COMPARATIVE EVALUATION OF ANTIOXIDANT STATUS AND MINERAL COMPOSTION OF DIPLOSCHISTES OCELLATUS, CALVATIA CANDIDA (ROSTK.) HOLLÓS, BATTARREA PHALLOIDES AND ARTEMISIA LERCHIANA IN CONDITIONS OF HIGH SOIL SALINITY

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ABSTRACT: Natural reserves play a fundamental role in maintaining flora and fauna biodiversity, though biochemical characteristics of such ecosystems have been studied in an extremely fragmentary way. For the first time mineral composition and antioxidant status of three systematic groups of organisms, lichens (Diplischistes ocellatus), mushrooms (Calvatia candida and Battarrea phaloides) and wormwood (Artemisia lerchiana), are described at the territory of Bogdinsko-Baskunchak Nature Reserve (the North-Eastern part of Astrakhan region, Russia), characterized by remarkable oxidant stress, i.e., high salinity and solar radiation, and water deficiency. Through ICP-MS, it was determined that scale lichen D. ocellatus accumulated up to 10-15% Ca, 0.5% Fe, 15 mg kg-1 d.w. iodine (I), 54.5 mg kg-1 Cr and was characterized by low levels of Na, K and P (873, 1389 and 302 mg kg-1 d.w., respectively). Battarrea phalloides demonstrated anomalously high concentrations of B, Cu, Fe, Mn Se, Zn, Sr and low Na levels, contrary to Calvatia candida mushrooms accumulating up to 10850 mg kg-1 Na and only 3 mg kg-1 Sr. The peculiarity of A. lerchiana plants was the high accumulation of B (22.23 mg kg-1 d.w.), Mn (57.48 mg kg-1 d.w.), and antioxidants (total antioxidant activity - 68.6 mg GAE g-1 d.w.; polyphenols - 21.0 mg GAE g-1 d.w. and proline - 5.45 мM g-1 d.w.). Diplischistes ocellatus and Calvatia candida demonstrated the lowest antioxidant status: 3.6-3.8 mg GAE g-1 d.w. total antioxidant activity, 1.73-2.10 mg GAE g-1 d.w. polyphenols and 2.0-5.3 mg g-1 d.w. proline. Overall, according to the elemental analysis of lichen from Baskunchak Nature Reserve and Southern Crimean seashore, the vicinity of Baskunchak salty lake was characterized by increased environmental levels of Cr, Si, Li, Fe, Co, Ni and Ca.

Keywords: Bogdinsko-Baskunchak Nature Reserve, lichen, mushrooms, wormwood, mineral elements, antioxidants

Bogdinsko-Baskunchak Nature Reserve is situated on the peripheral part of the Caspian lowland on the border with Kazakhstan and occupies the territory of 18 478 ha. In 2021, the reserve was classified by UNESCO as a World Heritage Site. Climate is continental with low precipitation (mean annual precipitation is 270 mm), differing from 150 to 400 mm in different years and the highest temperature range of -40oC in winter and up to +40oC in summer (mean values are -8.1oC and +24.8oC accordingly). Light chestnut weakly-, medium- and strongly alkaline soils with low amount of humus predominate. Sodium chloride concentration in Baskunchak lake reaches 300 g L-1 with surface salt deposit thickness of 10-18 m.

Gypsum outcrops, intense winds bringing dust and salt are additional factors inducing significant oxidative stress at the territory of the Reserve. In such extreme conditions there exist about 507 plant (Volobaeva, 2021) and 71 lichen species and about 100 families of primitive mushrooms (Chuikov, 1998).

Natural conditions of the Reserve provide a potential opportunity to study the effect of geochemical anomaly on the biochemical characteristics of Baskunchak flora representatives. In this respect, the comparison of the characteristics related to three systematic groups of flora differing greatly by metabolism intensity, life span duration and nutrition, i.e., lichens, mushrooms and vascular plants, is expectedly interesting, Among the mentioned organisms, Artemisia species are the most attractive due to drought and salinity tolerance and the ability to accumulate significant amount of essential oil, which are important components of natural antioxidants (Golubkina et al., 2020a). Among 13 identified Artemisia species of the Reserve, perennial Artemisia lerchiana Web is the most abundant, forming broad wormwood - fescue-feather grass communities (Orlova, 2009).

Among lichens of the Reserve crustose forms dominate and are confined mainly of stony substrates. The exception is epilithic lichens Diploschistes ocellatus (Vill.) Norman found both on limestones and soils. Lichens are poikilohydric organisms getting nutrients necessary for growth and development mainly from atmosphere. In a whole passive nutrient absorption by these organisms prevails above active one. Nevertheless, they are able to accumulate chemical substances also from substrate, and in particular with higher, toxic for higher plants and mushrooms concentrations (Purvis, Halls, 1996).

Higher fungi present the third group of organisms characterized by extremely intensive growth rate of fruiting bodies during short periods of water availability. All necessary nutrients for the development and growth of mushrooms fruiting body are accumulated from mycelium.

No species within these three groups of organisms inhabiting the Bogdinsko-Baskunchak territory have been characterized earlier in terms of ability to accumulate macro- and trace elements and of the intensity of antioxidant protection against unfavorable environmental conditions.

Taking into account the mentioned phenomena, the aim of the present investigation was the evaluation of mineral and natural antioxidants accumulation in wormwood Artemisia lerchiana, lichen Diploschistes ocellatus and mushrooms Calvatia candida and Battarrea phalloides, in the conditions of Bogdinsko-Baskunchak Nature Reserve.

Material and Methods

Twelve samples of lichen Diploschistes ocellatus, 9 – of puffball Calvatia candida fruiting bodies, 12 - of Battarrea phalloides and 18 samples of wormwood Artemisia lerchiana were gathered at Bogdinsko-Baskunchak Nature Reserve territory (Fig.1a) in May, 2021, 2022. Lichen thalli were collected on soil surface.

The Crimean samples of Artemisia lerchiana (Karadag Nature Reserve), and D. ocellatus (Novy Svet settlement), were used in the investigation to make a comparison (Fig. 1b).

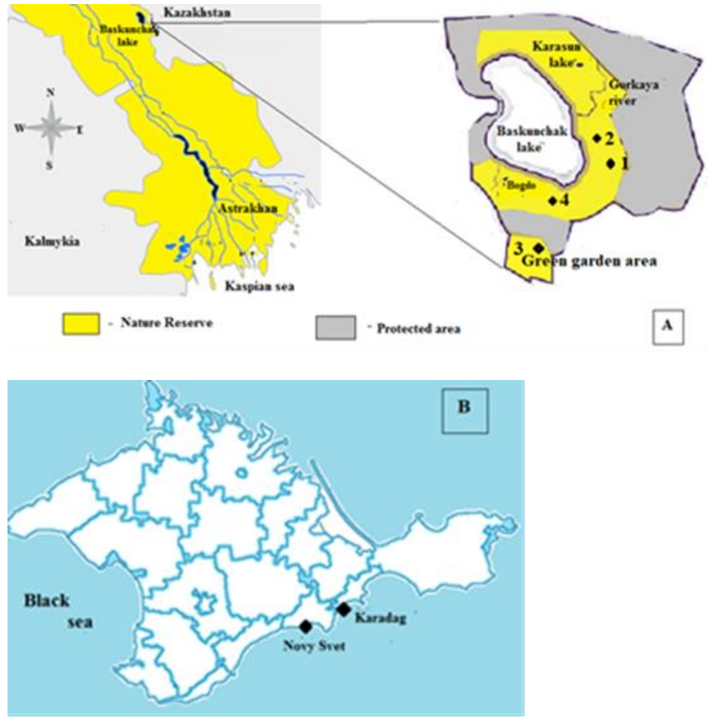


Figure 1: Sampling sites of lichens Diploschistes ocellatus (1), mushrooms Calvatia candida (Rostk.) Hollós (2) and Battarrea phalloides (Dicks.) Pers (3) and wormwood Artemisia lerchiana (4), at the territory of Bogdinsko-Baskunchak Nature Reserve (A) and at the Crimean Southern shore (B).

Gathered samples were grinded, dried at room temperature and homogenized.

Mineral composition

Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, I, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Si, Sn, Sr, V, and Zn contents in dried homogenized samples were assessed using ICP-MS on quadruple mass-spectrometer Nexion 300D (Perkin Elmer Inc., Shelton, CT, USA), equipped with the seven-port FAST valve and ESI SC DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA) at the Biotic Medicine Center (Moscow, Russia). Rhodium 103 Rh was used as an internal standard to eliminate instability during measurements. Quantitation was performed using external standard (Merck IV, multi-element standard solution); Perkin– Elmer standard solutions for P, Si, and V, and all the standard curves were obtained at five different concentrations. For quality control purposes, internal controls and reference materials were tested together with the samples daily. Microwave digestion of samples was carried out with sub-boiled HNO_3 diluted 1:150 with distilled deionized water (Fluka No. 02, 650 Sigma-Aldrich, Co., Saint Louis, MO, USA) in the Berghof SW-4 DAP-40 microwave system (Berghof Products + Instruments Gmb H, 72, 800 Eningen, Germany). The instrument conditions and acquisition parameters were: plasma power and argon flow, 1500 and 18 L min−1, respectively; aux argon flow, 1.6 L min−1; nebulizer argon flow, 0.98 L min−1; sample introduction system, ESI ST PFA concentric nebulizer and ESI PFA cyclonic spray chamber (Elemental Scientific Inc., Omaha, NE, USA); sampler and slimmer cone material, platinum; injector, ESI Quartz 2.0 mm I.D.; sample flow, 637 L min−1; internal standard flow, 84 L min−1; dwell time and acquisition mode, 10–100 ms and peak hopping for all analytes; sweeps per reading, 1; reading per replicate, 10; replicate number, 3; DRC mode, 0.55 mL min−1 ammonia (294993-Aldrich Sigma-Aldrich, Co., St. Louis, MO 63103, USA) for Ca, K, Na, Fe, Cr, V, optimized individually for RPa and RPq; STD mode, for the rest of analytes at RPa = 0 and RPq = 0.25 [60]. Trace levels of Hg in samples were not taken into account and, accordingly, they were not included in the tables.

Total polyphenols (TP)

Total polyphenols were determined in 70% ethanol extract using the Folin–Ciocalteu colorimetric method as previously described (Golubkina et al., 2020). One gram of dry homogenates was extracted with 20 mL of 70% ethanol at 80oC for 1 h. The mixture was cooled down and quantitatively transferred to a volumetric flask, and the volume was adjusted to 25 mL. The mixture was filtered through filter paper, and 1 mL of the resulting solution was transferred to a 25 mL volumetric flask, to which 2.5 mL of saturated Na_2CO_3 solution and 0.25 mL of diluted (1 : 1) Folin–Ciocalteu reagent were added. The volume was brought to 25 mL with distilled water. One hour later the solutions were analyzed through a spectrophotometer (Unico 2804 UV, Suite E Dayton, NJ, USA), and the concentration of polyphenols was calculated according to the absorption of the reaction mixture at 730 nm. As an external standard, 0.02% gallic acid was used. The results were expressed as mg of Gallic Acid Equivalent per g of dry weight (mg GAE g-1 d.w)

Antioxidant activity (AOA)

The antioxidant activity of samples was assessed using a redox titration method (Golubkina et al., 2020) via titration of 0.01 N $KMnO_4$ solution with ethanolic extracts of dry samples, produced as described in the 2.2 section. The reduction of $KMnO_4$ to colorless Mn^{+2} in this process reflects the quantity of antioxidants dissolvable in 70% ethanol. The values were expressed in mg Gallic Acid Equivalents (mg GAE g^{-1} d.w.).

Proline

Proline concentration was determined according to (Ouertani et al, 2021). with slight modification. About 50 mg of dry homogenized Artemisia leaves were grinded with 10 ml of 3% sulfur salicylic acid in a mortar. The mixture was filtered and 1 ml of the resulting filtrate, 2 mL of ninhydrin reagent and 2 ml of acetic acid were heated at 95oC during 1 hour. Proline concentration was evaluated using absorption value of the reaction mixture at 505 nm and a calibration curve with 5 different proline (Merck) concentrations.

Malonic Dialdehyde

Lipid peroxidation was measured by tracing malonic dialdehyde content using thiobarbituric acid method as described by Heath and Parker (1968) with a small modification. About 0.1 g of dried sample were mixed with 10 mL of 0.5% thiobarbituric acid solution and heated at 95 oC during half an hour. The mixture was cooled and absorption at 232 nm was evaluated. MDA content was calculated using the extinction value equal to 155 mM cm^{-1}.

Carbohydrates

The monosaccharides were determined using the ferricyanide colorimetric method based on the reaction of monosaccharides with potassium ferricyanide (Swamy, 2008). The total sugars were analogically determined after acidic hydrolysis of water extracts with 20% hydrochloric acid. The disaccharides content was calculated as a difference between total sugar and monosaccharides contents. Fructose was used as an external standard. The results were expressed in % per d.w.

Photosynthetic Pigments

Half a gram of dry samples was homogenized in a porcelain mortar with 10 mL of 96% ethanol. The homogenized sample mixture was quantitatively transferred to a volumetric flask, bringing the volume to 25 mL and the mixture was filtered through filter paper. The resulting solution was analyzed for chlorophyll-a, chlorophyll-b and carotene determination through a spectrophotometer (Unico 2804 UV, USA). Calculation of chlorophyll and carotene concentrations was achieved using appropriate equations (Lichtenthaler, 1987):

$Ch\text{-}a = 13.36A_{664} - 5.19A_{649}$;

$Ch\text{-}b = 27.43A_{649} - 8.12A_{664}$;

C c = (1000A470 − 2.13 Ch-a − 87.63 Ch-b)/209;

where A = Absorbance, Ch-a = Chlorophyll a, Ch-b = Chlorophyll b and C c = Carotene.

Statistical analysis

The data were processed by analysis of variance and mean separations were performed through the Duncan's multiple range test, with reference to 0.05 probability level, using SPSS software version 21. Data expressed as percentage were subjected to angular transformation before processing.

Results and Discussion

Among organisms tested only lichens (Diploschistes ocellatus) and wormwood (Artemisia lerchiana) were characterized in terms of biological activity, indicating high prospects of their utilization in pharmacology, cosmetics and medicine (Table 1). Up to date, no data regarding the biological activity of Battarrea phalloides (sandy stiltball) and Calvatia candida (puffball) have been reported. Indeed, the only

information available is devoted to the description and geographical distribution of Battarrea phalloides. Despite wide areas of habitat including European countries, America, Africa and Australia this mushroom may be found only episodically, which indicates the necessity of special species protection (Evans, 2001; Morren, 1995; Gargano, 2020). On the other hand, it was indicated that Calvatia species on a whole demonstrate antibacterial, antiviral, antitumor and wound healing properties (Hedawoo, 2020).

Table 1 Biological activity of Diploschistes ocellatus and Artemisia lerchiana.

	Characteristics	Biological effect	Biologically active compounds
Diploschistes ocellatus	Symbiotic organism of fungi and algae *Trebouxia,* calcephitis (Fernandez-Brime et al., 2013)	Photo-protective, anti-microbial to gram-positive and gram-negative bacteria, anti-fungal to *Enterococcus faecalis* (Mendili et al., 2019).	Polyphenols, flavonoids
Artemisia lerchiana	Haloxerophitis with high tolerance to water deficiency and soil salinity	allopathic, fungicidal, bactericidal and antioxidant (Orlova, 2009)	Essential oil (camphor derivatives)

Mineral composition

Biological activity and the level of tolerance to salinity, drought and high insulation in plants, lichens and higher mushrooms are determined by their physiological peculiarities, specific accumulation of biologically active compounds and mineral composition, which reflects both genetic peculiarities of the species and environmental biogeochemical characteristics (Golubkina et al., 2021; Kalać, 2012; Delmail et al., 2013).

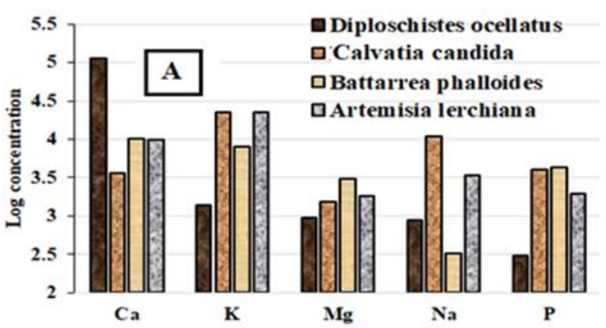
Data presented in Table 2 and Fig.2 are the first detailed mineral composition of Diploshistes ocellatus, Calvatia candida, Battarrea phalloides and Artemisia lerchiana belonging to three systematic groups: lichens, mushrooms and plants. Among the above enumerated organisms only D. ocellatus has been described as a natural hyperaccumulator of Ca (Piterance, 1975) while up-to-date no information exists about its total elemental profile and elemental profile of other species investigated. Comparative evaluation of Diploschistes ocellatus, Calvatia candida, Battarrea phalloides and Artemisia lerchiana biochemical and mineral composition indicates specific peculiarities of these organisms characterized by different nutrition character in conditions of intensive oxidative stress.

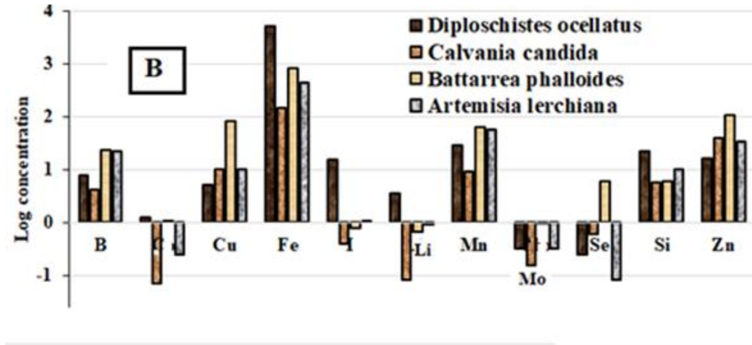
Table 2. Mineral composition of Diploschistes ocellatus, Calvatia candida, Battarrea phalloides and Artemisia lerchiana at the territory of Bogdinsko-Baskunchak Nature Reserve (mg Kg-1 d.w.).

Diploschistes ocellatus	***Calvatia candida***	***Battarrea phalloides***	***Artemisia lerchiana***
Macroelements			
Ca	114 617±11462a	3596±360c	10014±1001b
K	1389±139c	22543±2254a	8044±804b
Mg	934±93c	1514±151b	3052±305a
Na	873±87c	10851±1085a	328±33d
P	302±30c	4017±402a	4366±437a
Microelements			
B	7.9±0.79 b	4.1±0.41c	23.65±2.36a
Co	1.23±0.12a	0.07±0.011c	1.04±0.1a
Cu	5.18±0.52c	9.96±1b	82.14±8.21a
Fe	5072±507a	145±14d	837±84b
I	15.05±1.51a	0.4±0.048c	0.76±0.091b
Li	3.5±0.35a	0.08±0.012c	0.65±0.078b
Mn	29.2±2.92b	9.09±0.91c	62.46±6.25a
Mo	0.31±0.038b	0.15±0.018c	0.93±0.111a
Se	0.25±0.029b	0.59±0.071b	6.14±0.61a
Si	22.26±2.23a	5.68±0.57c	5.93±0.59c
Zn	16.08±1.61c	39.19±3.92b	107±11a
Al, As and heavy metals			

Al	3139±314 a	99.4±9.9d	193±19c
As	1.41±0.14 a	0.04±0.007 d	0.9±0.109b
Cd	0.06±0.01 d	0.84±0.10b	1.2±0.11a
Cr	54.46±5.45a	0.67±0.08d	3.39±0.34b
Ni	10.9±1.09 a	0.3±0.036b	3.77±0.38b
Pb	5.54±0.55 a	0.1±0.014d	1.09±0.11b
Sn	0.07±0.01 a	0.07±0.01a	0.1±0.014a
Sr	114±11a	2.79±0.28c	56±5.6b
V	6.78±0.68 a	0.31±0.038 c	2.89±0.29b

Along each line the values with the same letters do not differ statistically according to Duncan test at $p<0.05$.





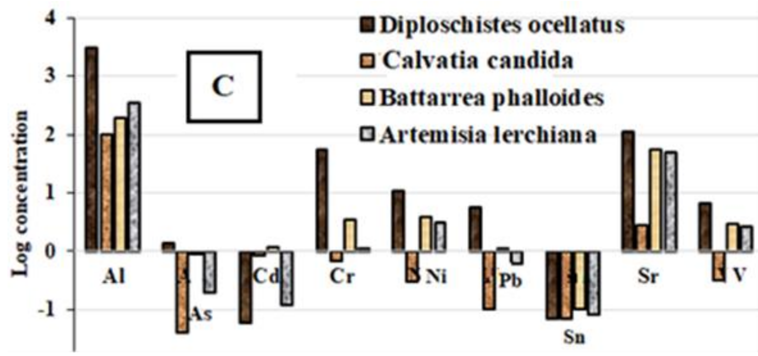


Figure 2: Accumulation of macro- (A), microelements (B), and As, Al and heavy metals (C) in lichens D. ocellatus, mushrooms Calvatia candida and Battarrea phylloides and plants A. lerchiana at the territory of Bogdinsko-Baskunchak Nature Reserve.

Ca-hyperaccumulation is typical in D. ocellatus and is recorded at different biogeochemical conditions: Baskunchak Nature Reserve and the Southern shore of the Crimea (Ca concentrations reached 11.5% and 10.0% accordingly) despite significant differences in other elements availability (Fig. 3).

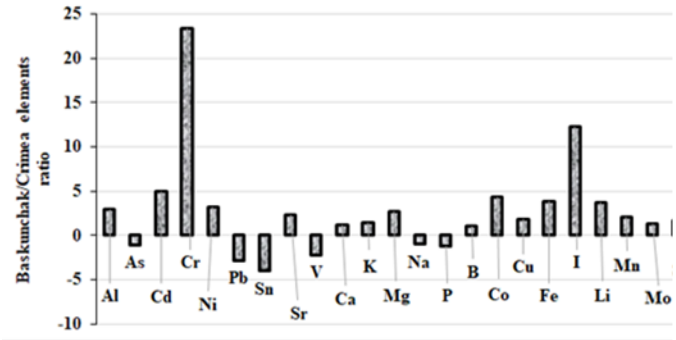


Figure 3: Differences in macro- and microelements accumulation in lichen D. ocellatus at the territory of Baskunchak Nature Reserve and the Southern Crimean shore (Novy Svet).

Table 2 data indicate that Ca content in A. lerchiana, Calvatia candida and Battarrea phalloides are 11.8 and 31.9 times lower respectively, than Ca level in lichen. Lichens Ca coating in a form of Ca oxalate crystals in dry habitats can be facilitated by the abundance of Ca in the substrate (Weber, 1962) and is supposed to contribute in the conservation of water in thalli (Wadsten, Moberg, 1985; Ručová et al., 2022). In this respect Ca hyperaccumulation by D. ocellatus may be considered as a specific form of protection against active oxygen forms and UV radiation excess (Cavigia, Modenesi, 1999; Modenesi et al., 2000, 2001).

In living organisms Ca is accompanied by Sr and there is a close relationship between these elements showing similar ionic radius and charge (Fig.4).

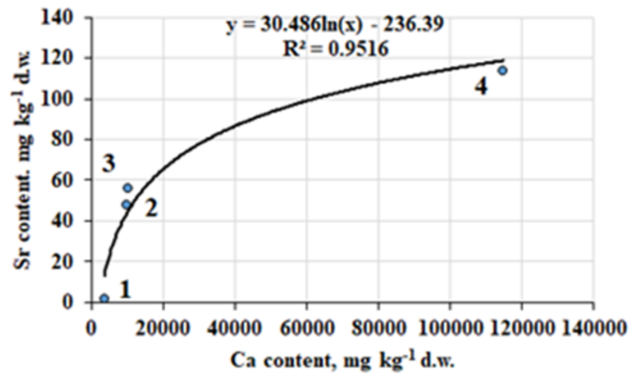


Figure 4: Correlation between Ca and Sr accumulation in Calvatia candida (1), A. lerchiana (2) Battarrea phalloides (3) and D.ocenalis (4) in Bogdinsko-Baskunchan Nature Reserve.

According to Fig. 4 data, Ca/Sr ratio changes from 1289 in Calvatia candida, to 1005 in lichen, and 179-202 Battarrea phalloides and A. lerchiana. In this respect both absolute Ca, Sr levels and Ca/Sr ratios for puffball (1) and stiltball (3) (Fig.4), reveal great interspecies variability between these mushrooms.

On the contrary, accumulation of other macroelements (K, Mg, and P) was the lowest in D. ocellatus receiving nutrients mainly due to the symbiosis with Trebouxia Puymaly (Fernandez-Brime et al., 2013). Low accumulation of Na seems to be typical for D. ocellatus, residing at the area of high salinity (EC 105 mS m-1) while low Na concentration in Battarrea phalloides seems to reflect decreased levels of total dissolves solids in soil of Green Garden area (EC 25 mS m-1; Fig.1). Taking into account that Calvatia candida and Battarrea phalloides are saprotrophic organisms, they accumulate the highest levels of P, reaching 4000-4366 mg kg-1 d.w.

Potassium and Mg levels were comparable in A. lerchiana and fruiting body of Calvatia candida and were the lowest in lichen, which indirectly indicates similar nutrient sources for the former organisms compared to lichen. On the contrary, among species studied Battarrea phalloides was the leader of Mg accumulation.

K/Na ratio in plant tissues is often used as an indicator of organism ability to maintain osmotic pressure in cells under salt uptake. This ratio was the highest in Battarrea phalloides fruiting body (24.5), while reached 6.6 in A. lerchiana, and did not exceed 2 in Calvatia candiida and D. ocellatus. A. lerchiana K/Na ratio in the vicinity of Ellton salt-lake in the neighboring Volgograd region was shown to be 2.8 (Rosentsvet et al., 2019), which indicates the existence of certain peculiarities in both salinity regions. A. lerchiana is known to maintain high water levels in tissues under salt supply up to 800 мM NaCl. Its photosynthetic apparatus tolerance to insulation excess and low water potential in soil and salinity is shown to relate to the peculiarities of K and Na accumulation (Orlova, 2009).

Microelements

The comparison of mineral composition of organisms investigated revealed that the highest levels of Fe, Mn, Li, I and Si are typical for D. ocillatus, while Battarrea phalloides accumulates predominantly B, Cu, Fe, Mn, Se and Zn (Fig.3b). The ability of certain mushrooms species to accumulate high levels of Se, Zn and Cu may protect them against oxidative stress caused by climate factors and heavy metals (Golubkina, Mironov, 2012; Falandysz, 2008). Mineral analysis revealed extremely high Fe concentrations in Baskunchak D. ocellatus compared to the appropriate data of the Crimean lichen, demonstrating 3.8 times excess. The phenomenon is in accordance with high Fe content in the Baskunchak environment reflected in the well-known fact of red water in Gorkaya river which flows into the lake.

At the same time, highly significant Fe accumulation in fruiting body of Battarea phalloides. and A. lerchiana, compared to lichen should be indicated (Table 2, Fig. 2c). Fe content

in lichen is 6-35 times higher than in mushroom and 11 times higher than in A. lerchiana. Higher levels of certain metals in D. ocellatus, compared to A. lerchiana and mushrooms, are connected with the ability of epigeic lichens, grown on substrates with high metals content, to accumulate them in higher concentration than in the substrate (Nöeske et al., 1970; Lawrey, Rudolph, 1975; Purvis; et al., 1990), and the precipitation phenomenon of dust containing lithospheric elements, such as Fe at the thallus surface with their subsequent assimilation. Values of Fe accumulation in fruiting bodies of Battarrea phalloides and Calvania candida are a good examples of wide inter species differences in the ability to accumulate Fe.

Bogdinsko-Baskunchak Nature Reserve proves also high accumulation of iodine (12 times higher than in Crimea), Li (3.6 times higher), Co (4.3 times higher) and Cu (almost twice higher) in D. ocellatus. The detected differences in elements accumulation reflect biogeochemical anomalies of the region investigated and also genetic peculiarities of the lichen. At the same time the phenomenon of Cu hyperaccumulation by Battarea phalloides was registered in the present work for the first time (Table 3). In general, the latter mushroom happened to be a powerful hyperaccumulator not only of Cu, but of B, Mn, Zn and Se. The latter phenomenon is of special importance as high salinity (registered at the territory of Bogdinsko-Baskunchak Nature Reserve) is known to inhibit the accumulation of Se (Mikkelsen et al, 1988).

Antioxidant properties of Se may be highly valuable for Calvatia candida and Battarrea phalloides grown in conditions of oxidant stress. The ability of Se hyperaccumulation is typical only for several mushrooms species (Falandyzs, 2008). The present results indicate that Calvatia candida and Battarrea phalloides at the territory of Baskunchak Nature Reserve accumulate 2-25 times higher Se levels than lichen and 7-77 times higher than A. lerchiana (Table 2).

At the same time, A. lerchiana demonstrated increased levels of B and Mn compared to D. ocillatus and Calvatia candida data. In the conditions of Baskunchak Nature Reserve, Fe/Mn ratio varied from 174 in D. ocellatus to 16 and 13.4 in fruiting bodies of Calvatia candida and Battarrea phaloides accordingly and 7.7 in A. lerchiana. Mn is an essential element for all living organisms, participating in photosynthesis, respiration, free radicals scavenging, protection from pathogens and in hormonal signaling (Alejandro et al., 2020). Manganese in plants is one of the 17 essential elements necessary for growth and reproduction.

Regardless of growing area, lichen is characterized by high levels of Ca, Fe, Al, Mg, which is connected with geochemical peculiarities of regions and with these elements' assimilation by thallus as a result of their air transfer from the soil surface with dust. Indeed, dust storms are typical in Summer in Astrakhan region. High levels of Al and Mg in lichens were recorded in regions rich with dolomites and lime substrates (Corapi et al., 2014). Significant amount of metals, causing damage to vascular plants. is present in lichens in non soluble forms (as oxalates), which results in physiological deactivation of these metals (Byazrov, 2002). Indeed, Fe is deposited at the cell walls of mycobiont in intercellular spaces (Nöske et al., 1970).

The comparison of lichen mineral composition of Bogdinsko-Baskunchak Nature Reserve and the Southern Crimean shore records indirectly that insignificant differences in As, Ca, K, Na, P, B, Mo, Se accumulation reflect both similarity of biochemical characteristics, and genetic peculiarities of the species (Fig.3). The accumulation levels of elements in different lichens may differ significantly and this characteristic may be used for species distinction (Bennett, 2008). On the other hand, at the territory of Baskunchak Nature Reserve the high levels of Al, Cd, Cr, Co, Fe, I, Li in lichens are predominantly

determined by wind, as it is known that except for the substrate, additional Al, Fe, Sc, Ti source relates to dust precipitation on lichen thallus (Nash III, 2010).

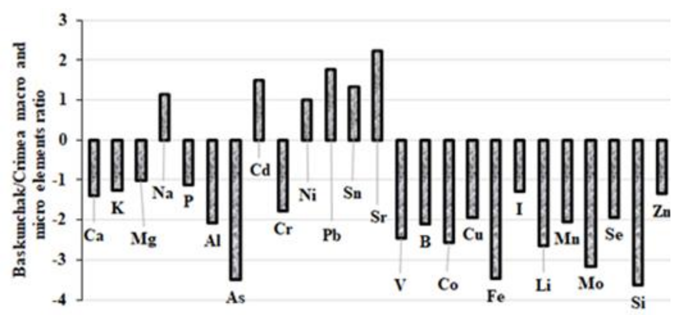


Figure 5: Differences in macro- and microelements accumulation in A. lerchiana in Baskunchak and Karadag Nature Reserves (Crimea).

Plants are able to accumulate heavy metals cations, absorbing them also from air and atmospheric precipitations, but the highest accumulation ability is typical in lichens. In conditions of background heavy metals concentrations in air, their mean concentrations in lichens are usually higher than in ground part of higher plants (Insarova, 1983). Comparison of A. lerchiana mineral composition from Baskunchak Nature Reserve and the territory of paleovolcano Karadag indicates the preferential accumulation of most elements in Karadag plants, which reflects the long-term effect of the volcanic activity at Karadag (Fig. 5). Thus, elevated levels of Li, B, Se in the environment as a result of volcanic activity were recorded (Benson et al., 2017; Winkel et al., 2015), including Karadag Nature Reserve (Golubkina et al., 2022). On the contrary, Cd, Pb and Sr levels in A. lerchiana in the vicinity of Baskunchak lake was significantly higher than the corresponding values recorded in Karadag plants.

Overall, among the species studied D. ocellatus was the leader in accumulation of Al, As, Cr, Ni, Pb, Sr and V. The ability of Pb hyperaccumulation in Diploschistes representative without harmful effect for the organism is connected with production of a certain mechanism of cell protection from toxic effect of this element. Due to increased synthesis of oxalates, Pb precipitates as insoluble salts which stimulates its high accumulation and exclusion from the metabolism as was shown earlier in Diploschistes muscorum (Osyczka et al., 2021; Sarret et al., 1998). On the other hand between mushrooms investigated Battarrea phaloides demonstrated unusually high ability to accumulate Sr, Ni, Cr, As and V.

Antioxidants

Stress is known to stimulate the formation of active oxygen forms and promote the biosynthesis of natural antioxidants. Among the latter polyphenols occupy the leading position, indicating to a large extent the adaptive capacity of plants, lichens, and mushrooms (Kosanić, Ranković, 2019; Novakovic et al., 2015; Orlova, 2019; Román et al., 2020). Difficulties in the total antioxidant activity and polyphenol content evaluation include choice of conditions, providing maximum extraction of active components, significant effect of biogeochemical conditions and phase of plant development. All these facts to a large extent complicate the production of the adequate results and comparison of the latter with the published data. The results of the present investigation obtained for D. ocellatus demonstrated that utilization of 70% ethanol using short-term heating is the most effective method for polyphenol (TP) extraction (Fig. 6).

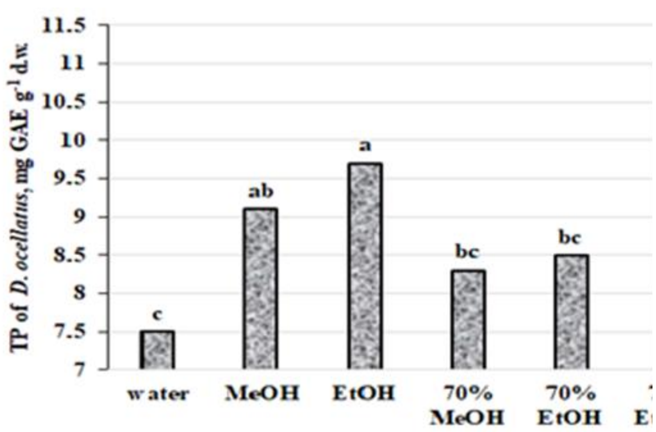


Figure 6: Effect of extraction conditions on polyphenol (TP) content in D. ocellatus (* - 80oC, 1 hour; in other cases 60oC, 1 hour). Values with similar indexes do not differ statistically according to Duncan test at P<0.05.

The efficiency of this extraction is determined by short duration, quick deactivation of enzymes at 80 oC, which prevents polyphenols destruction, stable at high temperature (Golubkina et al, 2020), and low toxicity of ethanol compared to methanol. In this respect it seems interesting to indicate that polyphenol content in Calvatia candida revealed in the present work was 10 times higher that values obtained earlier in conditions of prolonged extraction (24 hours) at room temperature (Novakovic et al., 2015), though such differences are undoubtedly connected also with different levels of oxidative stress.

The results obtained in selected conditions indicate that A. lerchiana is characterized by the highest total antioxidant activity (AOA) and polyphenol content (Table 3). Indeed, A. Lerchiana AOA was 32 times higher that AOA of lichen and mushrooms, while A. Lerchiana polyphenol content was double compared to the values detected for Calvatia candida and D. ocellatus. Despite different environmental conditions, these parameters did not differ significantly within both D. ocellatus samples gathered in the Baskunchak Nature Reserve and the Southern Crimean shore, and A. lerchiana. High values of A. lerchiana AOA may be connected also with high levels of essential oil (Golubkina et al., 2020a; Orlova, 2009). One should also note that in the list of investigated organisms Battarrea phalloides demonstrated the lowest antioxidant status, which partly was compensated by unusually high levels of Se accumulation. Indeed, Se is known to be another natural antioxidant capable to protect plants from different forms of oxidant stress, caused by biotic and abiotic factors (Golubkina et al., 2021).

Table 3 Antioxidant status of D. ocellatus, Calvatia candida, Battarrea phalloides and A. lerchiana.

Object	**Location**	**AOA**	**TP**	**Se**	**Proline**
		Mg GAE g-1 d.w.	µg kg-1 d.w.	mg g-1 d.w.	Mg GAE g-1 d.w.
Diploschistes ocellatus	Baskunchak Crimea *	19.0 ±1.6 b 18.2±1.6b	11.0 ±1.0 b 10.1±1.0b	250±20c 261±21c	1.03±0.10c 1.02±0.10c
Calvatia candida	Baskunchak	21.1±1.9b	12.1±1.0b	590±43b	1.31±0.12b
Battarrea phalloides	Baskunchak	7.9±0.7c	7.4±0.7c	6140 ±546a	1.12±0.10b
Artemisia lerchiana	Baskunchak Crimea **	68.6 ±5.1a 58.5±5.2a	21.0 ±1.8a 24.1±2.1a	135±11d 116±10.0d	5.45±0.44a 5.29±0.41a

*Novy Svet, **Karadag Nature Reserve. AOA - total antioxidant activity; TP - total polyphenols. Within each column, the values with the same letters do not differ statistically according to Duncan text at $P<0.05$.

This trace element is not essential for plants, though its accumulation levels are determined both by biogeochemical peculiarities of the territory and genetic characteristics (Gupta, Gupta, 2017). In his review Falandyzs (2008) indicated several hyperaccumulators of Se among edible and toxic mushrooms (Boletus, Amanita, etc). The ability to accumulate high Se levels by Calvatia candida was also demonstrated in this work. On the contrary, a powerful Se hyperaccumulation by Battarrea phalloides fruiting body was recorded by us for the first time. As far as D. ocealatus and A.lerchiana are concerned Se concentrations in these organisms did not differ significantly for Baskunchak and Crimea, which indirectly indicates similar bio accessibility of soil Se and aerosol transfer of the element. Nevertheless, the Se soil bio accessibility in Baskunchak Nature Reserve needs special investigations due to extremely high salinity caused predominantly by magnesium chloride ($MgCl_2$ – 90%, $MgSO_4$ – 9%, NaCl – 1%), gypsum deposits and water deficiency. Sulfur as a Se analog is known to prevent Se accumulation by plants (Lanza, dos Reis, 2021; Mikkelsen et al., 1988).

Osmoregulators

Amino acid proline is essential for plants in stress conditions. In addition to being an excellent osmoregulator, proline also acts as a metal chelator, antioxidant and signaling molecule. In stress conditions proline biosynthesis is enhanced for maintenance of osmotic balance. It stabilizes membranes, preventing electrolytes leakage, normalizing the levels of oxygen active forms, preventing oxidant stress. Proline supplementation is known to increase stress tolerance of plants to stress factors. It stabilizes subcellular structures (membranes and proteins) via scavenging free radicals. In many plants proline accumulation levels correlates with stress resistance (Ouertani et al., 2021; Hayat et al., 2012).

Table 3 data indicate that at the territory of Bogdinsko-Baskunchak Nature Reserve A. lerchiana accumulated 5 times higher proline levels than D. ocellatus and mushrooms. This phenomenon to a large extent is determined by peculiarities of organisms' development: A. lerchiana is characterized by long vegetation period and high salinity tolerance; D. ocellatus spend most time in physiologically inactive state, while fruiting body of mushrooms is ephemeric and exists in extremely narrow time interval. Close values of proline accumulation in Baskunchak Nature Reserve and the Southern Crimean shore indicate similarities in oxidant stress values at these territories.

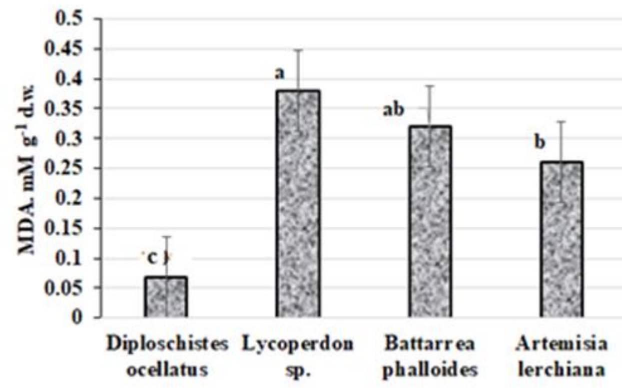


Figure 7: Malonic dialdehyde content in D. ocellatus, Calvatia candida, Battarrea phalloides and A. lerchiana in conditions of Baskunchak Nature Reserve. The standard error is shown

Differences in metabolism of these organisms are connected also with malonic dialdehyde (MDA) accumulation, reflecting the intensity of lipids peroxidation and the levels of oxidant stress (Fig. 7). Indeed, the highest MDA levels were registered in mushrooms, with the highest rate of metabolism and extremely limited duration of life cycle, and the lowest - in D. ocellatus, which may be connected, first of all. with the possibility of physiological processes development in wet thallus and complete cessation of physiological processes in dry thallus. Taking into account that most of time lichens are inactive, the stress factors effect for them is negligible. MDA level in Baskunchak A. lerchiana was close to that of plants grown in the vicinity of Elton lake (Volgograd region) (Rosentsvet et al., 2019). Products of lipids peroxidation are known to change ionic channels, including calcium (Schmidt et al., 2016).

Monosaccharides belong to another group of osmoregulators in plants. These compounds are practically absent in fruiting bodies of mushrooms and lichen. On the contrary, A. lerchiana was shown to accumulate up to 3.6% of monosaccharides per d.w., which indicates high adaptability level of this plant. In conditions of Elton lake vicinity A.lerchiana accumulated only 2% of monosaccharides (Rosentsvet et al., 2019).

Photosynthetic pigments

Table 3 data provide the first information on chlorophyll a, b and carotene content in D. ocellatus and A. lerchiana of Bogdinsko-Baskunchak Nature Reserve. Chlorophyll content in A. lerchiana was 6.6 times higher than in lichen, while carotene content was almost 30 time lower. The results of the present investigation are in accordance with the literature data of Shmakova, Markovskaya (2010), who investigating plants and lichens at the territory of Spitsbergen showed the increase in chlorophyll and carotene content, and chlorophyll/carotene ratio with the increase of organism organization with relative stability of chlorophyll a/b ratio. The latter parameter did not differ significantly between D. ocellatus and A. lerchiana (2.2) while, chlorophyll/carotene ratio reached 7 and 30.7 in A. lechriana and D. ocellatus accordingly. Notable, in the conditions of Bogdinsko-Baskunchak Nature Reserve A. lerchiana accumulates predominantly chlorophyll b while in the vicinity of Elton lake chlorophyll a predominates in wormwood leaves (Rosentsvet et al., 2019).

Table 3 Photosynthetic pigments content in D. ocellatus and A. lerchiana.

Parameter	***Diploschistes ocellatus***	***Artemisia lerchiana***
Chlorophyll a, mg 100 g-1 d.w..	6.18d	41.7a
Chlorophyll b, mg 100 g-1 d.w..	13.8c	91.0a
Total chlorophyll, mg 100 g-1 d.w..	19.98b	132.7a
Chl b/Chl a ratio	2.23e	2.18a
Carotene, mg 100 g-1 d.w..	0.65f	19.0a
Chlorophyll/carotene ratio	30.7a	7.0a

Along each line, the values with the same letters do not differ statistically according to Duncan test at $p<0.05$.

Lichens are symbiotrophic organisms, whereas photosynthesis is achieved by algae and/or cyanobacteria. In laboratory experiments photobiont Trebouxia sp. TR9 in salinity stress conditions decreased chlorophyll content with salt concentration increase (Hinojosa-Vidal et al., 2018). As lichens are poikilohydric organisms, high photosynthesis efficiency may take place only in conditions of optimal thallus water saturation (Green et al., 2010). Diploshistes species inhabit arid habitats (Piterance, 1975), thus their metabolic activity is restricted by a very short period of time, and active metabolism processes coincide with rain season. Laboratory experiments with D. diacapsis from arid habitats revealed a close relationship between photosynthesis, respiration intensity and chlorophyll content on thallus water saturation (Pintado et al., 2005). While many lichen species demonstrate photosynthesis decrease in conditions of high thallus water saturation, D. muscorum photosynthesis rate remains close to maximum even in conditions of extremely high thallus water saturation (Lange et al., 1997). D. scruposus demonstrates not significant photosynthesis depression in these conditions (Lange et al., 1997). It is supposed that such reaction of Diploshistes representatives relates to the presence of hydrophobic proteins in micobiont and interactions between the symbionts (Lange et al., 1997, 1999).

The restricted data on photosynthetic pigment accumulation in lichens and wormwoods in conditions of intensive oxidant stress, such as soil salinity, entails the necessity of further investigations at the territory of Bogdinsko-Baskunchak Nature Reserve.

Conclusion

The results of the first biochemical and mineral characteristics analysis of organisms from different systematic groups, such as lichens, mushrooms and plants, in conditions of increased salinity revealed the peculiarities of mineral composition and antioxidant status: in D. ocillatus, Ca, Al, Cr, Sr, Fe, Si hyperaccumulation with minimum levels of K, Na, P, Cd, Cu and Zn; in fruiting body of Calvatia candida, intensive accumulation of Se, Na, P and Cd and extremely low levels of Al, As, Cr, Ni, Pb, V, Co, Li and Mo, and the highest MDA accumulation; in fruiting body of Battarrea phalloides, hyperaccumulation of Fe, Mn, Se, Zn and Sr and the lowest accumulation of Na; in A. lerchiana, the highest antioxidant activity, proline and monosaccharides content and high Mn and B levels. Further investigations are needed to study plant responses to intensive oxidant stress in the conditions of Baskunchak Nature Reserve.

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