyll a, b, a+b, and carotenoids) in the leaves of *Geranium macrorrhizum* L., *Doronicum columnae* Ten., *Aegopodium podagraria* L. and *Tussilago farfara* L. from a beech forest that had undergone fire on Vidlič Mountain was determined. The same species of plants from a place that had not been exposed to fire were taken as controls. Chloroplast pigments were determined from acetone extracts of these plants spectrophotometrically. In the first year after the fire the content of chlorophyll a, b and a+b in *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. was greater than in the plants not exposed to fire. The results were the opposite for *Aegopodium podagraria* L. and *Tussilago farfara* L. These differences can be attributed to the different physiology of the plants and consequently their different adaptation patterns. The carotenoid content was higher in the plant species at the fire site than in the area not exposed to fire.

Key words: Chloroplast pigments, chlorophyll, carotenoids, fire site, unburned habitat, Vidlič Mountain

INTRODUCTION

Vidlič Mountain (Mt.) is located in the central area of the Balkan Peninsula on the northeastern edge between Pirot and the Sofia valley, and is predominantly in Serbia (Fig. 1) (Stankov-Jovanović et al., 2011). It is located in southeastern Serbia between Stara Planina Mt. and the river Visočica in the north, the final slopes of Suva Planina Mt., Vlaška Planina Mt. and Greben Mt., Nišava valley in the south, Gradašnička River in the west and the Sofia valley in the east. In geological terms, Vidlič Mt. is composed

mainly of limestone (Vidanović, 1960). The climate is continental with mountain climate characteristics (Marinkov, 1990). In the Republic of Serbia in the period 2003-2007, 579 wildfires were reported. Most of them (370) occurred in 2007 (Tabaković-Tošić et al., 2009), including the fire on Vidlič Mt. The consequences of fire are specific: on the one hand, the destruction of life, on the other new environmental conditions at the site of the previously formed phytocenoses or even entire ecosystems. Firstly, there is a drastic change in the qualitative and quantitative composition of vegetation, and soil on the surface forms

a thicker or thinner layer of ash (Janković, 2003). The fire on Vidlič Mt. that started on 20 July, 2007 lasted for 10 days, and was caused by human factor. The fire burned over 2500 ha of low vegetation, scrub and forests (Ministry of Environment, 2008). That year the vegetation was totally destroyed. In the area of beech forests, a biologically empty space was created. After the fire burned out, only a great amount of dust remained. Immediately after fire, the spring microclimate for plant growth could be characterized as one of high light and warm soil temperature relative to the unburned sites (Knapp, 1984). Fires cause a modification of organic matter, violation of the soil structure, significant losses of nutritive substances as well as qualitative and quantitative changes in the community of microorganisms and invertebrates (Garcia-Marco et al., 2008). Fire-affected landscapes are very specific in their ecological factors and soil compositions. Most of the characteristics of the soil are significantly altered by fire, such as conductivity, pH in water suspension, pH in KCl solution suspension and Cl content. Analysis of the total heavy metal concentration in the soil showed that the majority of samples from the post-fire area had an increased content of analyzed metals, which can be attributed to the local soil characteristic rather than the impact of the fire (Stankov-Jovanović et al., 2011). The soil is now enriched with mineral materials, which are accessible to plants. So, it is very interesting to examine the biochemical and physiological parameters in plants that inhabit this kind of landscapes. Ecosystems affected by fire are those with great changes in the variety of ecological parameters, and they can recover by natural succession. Post-fire areas are being occupied by pioneer plants that start one natural cycle. Plants on fire-affected forest regions are highly adaptive plants and have a characteristic metabolism and mechanism for survival. They can change the environment, and create favorable conditions for natural succession and invasion by other plant species (Nešić et al., 2010). Forest fires create the conditions favorable for certain plant species that were not present in that ecosystem earlier, or which were represented by very few numbers; they are known as pioneer plants. These plants are specific because they face many unfavorable ecological factors such

as high illumination, high temperature, low moisture, increased evaporation and, finally, significant changes in soil composition. Pioneer and autochthonous plants from habitats exposed to fire have to adapt very quickly and become competitive. The aim of our research was focused on testing the quantitative content of chloroplast pigments in changed conditions of increased light on the post-fire site. Plant pigments are significant for the biosphere. Chlorophylls are the earth's most important organic molecules as they are necessary for photosynthesis. Carotenoids are essential for the photosynthetic functions of plants and for mammalian survival through their nutritional functions; other pigment groups are key to the physiology of plants and the organisms with which they interact (Davies, 2004). Within leaf chloroplasts, the antenna pigments absorb solar radiation and transfer the energy to the reaction centre pigments, which initiate photosynthesis (Richardson et al., 2002). The most important of these pigments are chlorophylls, which are of interest in their own right, but, from a physiological perspective, chlorophyll concentration is important for several reasons. Chlorophylls have a dominant control over the amount of solar radiation that a leaf absorbs, and therefore, the foliar concentration of chlorophyll controls photosynthetic potential and, consequently, primary production (Blackburn, 2007). Carotenoids are the second major group of plant pigments, composed of carotenes and xanthophylls. Carotenoids absorb incident radiation and contribute energy to photosynthesis, as they are an essential structural component of the photosynthetic antenna and reaction centre complexes (Bartley et al., 1995). Chlorophylls generally decrease under stress and the ratio of chlorophyll a and chlorophyll b changes with an abiotic factor such as light (Fang et al., 1998), which increased in the fire site area in relation to the unburned beech forest. Measurements of total chlorophyll, and chlorophyll a and chlorophyll b individually, can provide useful insights into plant-environment interaction (Richardson et al., 2002), especially when comparing a plant's chlorophyll a, chlorophyll b, chlorophyll a+b and ratio of chlorophyll a and chlorophyll b at a fire site and the closest unburned habitat. While changes in chlorophyll are indicative of stress and the phono-

logical stage, presumably because of fire, the carotenoid concentration provides much complementary information on vegetation physiological status (Young et al., 1990). On this basis, besides the quantitative content of chlorophyll we examined the quantitative carotenoid content in plants at the fire site and the closest unburned beech forest on Vidlič Mt.

MATERIALS AND METHODS

The plant material was collected in the spring months (April and May) twice, in 2008 and 2009, and after the forest fire in 2007. A control group of plants was collected at the same time but from a forest that had not been affected by fire. The plants tested in the first year after the fire were: *Geranium macrorrhizum* L., *Doronicum columnae* Ten., *Aegopodium podagraria* L. and *Tussilago farfara* L. Plants tested in the second year after the fire were: *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. Plant samples were frozen and stored in a freezer until analysis. Determination of plant material was carried out using the keys from the regional flora reference (Josifović, 1970-1977; Velčev, 1982-1989). Voucher specimens of the analyzed plants were deposited at the Herbarium of the Faculty of Biology, Belgrade (BEOU) and voucher numbers are shown in Table 1. Representative samples of leaves of the plants were taken for the analysis, cut into very small pieces and precisely weighed to 0.5000 g. The measured plant material was placed in a mortar and homogenized with a pestle with the addition of 5-10 ml of acetone and a little quartz sand. To prevent acidification of the solution, a small amount of MgCO₃ was added. After homogenization for 3 min, the content of the mortar was quantitatively transferred to a glass filter and filtration was performed with the help of a vacuum-water jet pump. The mortar and pestle were washed several times with 2-3 ml of acetone and the content was also quantitatively transferred to the filter; if necessary, the filter was washed with acetone so that the rest of the filter was completely white. The resulting filtrate was an extract of pigments, which was transferred and diluted up to 25 ml with acetone. As the concentrations of pigments in most cases were high, the obtained extracts were diluted to enable

spectrophotometrical readings. Absorptions for the prepared extracts were read on the spectrophotometer at wavelengths of 662, 644 and 440 nm and then calculation using the formula of Holm and Wetstein (mg/l) was performed:

$$\text{Chlorophyll } a = 9.784 \cdot A_{662} - 0.990 \cdot A_{644}$$

$$\text{Chlorophyll } b = 21.426 \cdot A_{644} - 4.650 \cdot A_{662}$$

$$\text{Chlorophyll } a + b = 5.134 \cdot A_{662} + 20.436 \cdot A_{644}$$

$$\text{Carotenoids} = 4.695 \cdot A_{440} - 0.268 \cdot (a + b);$$

where A = absorbency at corresponding wave length, values 9.784, 0.990, 21.426, 4.650 and 0.288 is the molar absorptivity coefficient according to Holm (1954) and Wetstein (1957) for acetone (absorption of 1 cm).

After calculating the concentrations, the amounts of pigment per mg and g of fresh and dry matter were calculated applying the formula

$$c = \frac{C_1 \cdot V \cdot R}{G \cdot 100};$$

where C = the content of pigment (mg/g) of dry or fresh matter; C₁ = the concentration of pigment calculated by the previous formula (mg/l); V = the starting volume of extract (ml); R = dilution, if there is any; G = the weighed fresh (dry) plant (g); 1000 = the coefficient for recalculating g to mg.

In this study the content of chloroplast pigments (chlorophyll a, b, a+b, and carotenoids) in leaves of plants from the beech forest exposed to fire on Vidlič Mt. were determined. As controls the same kind of plants from an area not exposed to fire were taken. Analyses of the chloroplast pigments were done a year later. The chloroplast pigments were determined from acetone extracts of these plants spectrophotometrically at wavelengths 662, 644 and 440 nm.

RESULTS AND DISCUSSION

Fire has a chemical impact on plants through “ash-

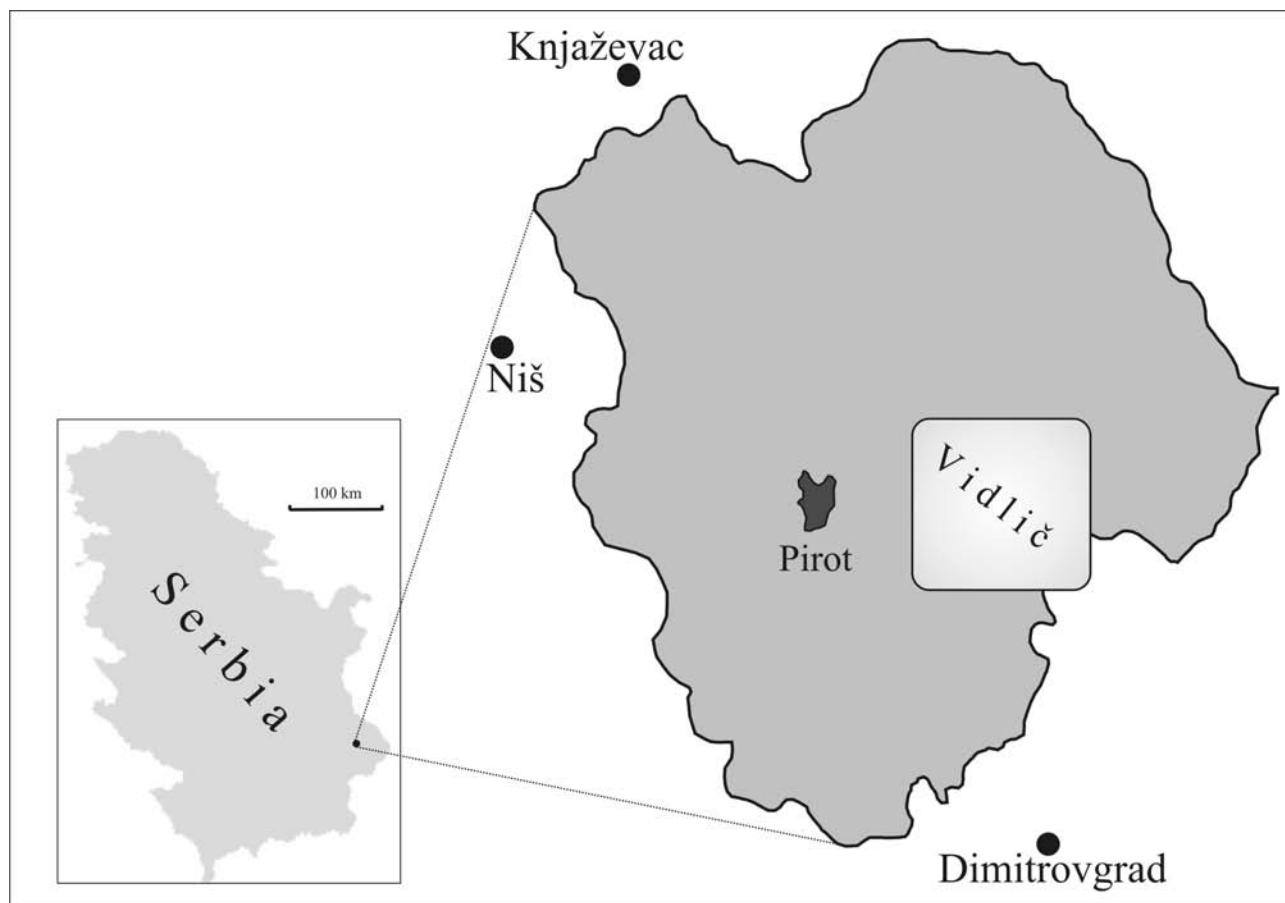
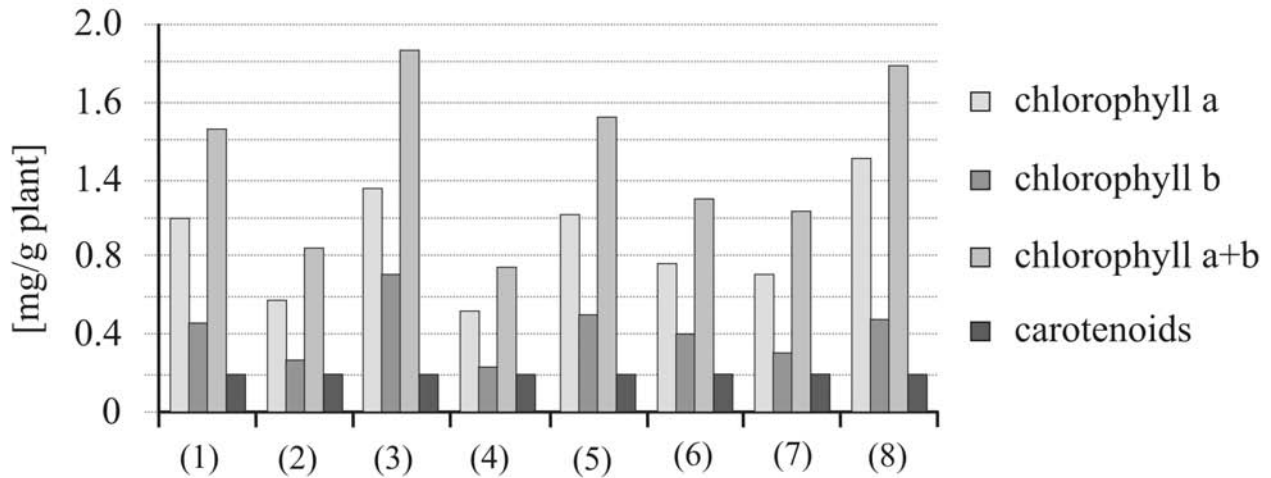


Fig. 1. Geographical locations of collected samples.

bed” effects and altered microclimate. After fire, erosion patterns affect the rates of vegetation. We looked for early post-fire flora elements. In these young species, we examined the chloroplast pigments that are associated with light absorption and photoprotection. In addition, these pigments are affected by leaf age and climatic conditions. Differences in pigment contents in cryptophytes *Geranium macrorrhizum* L., *Doronicum columnae* Ten., *Aegopodium podagraria* L. and *Tussilago farfara* L., were evident in Vidlič Mt. According to the chlorophyll content, we observed two patterns of response to post-fire conditions among these species. In spite of the fact that the plants collected at the same period of season, early in the spring the first year after the fire, they exhibit differences, because they were in different phenophases. At the moment of collecting the

plants, *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. were in full bloom, while *Aegopodium podagraria* L. hadn't yet bloomed, and *Tussilago farfara* L. had already finished blossoming. Different phenophases are followed by different morphology and physiology in a plant; hence alternative adaptive responses from the plants were not unexpected. The response to fire of the analyzed characters in *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. were consistent with the reported response of other species (Knapp et al., 1985). *Aegopodium podagraria* L. and *Tussilago farfara* L. exhibited opposite behavior. Early in the growing season, leaves of *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. in the post-fire site, had significantly lower chlorophyll content (mass/mass), however, the chlorophyll a:b ratio was greater than in leaves from the unburned



- (1) *Geranium macrorrhizum* L. beech forest 2008. (5) *Doronicum columnae* Ten. beech forest 2008.
 (2) *Geranium macrorrhizum* L. after fire site 2008. (6) *Doronicum columnae* Ten. after fire site 2008.
 (3) *Geranium macrorrhizum* L. beech forest 2009. (7) *Doronicum columnae* Ten. beech forest 2009.
 (4) *Geranium macrorrhizum* L. after fire site 2009. (8) *Doronicum columnae* Ten. after fire site 2009.

Fig. 2. The content of chloroplast pigments in *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. the first (2008) and second year (2009) after fire.

site. The carotenoid content remained on a similar level (Table 2). The total chlorophyll content of leaves from the post-fire site was 43% less in *Geranium macrorrhizum* L. and 27.2% in *Doronicum columnae* Ten. in relation to the control. The ratio of the chlorophyll to the carotenoid pigment content was almost twice as great in the unburned control populations in both *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. than in leaves from the post-fire plants. The higher values of Chla/Chlb ratio observed in the post-fire plants could indicate a higher potential for photosynthetic activity (Boardman, 1977; Edwards et al., 1983). On the other hand, the lower Chla/Chlb ratio in the native control plants is typical of shade plants and is thought to enable more efficient light absorption under shade conditions as a result of a relative increase in chlorophyll *b* (Boardman, 1977). The results for *Aegopodium podagraria* L. and *Tussilago farfara* L. were reverse. The total chlorophyll content of leaves in the unburned site was 45.8% less in *Aegopodium podagraria* L., and 22% less in *Tussilago farfara* L. The ratio of chlorophyll to carotenoid pigment content was

less in the unburned *Aegopodium podagraria* L. and *Tussilago farfara* L., than in plants from the post-fire site. Carotenoid content remained on a similar level, or showed a slightly greater value in all the analyzed plants from the post-fire site. These plants grow under high irradiance; therefore, this increase in carotenoid content is important to protect the chlorophyll from photo-oxidation or ultraviolet radiation damage (Goodwin, 1980). It is obvious that the biological responses of plants to post-fire conditions are variable and depend on many factors such as: a) plant species; b) microclimate factors (soil temperature, light irradiance, etc.); c) density of population growth; d) competition among plants; e) limited nutrient resources; f) fire intensities, and g) season. Post-fire plant growth conditions vary from natural habitat, and respond to stress conditions. Changed ecophysiology factors affect the morphology and physiology of plants. The observed variance in pigment content may be a response to altered morphological characters. The increase of ash in the soil surface indicates an increase of the soil mineral N. This nutrient increase correlated with certain increases in

Table 1. Inventory numbers of analyzed plant species and locality coordinates for plants from Vidlič Mountain (locality Vazganica)

Inventory number	Habitat	Subnomen	Coordinates	Altitude
16423	beech forest fire site	<i>Aegopodium podagraria</i> L.	43° 10' 37,2" N 22° 43' 29,5" N	1140
16426	beech forest fire site	<i>Doronicum columnae</i> Ten.	43° 10' 37,0" N 22° 42' 28,8" N	1080
16427	beech forest fire site	<i>Tussilago farfara</i> L.	43° 10' 41,2" N 22° 42' 37,1" N	1070
16431	beech forest fire site	<i>Geranium macrorrhizum</i> L.	43° 10' 41,1" N 22° 42' 49,1" N	1190

Table 2. Comparison of chloroplast pigment content of leaves (mg/g) from burned and unburned population on Vidlič Mt., first year (2008) after fire

Plant species	Chl a	Chl b	Chl a+b	Chl a/b, ratio	Carotenoids	Chl a+b/carotenoids, ratio
<i>Geranium macrorrhizum</i> L. control (unburned)	1.00	0.47	1.47	2.13	0.20	7.35
<i>Geranium macrorrhizum</i> L. test plant (burned)	0.58	0.26	0.84	2.23	0.20	4.20
<i>Doronicum columnae</i> Ten. control (unburned)	1.01	0.50	1.51	2.02	0.15	10.07
<i>Doronicum columnae</i> Ten. test plant (burned)	0.77	0.32	1.10	2.41	0.19	5.79
<i>Aegopodium podagraria</i> L. control (unburned)	0.51	0.20	0.71	2.55	0.15	4.73
<i>Aegopodium podagraria</i> L. test plant (burned)	0.82	0.49	1.31	1.67	0.20	6.55
<i>Tussilago farfara</i> L. control (unburned)	0.48	0.18	0.66	2.67	0.16	4.13
<i>Tussilago farfara</i> L. test plant (burned)	0.536	0.22	0.75	2.41	0.16	4.69

Table 3. Comparison of chloroplast pigment content of leaves (mg/g) from burned and unburned population on Vidlič Mt., second year (2009) after fire

Plant species	Chl a	Chl b	Chl a+b	Chl a/b, ratio	Carotenoids	Chl a+b/carotenoids, ratio
<i>Geranium macrorrhizum</i> L. control (unburned)	1.16	0.71	1.87	1.63	0.12	15.58
<i>Geranium macrorrhizum</i> L. test plant (burned)	0.52	0.23	0.75	2.26	0.19	3.95
<i>Doronicum columnae</i> Ten. control (unburned)	0.72	0.31	1.03	2.23	0.17	6.06
<i>Doronicum columnae</i> Ten. test plant (burned)	1.31	0.48	1.79	2.73	0.17	10.53

morphological parameters during the year following exposure to fire. Increased surface temperature and light during growth also alter the anatomical and physiological characteristics of plants (Hulbert, 1988). According to previous records (Knapp et al., 1998), plants that grow at higher illumination in a post-fire environment have thicker and wider leaves, higher specific leaf mass, higher N concentration and greater stomatal density. Consistent with these parameters, leaf-level photosynthetic rates as well as photosynthetic water- and nitrogen-use efficiencies are greater in burned sites (Svejcar et al., 1988). The increase in leaf thickness may contribute to an increase in the ratio of mesophyll cell surface area to leaf surface area, which leads to increased mesophyll conductance to CO₂ and photosynthetic rates (Nobel et al., 1975). *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. were tested twice, in the first and second years after the fire (Table 3, Fig. 2). The relationships in chlorophyll content in the first and second years between the post-fire and unburned plants were similar in *Geranium macrorrhizum* L. We noticed that *Doronicum columnae* Ten. had a greater chlorophyll content in the post-fire plants than in the control group during the second year after the fire. This could be due to greater population density. The Chla/Chlb ratio, for both species, remained higher in the post-fire plants all the time, during first and second years, indicating that these plants have higher photosynthetic activity. It seems that *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. have very analogical adaptation patterns in stress conditions.

CONCLUSION

Altered post-fire conditions require from plants anatomical, physiological and biochemical acclimation of leaves. The ability to adapt to stress conditions is crucial for plant establishment after fire under natural conditions. Different plants may show different responses to stress conditions, depending on their different genetic origin, different phenophases and different morpho-anatomical potentials to show plasticity. The plant pigment dynamics are closely related to the physiological status of a plant. Accord-

ing to the presented results, we concluded that *Geranium macrorrhizum* L., *Doronicum columnae* Ten., *Aegopodium podagraria* L. and *Tussilago farfara* L. possess good adaptation potentials to post-fire stress conditions.

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