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A comparison of the biochemical parameters and micronutrient concentrations in the leaves of two Balkan endemic *Scilla* species from a karst area

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ABSTRACT:
Karst areas represent challenging habitats for plants, confronting plants with various abiotic and biotic stresses. This study aimed to compare the concentrations of certain primary (photosynthetic pigments and proteins) and secondary metabolites (phenolic compounds) and essential metals: iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), and nickel (Ni) between the two Balkan endemic *Scilla* species: *S. lakusicii* and *S. litardierei* inhabiting karst areas. The metal concentrations in the leaves and soil were determined by atomic absorption spectrometry, while the concentrations of metabolites were determined spectrophotometrically. Significant differences were observed both between the localities and the plant leaves. The concentrations of metals in the leaves of *S. lakusicii* were: Fe 63.8 mg/kg; Zn 46.6 mg/kg; Mn 23.2 mg/kg; Cu 6 mg/kg and Ni 0.757 mg/kg, and in the leaves of *S. litardierei*: Fe 140 mg/kg; Zn 33.46 mg/kg; Mn 25.2 mg/kg; Cu 3.6 mg/kg and Ni 0.963 mg/kg. The concentration of soluble proteins in the leaves of *S. lakusicii* was 2.936 mg/g, compared to 3.459 mg/g in *S. litardierei*, while the concentration of phenolic compounds was 5.803 mg GAE/g in the leaves of *S. lakusicii* and 6.28 mg GAE/g in *S. litardierei*. The Chla/b ratio was slightly higher in the *S. litardierei* leaves, 1.8 compared to 1.63 in *S. lakusicii*. However, a significantly higher ratio of Chl (a+b)/Car (4.4) was measured in the leaves of *S. litardierei* compared to (2) in *S. lakusicii*. The differences in the concentrations of primary and secondary metabolites observed between the two *Scilla* species may be attributed to the influence of the habitat. The results are discussed in relation to the role of the analysed metabolites in the adaption of the two *Scilla* species to the karst environment.

Keywords: phenolic compounds, metals, *Scilla lakusicii*, *Scilla litardierei*, proteins

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INTRODUCTION

Flattened depressions with steep surrounding slopes and a particularly developed drainage system are known as karst fields (Nicod 2023), representing a specific, challenging terrain marked by various stress factors. They are mostly elongated depressions, which in the Dinarides extend in a northwest-southeast direction. Since these depressions are deep, the karst fields receive water from underground channels and the surrounding terrain. The Dinarides primarily consist of dry terrains where water flows through hundreds of meters of thick carbonate deposits, with no impermeable parental rock to retain the water. The highest precipitation occurs during autumn and winter, occasionally causing floods due to the limited space in the underground channels. The flooding period usually lasts from October-November to April-May, while

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during the summer the karst fields are dry (Riter-Studnička 1954; Škondrić *et al.* 2024a, b). Karst characteristics such as the high-water permeability of the terrain, long dry seasons, and high light intensity, lead to the development of a specific combination of morphological, physiological, and biochemical traits in those plants which inhabit such environments. Plants employ different mechanisms in response to unfavourable environmental conditions, such as alternations in the metabolism of primary and secondary metabolites and their employment in different adaptive processes (Zhang *et al.* 2020; Thapa & Shrestha 2022). Exposure to various environmental stresses disrupts cell redox homeostasis, leading to oxidative stress. Oxidative stress results from increased production of reactive oxygen species (ROS: superoxide anion radical, $O_2^{\cdot -}$; hydrogen peroxide, H_2O_2; hydroxyl radical, $\cdot OH$) and/or impaired ROS elimination mechanisms (Mittler 2002; Mishra *et al.* 2023).

Proteins are among the most important biomolecules in plant cells, involved in all plant growth and development processes. Under conditions of exposure to different types of stress, ROS can trigger protein degradation. On the other hand, the synthesis of antioxidant enzymes (superoxide dismutase, catalase, and Class III peroxidase), heat shock proteins, and Lea proteins are activated in response to conditions of drought and high temperatures (Augustine 2016; Guo *et al.* 2018; Škondrić *et al.* 2024b).

Phenolic compounds are essential non-enzymatic antioxidants in plants which remove ROS and reduce the intensity of oxidative stress. The antioxidant properties of phenolic compounds depend on their structure (the number and position of OH groups, the number and position of double bonds, and glycosylation) (Rice-Evans 1996; Liu *et al.* 2020). Phenolic compounds can eliminate ROS in direct reactions, chelate metals involved in Fenton-type reactions, or serve as substrates for enzymes (class III Peroxidases) (Michalak 2006; Vidović *et al.* 2017; Veljović Jovanović *et al.* 2018).

Micronutrients play an important role in plant metabolism, and are necessary in concentrations lower than 1000 mg/kg dry weight. Micronutrients including iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), and nickel (Ni) serve as enzyme cofactors and are involved in chlorophyll biosynthesis, the assimilation of nitrogen (N) and sulphur (S), photosynthesis, ROS scavenging, lignification, protein synthesis, and the detoxification of reactive carbonyl, etc. (Krämer & Clemens 2005; Rai *et al.* 2021; Lilay *et al.* 2024). *Scilla lakusicii* Šilić and *S. litardierei* Breistr. (Asparagaceae) are closely related Balkan endemic plant species which inhabit different types of karst areas in the Dinarides. *Scilla lakusicii* inhabits open rocky limestone, grasslands, and crevices in the Southeast Dinarides, while he most numerous populations of *S. litardierei* were recorded in wet meadows, which develop on the flat floors of the karst fields of the Dinarides (Škondrić *et al.* 2024b).

This study aimed to analyse the concentrations of certain primary (photosynthetic pigments and protein concentrations) and secondary metabolites (phenolic compounds) and micronutrients (Fe, Cu, Zn, Mn and Ni) in the leaves of two *Scilla* species: *Scilla lakusicii* and *S. litardierei* from karst environments to obtain more information about their metabolism, which remains insufficiently explored.

MATERIAL AND METHODS

Plant material. The leaves of *Scilla lakusicii* and *S. litardierei* were collected in eastern Herzegovina (Bosnia and Herzegovina) during the flowering phase in 2020. *Scilla lakusicii* was collected on the karst edges of Popovo Polje, and *S. litardierei* in Nevesinjsko Polje (Table 1, Fig. 1.). To determine the metal concentrations and the total concentration of phenolic compounds, one leaf was collected from each of 30 individuals (a mixture of plants) per species.

Table 1. Sampling sites of the *Scilla* species used in the research. Data on the mean annual air temperature and annual precipitation are taken from Škondrić (2019) and Dragić *et al.* (2021)

Species	Sampling sites	Longitude (°N)	Latitude (°E)	Altitude (m a. s. l.)	Habitat	Mean annual air temperature (°C)	Annual sum of precipitation (mm)
Scilla lakusicii	Bosnia and Herzegovina, Popovo Polje, Gojšina	42.76472	18.13004	276	Rock crevices	15.4	1124.4
Scilla litardierei	Bosnia and Herzegovina, Nevesinjsko Polje, Ružica	43.34091	18.13793	837	Wet meadows	8	1715

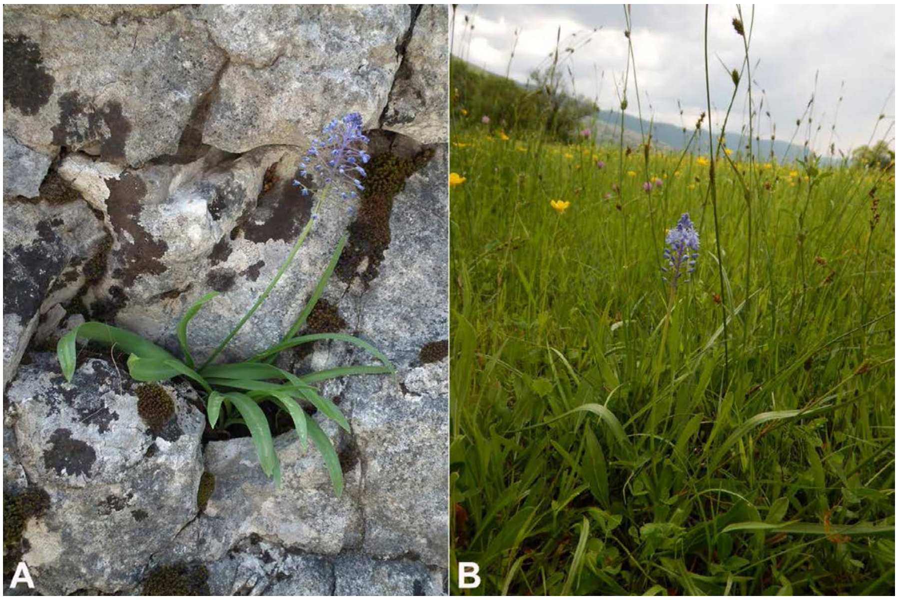

Fig. 1. *Scilla lakusicii* (A) in rock crevices and *Scilla litardierei* (B) in wet meadows (photos by S. Škondrić)

The leaves were dried at room temperature in a well-ventilated area for several days. For photosynthetic pigment and protein concentrations one leaf was collected from each of 10 individuals (a mixture of plants) per species. After collection, the leaves were washed with distilled water, placed in aluminium envelopes, frozen in liquid nitrogen, and stored at -20˚C prior to extraction. The populations of these species are abundant at the both sampling locations, so the distance between the collected individuals ranged from 2 to 5 meters. The identification was carried out using the relevant literature (Nikolić 2020). The herbarium specimens are deposited in the Institute for Nature Conservation of the Vojvodina Province collection, Novi Sad, Serbia (PZZP; Thiers 2024).

Soil sampling. Soil samples were taken from the top soil layer (depth 0–20 cm) using an agrochemical probe. The average sample, weighing approximately 1.5 kg, was bulked in the field by combining 5–10 individual samples. Before the analysis, the samples were air-dried, ground and sieved at 2 mm.

Determination of the soil pH. The pH was measured in distilled water following intensive mixing (10 minutes) of 10 g of soil sample with 50 mL of distilled water (Kalra 1995). The soil particles were then left to settle, and the pH of the supernatant was measured with a pH meter (WTW inoLab pH 7110).

Determination of the metal concentration in the soil and plants. The soil samples were digested according to the ISO 11466 method (1995), with concentrated HNO_3:HCl=1:3 (aqua regia). The plant tissues were digested using a mixture of concentrated HNO_3 with 30% H_2O_2 and 70% $HClO_4$ (Pequerul *et al.* 1993). The metal concentrations were quantified by atomic absorption spectrophotometry (AAS, AAnalyst 400 Perkin Elmer USA). Standard solutions were prepared using a single-element stock solution (1000 mg/L, Perkin Elmer, USA) and appropriate diluent extractant. The selected wavelengths for the measured elements were as follows: Cu: 324.75 nm, Fe: 248.33 nm, Mn: 279.48 nm, Ni: 232.00 nm, and Zn: 213.70 nm. The examined elements were quantified in an air/acetylene flame with an additional deuterium background correction system for Ni and Zn.

Determination of the concentration of photosynthetic pigments. The photosynthetic pigments in the leaves of two *Scilla* species were determined following extraction in acetone. After grinding the plant tissue with liquid nitrogen, the photosynthetic pigments were extracted with acetone (the tissue to solvent ratio was 1:10) and centrifuged at 10,000 rpm for 10 minutes at 4°C (Centric 200R, Tehtnica). Absorbance in the supernatant was measured at 662 nm (chlorophyll a; Chl a), 645 nm (chlorophyll b; Chl b) and 470 nm (carotenoid; Car) using a UV-VIS spectrophotometer (Shimadzu UV-160, Kyoto, Japan) (Lichtenthaler & Wellburn 1985; Dere *et al.* 1998). The concentrations of photosynthetic pigments were calculated based on the equations given below.

$$\mathrm{Chl_a} - 11.75\mathrm{A}_{662} - 2.35\mathrm{A}_{645}\ \mathrm{mg/L}$$

$$\mathrm{Chl_b} - 18.61\mathrm{A}_{645} - 3.96\mathrm{A}_{662}\ \mathrm{mg/L}$$

$$\mathrm{Car} = \frac{1000\mathrm{A}_{470} - 2.27 - 81.4\mathrm{Chl_b}}{227}\ \mathrm{mg/L}$$

Protein isolation. To isolate soluble proteins, the plant tissue (fresh leaves) was homogenised in an extraction buffer (0.5 g of tissue: 4 mL of extraction buffer). The extraction buffer, 0.1 M Na-phosphate buffer, pH 6.4, contained 0.1% TWEEN and 1 mM PMSF (phenylmethylsulfonyl fluoride). Following centrifugation at 10,000 rpm for 10 minutes, the supernatant was used to determine the concentration of the soluble proteins. The remaining pellet was homogenised in 1 mL of 0.1 M Na-phosphate buffer containing 1 M NaCl, and incubated at room temperature with constant stirring for 30 minutes. The homogenate was centrifuged, and the resulting supernatant was labelled as ionically bound proteins. The protein concentration was determined according to the method proposed by Lowry *et al.* (1951).

Determination of the total phenolic compound concentration in the ethanolic extracts of the leaves. The plant leaves were dried at room temperature in the shade in an airy place for several days. After drying, the leaves were ground using an electric mill and subjected to extraction with ethanol (ratio of 10 g of leaves to 200 mL of 80% ethanol). The homogenate was then sonicated for 5 minutes and mixed for 30 minutes on a magnetic stirrer (Velp Scientifica Magnetic). After filtration, the obtained filtrate was designated as E1. The remaining precipitate was re-extracted with 100 mL of 80% ethanol and the same procedure was repeated to obtain extract E2. The final plant extract was obtained by combining extracts E1 and E2 and evaporating them on a vacuum evaporator (IKA Rotary evaporator RV 8 V-C) to 30 mL. The extracts were stored at -18°C prior to analysis and were centrifuged (Tehtnica, Centric 200R) for 10 minutes at 10,000 rpm before each analysis. The concentration of total phenolic compounds (TPC) was determined according to Singleton & Rossi (1965), by measuring the absorbance at 724 nm using a UV/VIS spectrophotometer (Shimadzu UV-1800). The quantification of phenolic compounds was calculated based on the calibration curve for gallic acid and the results were expressed as gallic acid equivalents (GAE) per g of plant dry weight.

Data analyses. Data analyses were performed using GraphPad Prism 8. The results were analysed with One-way ANOVA and paired t-tests to determine any statistical significance. The results are presented as mean values ± SD (n=3). The significance levels were set as follows: *$p < 0.05$, ***$p < 0.001$, and ****$p < 0.0001$.

RESULTS AND DISCUSSION

The concentrations of metals in the soil and the plant's leaves. Plants obtain essential micronutrients from the soil through root systems. Uptake can be influenced by numerous factors related to the chemical properties of the elements, soil properties, and adaptive mechanisms of plant species (Morgan & Connolly 2013). Soil properties which affect metal uptake are water content, pH, organic matter content, redox potential, and soil structure (Kabata-Pendias 2010; Morgan & Connolly 2013). As our results showed, in both localities, the concentration of metals decreased in the following order: Fe > Zn > Mn > Ni > Cu (Table 2). The concentrations of Cu, Fe, Mn, and Ni were significantly higher in the soil from the edges of Popovo Polje (*S. lakusicii* habitat), while the concentration of Zn was higher in the soil from Nevesinjsko Polje (*S. litardierei* habitat) (Table 2). In the karst areas of Croatia and Slovakia, the concentrations of micronutrients were as follows: Cu (24–52 mg/kg), Zn (86–392 mg/kg), Mn (686–1452 mg/kg), and Ni (35–145 mg/kg) with a higher concentration of Mn then Zn, thus differing from our samples (Miko *et al.* 2003).

Metals Fe, Cu, Zn, Mn, and Ni are vital for plant metabolism. The optimal ranges of concentrations for those metals in plants are 50–500 mg/kg for Fe, 2–20 mg/kg for Cu, 30–100 mg/kg for Zn, 20–200 mg/kg for Mn, and 0.01–10 mg/kg for Ni (Lilay *et al.* 2024). A deficiency or excess of metals can lead to protein and nucleic acid metabolism disorders, chlorosis, sterility, reduced growth and productivity, and reduced infection resistance (Rai *et al.* 2021). In addition, redox-active metals (Fe, Cu, Mn) can induce the production of highly reactive hydroxyl radicals in Fenton-type reactions, damaging the structures of biomolecules and the cells themselves (Grotz & Guerinot 2006). To maintain adequate ion homeostasis, plants regulate the intake, use, and storage of metals (Grotz & Guerinot 2006).

Table 2. Metal concentrations in the soil and the leaves of two *Scilla* species (in mg/kg). The results are presented as mean values ± SD (n = 3). The asterisks indicate statistically significant differences in the concentrations of individual metals between the research sites and the studied species. ***$p < 0.001$; **** $p < 0.0001$

Soil					
Locality	Cu	Fe	Zn	Mn	Ni
Popovo Polje	20.775±0.395	53946.86±84.40****	834.14±15.48	159.38±0****	61.785±1.764****
Nevesinjsko Polje	4.54±0.11****	22486.62±302.14	915.05±7.36****	103.56±5.88	49.4±0.88
Leaves					
Sample	Fe	Cu	Zn	Mn	Ni
Scilla lakusicii	63.795±2.826	6.027±0.369***	46.576±1.642****	23.163±0.614	0.757±0.0484
Scilla litardierei	140.583±2.836****	3.635±0.382	33.41833±1.186	25.163±0.7631***	0.963±0.060****

The concentrations of Fe, Cu, Zn, Mn, Ni in the leaves of the two *Scilla* species are presented in Table 2. Statistically significantly higher concentrations of Fe, Mn, and Ni were measured in the *S. litardierei* leaves, while significantly higher concentrations of Cu and Zn were recorded for the *S. lakusicii* leaves (Table 2). The concentrations of all metals in the leaves of the two *Scilla* species were within optimal ranges for plant tissue (Table 2; LILAY *et al.* 2024). In a related species, *Drimia maritima* (L.) Stearn, which belongs to the same family as the two species of the genus *Scilla* we investigated, KARAHAN (2023) found higher concentrations of Fe (885.232 mg/kg) and Cu (32.494 mg/kg) compared to our results. In the karst regions of Hungary, in the selected woody plants (*Carpinus betulus* L., *Quercus petraea* (Matt.) Liebl., and *Cornus mas* L.), the Cu concentration was in the range of 3.23 to 9.38 mg/kg and Zn in the range of 7.65 to 93.73 (KASZALA *et al.* 2003). These differences may be attributed to specific characteristics of the soil, but also specificities related to the plant species (mechanisms of metal uptake and distribution) (WAIRICH *et al.* 2024). The concentration of Fe was 2.3 times higher in Popovo Polje compared to Nevesinjsko Polje, but the concentration of Fe was higher in the leaves of *S. litardierei* compared to *S. lakusicii*. The differences in the metal concentrations in the soil and those taken up by the plants can be attributed to a slightly lower pH, the increased presence of water, organic matter, and the dense stands of the plants in Nevesinjsko Polje, compared to Popovo Polje. The soil pH was 7.5 for the karst edges of Popovo Polje and 7.1 for Nevesinjsko Polje. As pH decreases, the availability of metals generally increases, so this may be one of the reasons for the increased concentration of metals in the leaves of *S. litardierei*, even though the difference in pH is not significant. The increase of the pH by one unit decreases the availability of Fe by 1,000-fold as a result of the formation of insoluble Fe(III)-hydroxide (WAIRICH *et al.* 2024). In addition, water availability (e.g. the impact of flooding) can also increase Fe availability (WAIRICH *et al.* 2024). Increased concentrations of Fe can occur in different soils, including those with high pH and high levels of organic matter (HARISH *et al.* 2023). Plants can adapt to changes in metal concentrations in the soil. Thus, rice plants have been shown to possess several strategies (Fe exclusion by roots, Fe retention in roots and suppression of Fe translocation to shoots, Fe compartmentalisation in shoots or to reduce the consequences of Fe toxicity (ROS detoxification)) to respond to elevated Fe concentrations (WAIRICH *et al.* 2024). A similar trend was observed for Zn, with higher concentrations of metal in the soil but lower concentrations in the leaves (Table 2). According to BROADLEY *et al.* (2007), the Zn content in the lithosphere is usually 70–80 mg/kg, in sedimentary rocks 10–120 mg/kg, in mineral and organic soils 50–66 mg/kg, while agricultural soils contain 10–300 mg/kg. We measured a higher content of Zn in both the investigated localities (Table 2). Plants respond to fluctuating Zn availability in soil by regulating the levels of Zn transporters and chelators maintaining Zn homeostasis (STANTON *et al.* 2022). When the pH of the soil is more alkaline, the absorption of Zn can be reduced and plants can then synthesise components which solubilise Zn and chelators (STANTON *et al.* 2022). The *Scilla* species we investigated likely employ certain known metabolic mechanisms to maintain metal homeostasis, depending on the type of soil. However, based on our results, we cannot determine which specific mechanisms are involved. This aspect will be further investigated in our future studies.

The concentration of metals in plants can also be species-specific. Depending on the plant species and the age of the leaves, the Fe concentration can vary significantly. In their study encompassing 632 species, ANCUCEANU *et al.* (2015) found that Fe concentrations range from a minimum of 0.1 mg/kg to a maximum of 24,070 mg/kg.

Concentrations of photosynthetic pigments and proteins. The differences in water content in the habitats of the two *Scilla* species are very noticeable: *S. lakusicii* occurs in a rocky habitat with pronounced water permeability and high temperatures, while *S. litardierei* inhabits wet meadows characterised by significant seasonal fluctuations in soil moisture. The karst edges of Popovo Polje are characterised by higher average temperatures and less precipitation (Table 1). In addition, *S. lakusicii* and *S. litardierei* are exposed to high light intensity.

A significantly higher concentration of all photosynthetic pigments was measured in the leaves *of S. litardierei* (Table 3). *Scilla litardierei* grows in dense stands and shares its habitat with individuals of other plant taxa, so competition with other plants may be the reason for the synthesis of higher levels of photosynthetic pigments. On the other hand, *S. lakusicii* grows in rocky habitats and is directly exposed to sunlight, which is why even lower concentrations of photosynthetic pigments can be sufficient for its efficient photosynthesis. The carotenoid concentration was higher in the leaves of *S. litardierei* compared to *S. lakusicii* (Table 3). However, the ratio of total chlorophyll to carotenoid concentration was significantly greater in the *S. litardierei* leaves. The difference may be attributed to *S. lakusicii* being more directly exposed to sunlight, where increased carotenoid levels likely serve to protect the photosynthetic apparatus from photodamage (Table 3). Liu *et al.* (2011) observed an increased concentration of Car in the studied karst plants (*Pyracantha fortuneana* (Maxim.) H.L.Li, *Rosa cymosa* Tratt., *Cinnamomum bodinieri* H.Lév., *Broussonetia papyrifera* (L.) Vent., *Platycarya longipes* Y.C.Wu, and *Pteroceltis tatarinowii* Maxim.) which play an important role in protection from photooxidative damage.

Zheng *et al.* (2024) studied the photosynthetic pigment composition in the seedlings of *Pinus yunnanensis* Franch. and *P. elliottii* Engelm. under different karst fissure thicknesses and varying rainfall durations. The experiment included three types of karst habitats (stone-free soil, less stone with more soil, and half stone-half soil) and three rainfall scenarios (reduced, natural, and extended rainfall duration). Significant differences were observed between the two species regarding photosynthetic pigment concentration, influenced by karst fissures and rainfall duration. In summary, the authors concluded that mild and moderate rocky desertification favoured pigment synthesis in *P. yunnanensis*, while *P. elliottii* preferred less rocky, more soil-rich environments. The soil type and the rainfall duration significantly influenced the synthesis of photosynthetic pigments in both species.

A significant increase in total chlorophyll concentration was observed in the leaves of two alfalfa (*Medicago sativa* L.) varieties after 8 days of drought and temperature stress, with notable differences between the varieties (Xu *et al.* 2020). The authors also demonstrated that the ratio of carotenoid to total chlorophyll concentration fluctuates in response to the duration of stress exposure, highlighting that these changes are influenced not only by the stress conditions but also by the plant variety. The concentration of chlorophyll a and chlorophyll b, total chlorophyll, and carotenoids significantly decreased in the seedlings of eleven sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) genotypes under 10-day drought conditions (Islam *et al.* 2020). Although a decrease in the concentration of photosynthetic pigments was shown for all the genotypes, differences in the response to drought were observed between genotypes.

In addition to drought, plants in karst areas are also faced with the challenges posed by high temperatures. A study on the effects of high temperature and drought stress on epilithic moss *Homomallium simlaense* (Mitt.) Broth. showed that chlorophyll concentration decreased with decreasing moss water content and increasing temperature and drought treatment duration (Dang *et al.* 2018).

Table 3. The concentration of photosynthetic pigments, proteins, and total phenolic compounds (TPC) in the leaves of two *Scilla* species. Abbreviations: chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b), ratio of chlorophyll a and chlorophyll b (Chl a/b), ratio of total chlorophyll and carotenoids (Chl a+b/Car), soluble proteins (SolP), proteins ionically bound to the cell wall (IonP), gallic acid equivalents (GAE). The results are presented as mean values ± SD (n=3). The asterisks indicate statistically significant differences between the studied species. * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$

Sample	Chl a (mg/g)	Chl b (mg/g)	Chl (a+b) (mg/g)	Car (mg/g)	Chl a/b	Chl(a+b)/Car	SolP (mg/g)	IonP (mg/g)	TPC (mg GAE/g)
Scilla lakusicii	0.00238±5.16E-05	0.00146±3.23E-05	0.00384±7.72E-05	0.00187±0.000114	1.63±0.03	2.06±0.09	2.936±0.055	0.146±0.035	5.803±0.120
Scilla litardierei	0.01072±0.00111****	0.00598±0.00040****	0.01670±0.00147****	0.00379±0.00010****	1.79±0.10*	4.41±0.40****	3.459±0.161***	0.226±0.052*	6.28±0.203*

A higher concentration of soluble and cell wall ionically-bound proteins was measured in the leaves of *S. litardierei* (Table 3). These differences in protein concentration may be a consequence of the habitat and differences in the intensity of abiotic stresses (high temperatures, high light intensity, drought) to which the plants were exposed. Drought impairs the incorporation of amino acids into proteins, leading to a reduction in the protein concentration of tissues. Water deficit disrupts protein synthesis at the ribosomal level, where certain proteins are synthesised and inactivated rapidly, while others remain relatively stable (Sobhkhizi *et al.* 2014). A decrease in total soluble protein concentration under drought conditions was measured in two cultivars of *Sesamum indicum* L. (Fazeli *et al.* 2007), the leaves of *Levisticum officinale* Koch (Akhzari *et al.* 2016), and two alfalfa (*Medicago sativa* L.) cultivars (Liu *et al.* 2018). Ramanjulu *et al.* (1999) demonstrated that drought cycles which mimic natural conditions of daytime wilting in the light followed by recovery of turgor during the night lead to a decrease in the concentration of apoplastic proteins. Proteomic analysis of *Chlamydomonas reinhardtii* P.A.Dangeard showed the effect of high light intensity on 320 proteins compared to the control (Yadav *et al.* 2023), highlighting changes in those proteins involved in the photosynthetic electron transport chain, protein synthesis, metabolic processes, glycolysis, and cytoskeletal assembly. The concentration of total soluble proteins in strawberry (*Fragaria* × *ananassa* Duch) cultivars decreases at high temperatures, which may be a consequence of denaturation (Ergin *et al.* 2016). However, the authors also observed the synthesis of new proteins which are possibly involved in increasing thermostability.

Total phenolic compound concentration. The role of phenolic compounds in the response of plants to various types of biotic and abiotic stress has been demonstrated in the literature (Naikoo *et al.* 2019; Chowdhary *et al.* 2021; Kumar *et al.* 2023). The protective role of phenolic compounds depends on their qualitative and quantitative composition. A higher concentration of TPC was measured in *S. litardierei* (6.28 ± 0.203 mg GAE/g) than in the leaves of *S. lakusicii* (5.803 ± 0.120 mg GAE/g) ($p < 0.05$) (Table 3). For comparison, in the ethyl acetate extract of *Scilla mesopotamica* Speta and the methanol extract of *Scilla siberica* subsp. *armena* (Grossh.) Mordak, the TPC concentrations were 62.24 µg GAE/mg and 53.592 µg GAE/mg, respectively (Aktepe *et al.* 2021; Aydin *et al.* 2023). Variations in the concentration of phenolic compounds may be species-specific or may originate from the sampling period and habitat specificity.

As previously stated, *S. lakusicii* is exposed to the effects of drought, while *S. litardierei* is exposed to a variable water regime and high temperatures. In addition, the two *Scilla* species are also exposed to the influence of various biotic stresses (e.g. insects and microorganisms), especially *S. litardierei* which grows in meadows used for cattle grazing. Phenolic compounds may be an important element in the adaptation of the two *Scilla* species to environmental pressures (Tak & Kumar 2020; Kumar *et al.* 2023). Plants grown under drought conditions typically produce higher concentrations of bioactive compounds which protect them from free radicals, reactive oxygen species, and damage to the photosynthetic apparatus (Albergaria *et al.* 2020). However, the synthesis of secondary metabolites does not occur uniformly across all tissues and organs and can vary between different parts or developmental stages of the same plant. Additionally, factors such as the duration of exposure and the amount of water supplied play key roles in modulating the metabolic response to stress. Moreover, in natural environments, plants are often subjected to multiple stressors beyond drought, meaning the increased accumulation of phenolic compounds may result from a combination of stresses (Albergaria *et al.* 2020).

Flavonoids and phenolic acids are among the most important groups of phenolic compounds. Flavonoids also play a role in detoxification, have allelopathic and antimicrobial effects, and act as phytoalexins, and UV filters (Shomali *et al.* 2022). In the leaves of *Cistus clusii* Dunal, grown under Mediterranean field conditions, drought induced an increase in the concentration of flavonols (Hernandez *et al.* 2004). Phenolic acids are involved in plant responses to different types of abiotic stresses including light stress, and drought (Naikoo *et al.* 2019; Chowdhary *et al.* 2021). Also, phenolic acids are included in defence against biotic stress: chlorogenic acid against weeds, and protocatechuic acid against fungi (Tak & Kumar 2020).

The increased concentrations of Zn detected in the soil at both localities, despite not leading to an elevated Zn content in the leaves (Table 2), may have contributed to the elevated TPC, as observed in the study carried out by Badiaa *et al.* (2020). Phenolic compounds can be incorporated into the cell wall and form a physical barrier restricting the passage of metals, and they can also bind Zn in the root cell wall preventing translocation to the leaves (Chen *et al.* 2019; Badiaa *et al.* 2020). In addition to phenolic compounds, glutathione and phytochelatins also play an important role in adaptation to increased Zn concentrations in leaves via the formation of complexes with Zn, resulting in reduced mobility and the formation of ROS (Kaur & Garg 2021). Furthermore, phenolic compounds can chelate Fe and Cu, affecting their mobility and toxicity as redox-active metals (Kumar *et al.* 2023).

CONCLUSION

The concentration of Fe, Cu, Zn, Mn, and Ni in the leaves of *Scilla lakusicii* and *S. litardierei* and their rhizosphere soil were determined for the first time. Differences in the concentration of micronutrients, photosynthetic pigments, soluble and ionically bound proteins, and phenolic compounds were observed between the two species. These variations may reflect the distinct habitat requirements and environmental adaptations of *S. lakusicii* and *S. litardierei*.

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REZIME

Poređenje koncentracija biohemijskih parametara i mikronutrijenata u listovima dve balkanske endemične vrste roda *Scilla* poreklom iz kraških predela

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Kraški predeli predstavljaju izazovna staništa za biljke, koje su izložene različitim abiotičkim i biotičkim stresovima. Cilj ovog istraživanja bio je uporedno istraživanje koncentracija određenih primarnih (fotosintetički pigmenti i proteini) i sekundarnih metabolita (fenolna jedinjenja), kao i esencijalnih metala: gvožđa (Fe), bakra (Cu), cinka (Zn), mangana (Mn) i nikla (Ni) između dve balkanske endemične vrste roda *Scilla* poreklom iz kraških predela: *S. lakusicii* i *S. litardierei*. Koncentracije metala određivane su u listovima i zemljištu atomskom apsorpcionom spektrometrijom, dok su koncentracije metabolita određivane spektrofotometrijski. Koncentracije metala značajno su se razlikovale, kako između lokaliteta, tako i između listova različitih vrsta. Koncentracije metala u listovima vrste *S. lakusicii* bile su Fe 63,8 mg/kg; Zn 46,6 mg/kg; Mn 23,2 mg/kg; Cu 6 mg/kg i Ni 0,757 mg/kg, a u listovima vrste *S. litardierei*: Fe 140 mg/kg; Zn 33,46 mg/kg; Mn 25,2 mg/kg; Cu 3,6 mg/kg i Ni 0,963 mg/kg. Koncentracija rastvorljivih proteina u listovima vrste *S. lakusicii* iznosila je 2,936 mg/g, a kod vrste *S. litardierei* 3,459 mg/g, dok je koncentracija fenolnih jedinjenja iznosila 5,803 mg GAE/g u listovima vrste *S. lakusicii* i 6,28 mg GAE/g kod vrste *S. litardierei*. Odnos Chla/b bio je nešto veći u listovima vrste *S. litardierei* (1,8) u poređenju sa vrstom *S. lakusicii* (1,63). S druge strane, značajno veći odnos Chl (a+b)/Car je izmeren u listovima vrste *S. litardierei* (4,4) u poređenju sa vrstom *S. lakusicii* (2). Razlike u koncentracijama primarnih i sekundarnih metabolita uočene između dve proučavane vrste roda *Scilla* mogu biti rezultat uticaja staništa. Rezultati su diskutovani u vezi sa ulogom analiziranih metabolita u prilagođavanju dve vrste roda *Scilla* na uslove u kraškim predelima.

Ključne reči: fenolna jedinjenja, metali, *Scilla lakusicii*, *Scilla litardierei*, proteini