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Features Of Formation Of Physiological And Biochemical Protective Reactions In The Population Of *Physcia Stellaris* (L.) Nyl. Growing In Town And Different Natural-Climatic Zones.

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ABSTRACT

Lichens species *Physcia stellaris* (L.) Nyl were collected in industrial town, in mountain-forest and forest-steppe zone. Representatives from the town had a smaller dry mass, area, average length, and number of apothecia and lobes, compared to lichens growing in mountain-forest and forest-steppe zones. Significant differences in morphometric parameters between samples grown in the mountain-forest and forest-steppe zones were not established. A higher activity of the catalase in lichens from town was established, which indicates the participation of antioxidant system in the processes of adaptation to anthropogenic impact. Different levels of phytohormones (IAK, ABA, sum of cytokinins and zeatin derivatives) were shown. Samples collected in town showed high levels of ABA and low concentrations of auxins and cytokinins. It is suggested that low growth activity of lichens collected in town may be associated with the reaction of hormonal system. It was found that the hormonal status of lichens growing in different natural areas also differed. It was established that lichens from the mountain-forest zone had a higher level of auxin content, which correlated with larger cell sizes of the photobiont. Conversely, smaller photobiont cells were established against the background of high levels of cytokinins in lichens from the forest-steppe zone.

Keywords: *Physcia stellaris* (L.) Nyl, cell size, catalase, auxins, ABA, cytokinins.

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INTRODUCTION

Lichens have been actively studied for a long time as indicators of the state of the environment, and it is known that the degree of lichens growth inhibition is a marker of anthropogenic load level of [1]. The study of the morphological state of lichens (reduction in size, biomass, area, number of blades and apotecia) is the first visually distinguishable feature that indicates the impact of pollutants on lichens [2, 3], but it is just summarizing features that do not demonstrate the mechanisms of lichen adaptation to certain environmental conditions. Physiological and biochemical mechanisms play an important role in adaptation of lichens to habitat conditions [4, 5]. From the point of view of ecological physiology of plants it was interesting to find physiological and biochemical mechanisms of adaptation of one species of lichens to different habitats and their role in the formation of morphological features.

MATERIALS AND METHODS

The object of the study was a widespread throughout Russia lichen *Physcia stellaris* (L.) Nyl. This species of lichen belongs to the order TELOSCHISTALES Nannf., the family Physciaceae Zahlbr., and the genus Physcia (Schreb.) Michx. *Physcia stellaris* in natural conditions grows on the bark rich in minerals at pH<7, i.e. refers to acidophilic and neutrophilic species [6].

The studies were conducted in 2013-2018 on the territory of Sterlitamak town and at 50±5 km from it in the mountain-forest and forest-steppe zone of the Republic of Bashkortostan of the Russian Federation. The number of test sites at each point was 5. When determining the test site (10 × 10 m), the following was taken into account: the similarity of tree species, the density of forest plantations, the proximity of roads and ponds, the terrain, and the coefficient of occurrence of *P. stellaris* in the study area [6].

Lichens for research were collected at the generative age in dry weather on single-stemmed trees of small-leaved Linden (Tilia cordata Mill.) with a trunk circumference of 70-160 cm at a height of 0.9-1.65 m, without mechanical damage to the bark. The average weight of the harvested material of each variant in the dry state amounted to 250 ± 5 g. The following morphometric characteristics were determined in the thalli of *P. stellaris*: size of the thallus, maximum linear size, biomass, number of blades and number of apothecia, as well as the color of the apothecia, damage, color of the thalli, the density of ryzins [6, 7]. The collected plant material was dried at room temperature (22-26 ºC) to an air-dry state (10-12% water) to stop the activity of physiological processes and equalize the state of samples collected in different growing conditions.

Prior to determination of physiological and biochemical parameters, lichens were pre-incubated in a climatic chamber at a temperature of 10±2 °C for 14 hours [8]. To determine the level of phytohormones, extraction, purification, concentration and their subsequent determination by solid-phase enzyme immunoassay were carried out [8]. To determine the enzyme activity, preincubation of 0.2 g of dry sample was performed, then the sample was homogenized, centrifuged twice at 10000 g at 4 ºC. Catalase activity was determined by the residual amount of hydrogen peroxide forming a complex with ammonium molybdate [9].

Temporary samples were prepared to determine the size of fungal hyphae cells and algae cells. 0.5 g of pre-moistened sample of lichen was cut and gently rubbed in 5 ml of water, the resulting homogenate was used to prepare temporary samples, which were viewed using a light microscope Axio Imager, and photographed with a digital camera AxioCam MRc 5 (Carl Zeiss, Jena, Germany). In each temporary sample at least 50 cells of algae and fungi were measured, the volume of algae cells was calculated by the formula $V=(1/6)\pi d^3$, where “d” is the cell diameter. The cell volume of fungal hyphae was calculated using the ellipsoid formula: $V=(4/3)\pi ab^2$, where “a” is half a length; “b” is half a width of the cell. For cytological studies, samples were taken from the virginal and generative ontogenetic spectrum of the population. Individuals of earlier and later age were not considered.

X-ray fluorescence analysis of dried lichen samples for determining the SO^3 content was carried out using energy dispersive x-ray fluorescence spectrometer of EDX type (Schimadzu, Japan).

To determine the level of phytohormone content, their extraction, purification, concentration and subsequent determination were carried out using solid-phase enzyme immunoassay [7]. To determine the activity of the catalase enzyme, 0.2 g of dry sample was rehydrated, then the sample was homogenized,

centrifuged twice at 10000 rpm at 4 ºC. Catalase activity was determined by the residual amount of hydrogen peroxide forming a complex with ammonium molybdate [10, 11].

The rate of respiration was measured by open manometry [12], the content of free proline was determined by the Bates method [13], the content of lectins – by the method proposed by Yamaleeva [14].

All experiments were carried out in at least three biological repeats, physiological and biochemical analyses – in five repeats of each case. The figures show the arithmetic means and their standard deviations from two independent experiments. Statistical processing of the obtained data and plotting were performed in Microsoft Excel 2010.

RESULTS AND DISCUSSION

The most common in lichenoindication is the analysis of species diversity, saturation and density of lichen populations [3]. As can be seen from Table 1, the species richness of the studied sites ranges from 6 to 15 species, the smallest number of species we observed in Sterlitamak town, and there are close values in the mountain-forest and forest-steppe zone. The population density of *Physcia stellaris* was less in the city and the densest in the mountain-forest zone.

Analysis of the variability of individuals by morphometric features allows us to obtain information about such important characteristics of the population as its vitality and age structure [1, 2, 3]. The size of the thallomas and the number of apothecia depend primarily on the age of the thalloma and environmental conditions. It is known that the growth rate of lichens depends on a large number of natural factors (temperature, humidity, illumination, etc.) [3, 15].

As can be seen from Table 2, the light levels at the sampling sites differed. The places of lichen growth in the forests of the mountain-forest zone were the most darkened, and the trees in the parks of the town were the most illuminated. Determination of the degree of illumination of lichens from the forest-steppe zone showed that this parameter is closer to the values obtained in urban parks. Relative humidity also varied, it was the most in the forest of the mountain-forest zone, and the least - in the parks of town (Table 2). We have not established any significant differences in the air temperature at the sampling points.

It is known that the accumulation of sulfur in lichen thallomas has a negative impact on the growth and development of lichens [16]. Determination of sulfur content in lichen thallomas was performed due to the fact that urban enterprises and vehicles emit various sulfur compounds in dust and aerosol form [16]. As can be seen from Table 2, in the samples collected in the city there is a maximum accumulation of sulfur (VI) oxide. In the forest-steppe and mountain-forest zone, a smaller accumulation of sulfur compounds in lichens was found (Table 2).

Thus, the growth of lichens of the "urban" population took place in the conditions of negative action of sulfur compounds, less humidification of the air and more light. The most favorable conditions for lichen growth were in the mountain-forest zone.

In the wet temporary samples prepared by us, we determined the size of the cells of fungi and algae that are part of the symbiotic organism of lichen *P. stellaris*. As can be seen from Table 3, in lichens grown in different natural conditions, the size and volume of cells are different. The average cell sizes of fungi hyphae of lichens collected in town parks are essentially smaller than in the forest-steppe and mountain-forest zones (Table 3). Much greater comparative inhibition of growth processes was observed in phytobiont cells. Thus, the size of the algae cells of lichen in town is 3.7 and 5.2 times smaller than in the forest-steppe and mountain-forest zones, respectively. The difference in the samples collected in the forest-steppe and mountain-forest zones, probably, may be a result of abiotic factors, in particular humidity and illumination of lichen growing places.

An important indicator for assessing the level of influence of external environmental factors on the plant is the activity of enzymes of the antioxidant system of the plant [4, 5]. The main element of antioxidant protection of plants are enzymes (for example, catalase), eliminating hydrogen peroxide, detoxifying xenobiotics [9], heavy metals [16] and providing adaptive flexibility of organism [17].

We determined the activity of the catalase, which showed that the samples from the forest-steppe and mountain-forest zones had similar activity, and the "urban" samples enzyme activity is 1.5 times higher (Table 4). As can be seen from the Table 3, in samples from the town there was an accumulation of sulfur oxide (VI), this can lead, according to the literature, to overproduction of reactive oxygen species (ROS) and cause damage to proteins, lipids, carbohydrates, DNA and symbiont cells themselves [18]. Accordingly, an increase in the activity of the catalase may indicate, according to the literature, the active participation of the antioxidant system of *P. stellaris* lichens in the processes of adaptation to the aggressive environmental factors [18].

Data on the role of phytohormones in lichen growth processes are few [15, 19]. This is due to the fact that hormonal regulation is usually associated with fairly rapid physiological processes (from seconds to days), while growth processes in lichens sometimes last for decades. However, it is possible to talk about the hormonal background or status, which is formed during the episodic "awakening" of lichens and is necessary for the launch and regulation of physiological and biochemical processes and, ultimately, growth processes.

As can be seen from Table 5, the determination of the levels of auxin showed relatively low levels in “urban” samples compared to the samples collected in the mountain-forest and forest-steppe zone. In samples from the mountain-forest zone, the level of IAA content is higher than in lichens from the forest-steppe zone. Taking into account the literature data that auxins stimulate cell growth by stretching in plants [5] under the influence of environmental factors [20, 21], it can be assumed that the formation of larger lichen cells in the mountain-forest zone took place against the background of a relatively high level of auxins.

Levels of abscisic acid (ABA) in the thalli of lichen species differed significantly (Table 5). In the samples from town there is a significantly higher level of ABA content than in the samples collected in the mountain-forest and forest-steppe zone. It is obvious that the growth and development of lichens in the "urban" environment took place against the background of a decrease in the activity of many physiological and biochemical processes and this is due, among other things, to the accumulation of "stress" hormone [21, 22]. In samples from the forest-steppe zone, the level of ABA in lichen tissues was significantly higher than in the mountain-forest zone. It is known that with a decrease in the degree of hydration of plant tissue there is an increase in the level of ABA [21], and as can be seen from Table 2, the hydration of lichens in samples from the forest-steppe zone is 2 times lower than in samples from the mountain-forest zone. Although this did not affect the external parameters of lichens (Table 1), but this could lead to the formation of smaller cells in lichens from the forest-steppe zone (Table 2).

Determination of the total content of cytokinins in the samples showed the presence of a relatively high level of their content in the samples of lichens from the forest-steppe zone, samples from the mountain-forest zone had the intermediate value and, finally, the minimum value of the content of cytokinins was established in the town samples. It is known that cytokinins have a stimulating effect on cell division, and with many adverse factors, their content decreases [21].

Given that the value of the total content of cytokinins consists of the sum of the amounts of free zeatin and its associated forms [24], and their interconversion affects the nature and speed of physiological processes [8, 23, 24, 25], we analyzed the spectrum of zeatin derivatives available to us. As can be seen from the table, there is a relatively high level of free zeatin in the samples from the forest-steppe zone, a lower value in the "urban" lichens and a minimum in the samples from the mountain-forest zone. The presence of a relatively high level of free-form zeatin may indicate a possible faster activation of fungi and algae cell division processes by cytokinins [24, 25] in the forest-steppe zone. Thus, it is possible that the formation of approximately the same external size of lichens grown in the mountain-forest and forest-steppe zone (Table 1), at different cell volumes (Table 2), could be realized by enlarging the size of lichen cells from the mountain-forest zone under the action of auxins and stimulation of cell division of lichens of the forest-steppe zone under the action of zeatin (Table 5).

Comparing data on zeatin content in samples from the town and mountain-forest zone, we see that the level is lower in samples from mountain-forest zone than in the town. This is probably due to the relatively high level of IAA in these samples; it is known that IAA often acts as an antagonist to cytokinins in the regulation of growth processes [24, 25].

The content of zeatin nucleotide in lichen samples also differed, but not so pronounced (Table 5). The level of zeatin nucleotide content is higher in the samples from the mountain-forest and forest-steppe zone compared to the values of this hormone in the samples collected in the town. Given the multifunctionality of cytokinins and their pleiotropy of action [24] it can be assumed that a higher content of bound forms of zeatin can be considered as a reserve pool of phytohormone, which can be used as it is necessary to stimulate growth and physiological processes [25].

Determination of the level of zeatin riboside showed a relatively high level of its content in samples from the mountain-forest zone, with a relatively low level of zeatin (Table 5), which can also be considered as a potential pool of cytokinins necessary for the activation of cell division processes when certain environmental parameters change [8].

Based on the above, it can be assumed that equal growth rates were formed in lichens from the forest-steppe zone due to more frequent cell division against the background of high levels of cytokinins, and in lichens from the mountain-forest zone due to cell growth stimulated by auxins, larger cells were formed by stretching. The accumulation of ABA in the samples from town, as well as the low level of auxins and cytokinins in the tissues, inhibited growth processes in lichens.

As shown by the measurement of the proline content (Table 6), higher content was found in samples collected in the town and somewhat less in the forest-steppe zone. According to the literature, lichens react to various pollutants by the accumulation of free proline [26, 27], which can thus serve as one of the indicators of stress. We have found the increase in free proline content in the thalli of lichens collected in the town, which grew in conditions of elevated concentrations of toxic compounds (Table 2). The lowest level of accumulation of proline was found in samples collected in the mountain-forest zone, and in the forest-steppe zone we found a value close to the results obtained in the town (Table 6). We suggest that this may be due to a sufficiently low degree of hydration of lichen thallomas collected in the forest-steppe zone (Table 2).

It is possible to observe a decrease in the intensity of photosynthesis and, conversely, an increase in respiratory capacity as the level of pollution increases [28]. The ratio of the respiratory capacity of lichens to the potential intensity of photosynthesis increases under the stress, and sometimes exceeds the control level by 2-3 times [28]. Our measurement of respiration rate showed that lichens grown in urban conditions had the lowest level of oxygen absorption, while samples collected in the forest-steppe zone showed a highest rate of respiration (Table 6). It can be assumed that in urban conditions, lichens were exposed to a complex of damaging factors (for example, low moisture content and high SO_3 content), which led to inhibition of total respiration.

Determination of the content of lectins in the thallomas of lichens growing in the town showed an increase in hemagglutinating activity of lectins (Table 6). It is known that when exposed to adverse conditions, there is a significant increase in lectin activity, which is associated with the stabilizing and adaptogenic role of lectins, which also proves the influence of adverse conditions on *P. stellaris* lichen [14, 29].

Table 1: Characteristics of lichen cover in Sterlitamak town and in the directions of the dominant wind movement

Parameter	Forest-steppe zone	Town	Mountain-forest zone
Total species richness	14	6	15
Species saturation, species / trunk	3.9±0.6	3.2±0.2	4.2±0.49
P. stellaris population density, ex. / trunk	60.3±6.5	38±8.1	64.2±9.1

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Table 2: Morphometric indices of coenopopulations *Physcia stellaris* (L.) Nyl. located in different natural areas

Parameters	Location		
	Town	Forest-steppe zone	Mountain-forest zone
Biomass, g	0.03 ±0.01	0.15±0.06	0.12±0.05
Length, mm	13.8 ±2.7	15.8±2.5	16.4±3.4
Area, mm²	118.7±18.4	168.1 ±16.5	161.2±18.3
Fruiting bodies, pcs	39.7±6.5	87.1 ±8.9	84.3±11.4
Number of lobes, pcs	20.0 ±2.6	31.0±1.9	33.1±2.1
The water content in the raw lichens, %	11.1±1.19	20.3±2.46	46.4±6.02
Decrease in illumination*, %	58±1.3	62±2.2	78±1.9
Increase in relative air humidity**, %	2±1	6±1	8±2
Content of SO_3 , % of mass	1.801±0.053	1.306±0.043	1.285±0.036

* the difference between illumination under the forest canopy and open space

** the difference between the relative humidity of air under the canopy of the forest and the open space

Table 3: Volumes of hyphae cells of fungi and algal cells of lichens *Physcia stellaris* (L.) Nyl, µm³

Location	Cells of fungi	Algae cells
Town	28.7±2.2	469±55
Forest-steppe zone	41.6±5.1	1767±207
Mountain-forest zone	69.4 ±7.2	2438±378

Table 4: Catalase enzyme activity in *Physcia stellaris* (L.) Nyl lichens, mcat / g wet mass

Location	Activity
Town	0.73±0.01
Forest-steppe zone	0.47±0.04
Mountain-forest zone	0.42±0.03

Table 5: The contents of phytohormones in lichens *Physcia stellaris* (L.) Nyl

Location	Phytohormones content, ng / g wet mass					
	IAA	ABA	The total content of cytokinins	Zeatin nucleotide	Zeatin riboside	Zeatin
Town	12±2	147±21	46±2	10±0.6	13±2	17±2.7
Forest-steppe zone	37±3	106±8	98±2	16±2	27±2.3	34±4
Mountain-forest zone	45±3	74±6	72±2	24±1.8	44±3.8	6±0.2

Table 6: Proline content, respiration rate and hemagglutinating activity (HA) of lectins in lichens *Physcia stellaris* (L.) Nyl

Location	Proline content, mg / g dry weight	Respiration rate, µl O_2 / g h	HA of lectins
Town	4.45±0.39	373.76 ± 40.58	8
Forest-steppe zone	3.88±0.19	760.92 ± 47.89	4
Mountain-forest zone	2.9±0.29	549.08 ± 25.97	4

CONCLUSIONS

Thus, in the course of research *P. stellaris* lichens, the significant difference in physiological and biochemical parameters between samples collected in "urban" and "country" conditions was established. In the latter, there were no significant differences in the external morphometric values among themselves, however, in samples from the mountain-forest zone, the size of algae and fungi cells is larger than in the forest-steppe zone. It is established that to a certain extent the formation of larger lichen cells in the mountain-forest zone is associated with the accumulation of auxins in the lichen thalloma. In the forest-steppe zone, the formation of smaller cells occurred against the background of an increased content of free and bound cytokinins. Lichens grown in the conditions of the town park were found to have an increased content of ABA and at the same time low levels of IAA and cytokinins. Increased activity of the catalase was found in "urban" lichens, which indicates the participation of the antioxidant system in the processes of adaptation to the aggressive action of sulfur (VI) oxide.

Consequently, the growth of *P. stellaris* lichens in various environmental conditions is accompanied by a number of changes in physiological and biochemical processes that lead to the formation of certain morphometric parameters of lichens. Assessment only the external growth of the indicators is poor in the conduct of lichenoindication. More informative for lichenoindication are parameters such as the size of lichen cells, the content of pollutants in tissues and the state of the antioxidant and hormonal systems of lichens.

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