

## Physiological Responses of Epiphytic Lichens to Anthropogenic Activities at Kirazlyayla and Sarialan Camping Areas in Uludağ National Park (Bursa, Turkey)

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### ABSTRACT

The aim of this study was to determine the effects of anthropogenic activities on the physiology of epiphytic lichens in Kirazlyayla and Sarialan picnic areas at Uludağ National Park. Chlorophyll a, b, carotenoids contents and OD435/415 ratio of all the species collected from Kirazlyayla and Sarialan were higher in picnic areas compared to the control areas. Chlorophyll contents of the samples collected from the picnic areas at the end of summer were higher than that of collected in early summer. Chlorophyll a content of *Parmelia sulcata* collected from Kirazlyayla was lower at the end of summer than the value measured at the beginning of summer. The OD435/415 ratio of *Evernia prunastri* and *Pseudevernia furfuracea* samples collected from Kirazlyayla, and *Hypogymnia physodes* and *P. furfuracea* samples collected from Sarialan were higher in picnic area than the control area both in early summer and early autumn. Chlorophyll a was positively correlated with chlorophyll b, carotenoid, and OD435/415, and was negatively correlated with relative humidity and altitude. OD435/415 ratio was positively correlated with the other photosynthetic pigments, whereas it was only negatively associated with the altitude.

**Keywords:** Anthropogenic activities, epiphytic lichen, air pollution, Uludağ National Park, Bursa

### INTRODUCTION

Uludağ National Park is located 36 km south of Bursa city. Due to its geographical location and structural characteristics, the national park is intensively used during summer and winter seasons. The heavy pressure of anthropogenic activities in these areas not only causes problems such as environmental pollution but it also threatens the natural life of the national park. In order to prevent these threats, several ecological planning applications have been put to use. Due to ecological instability of Kirazlyayla and Sarialan picnic areas, were chosen as the study areas in these ecological planning applications (Eltan *et al.* 2016). Deforestation and increased anthropogenic activities in picnic areas alter microclimatic conditions and cause environmental problems.

Lichens do not have a protective cuticle layer like high plants; therefore, they are continuously exposed to pollutants in the air. Therefore for a long time lichens have been used as biological indicators to monitor the air quality of both urban and rural environments. For over 140 years, lichens have been known to be extremely sensitive to air pollution due to the adverse effects of pollutants on the primer metabolism of both the alga and fungal partners in the lichen thalli (Brodo *et al.* 2001).

Epiphytic lichen diversity is strongly affected by altered microclimate, resulting from changes in forest structure and anthropogenic effects on forest ecosystems (Aragon *et al.* 2010, Svoboda *et al.* 2010, Garrido-Benavent *et al.* 2015). Total species richness increases as a result of the increasing the number of eutrophication tolerant species due to the increase in the intensity of land usage (Pinho *et al.* 2012). The fully grown form and viability of epiphytic lichen thallus were reliable indicators for evaluating and comparing their response to climate, human disturbance, and stand structure-related conditions in forest ecosystems (Giordani *et al.* 2012). Lichens of sun-exposed habitats have generally higher chlorophyll contents than the lichens of shady habitats (Piccotto and Tretiach 2010). The influence of sun-irradiance and water availability on lichen photosynthetic pigments during Mediterranean summer season has been demonstrated by measuring the high chlorophyll deterioration and low pigment concentration in the samples from south facing sites rather than the examples from north facing sites of *Evernia prunastri* (Paoli *et al.* 2010). Chlorophyll a and b content was found lower in *Cetraria islandica* populations collected from the highly irradiated habitats of central Europe compared to the populations of shady habitats by Hajek *et al.* (2001).

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Decrease in the lichen diversity in response to environmental conditions is widely used for a long time as an indicator of air pollution (Munzi *et al.* 2014, Ozimec *et al.* 2016). Photosynthetic pigment contents of epiphytic lichens in the urban and residential sites were decreased as compared to the rural site (Sujetoviene and Galinyte 2016). A positive correlation was found between the lichen diversity and OD435/OD415 ratio, chlorophyll a, total chlorophyll contents in the thalli of *Evernia prunastri* in response to urban environmental conditions after the decrease in air pollution (Lackovicova *et al.* 2013). In several studies, an inverse relationship was observed between air pollution and photosynthetic pigment content of lichens (Riddell *et al.* 2012, Seed *et al.* 2013). Physiological parameters reflect the healthiness of lichen thalli and provide quick information on the effects caused by the stress, and allow monitoring the biological effects of air pollution (Munzi *et al.* 2009).

The aim of this study is to determine the effects of anthropogenic activities on the physiology of epiphytic lichens collected from Kirazlıyayla and Sarıalan picnic areas in Uludağ National Park.

## MATERIALS AND METHODS

### The study area

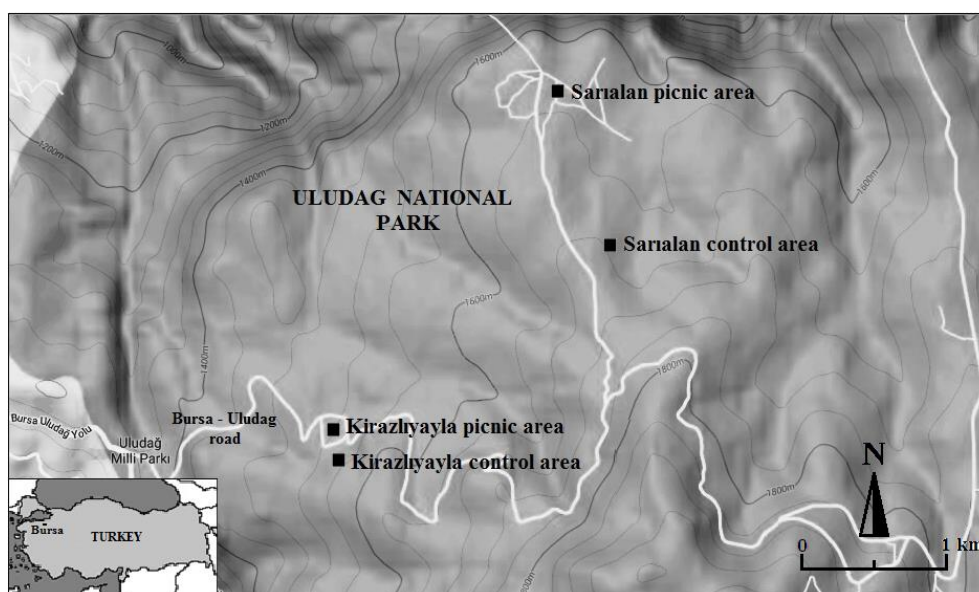
Uludağ is one of the largest winter sports centers in Turkey and has the highest peak in the Marmara region (Alt. 2453 meter). Uludağ was declared a National Park in 1961. Uludağ National Park is located between 40°03'17"–40°10'15" northern latitudes and 29°03'42"–29°19'11" eastern longitudes at 36 km south of Bursa City. In addition to winter tourism, it is also highly preferred for camping, trekking and daily picnic activities by visitors during the summer months.

Uludağ has a very rich and diverse habitat; it contains forest areas, maquis, subalpine shrubs, alpine steep areas, and open fields. A total of 1320 plant species are found at the Uludağ Mountain. Out of these, 33 species are endemic to Uludağ and 138 species are endemic to Turkey. The climate type gradually varies at low to high altitudes. The Mediterranean climate which is usually observed at low altitudes turns into the Black Sea climate as altitude increases. Towards the summit, it turns into a moist micro-thermal climate. The mean annual temperature in Bursa (alt. 100 m) is 14.6°C, and the mean annual rainfall is 696.3 mm. The mean annual temperature at Sarıalan (alt. 1620 m) on the northern slope of Uludağ is 5.5°C and the mean annual rainfall is 1252.1 mm. The mean annual temperature at Uludağ Hill (alt. 1877 m) is 4.6°C and the mean annual rainfall is 1483.6 mm. Kirazlıyayla and Sarıalan which were selected for the study area are located on the northern slope of Uludağ Mountain within the borders of Uludağ National Park. These two highlands are mostly preferred by visitors for daily picnic activities. Karabelen Entrance Gate of Uludağ National Park is 22 km away by road from the Bursa city. After entering into the National Park, Kirazlıyayla picnic area is 3 km and Sarıalan camping and daily picnic area is 10 km away. There is also a cable car to Sarıalan. Sarıalan is important for medical tourism due to the presence of picnic areas, excursions, hiking, as well as tents and highland guesthouses. Kirazlıyayla is mostly used as a daily picnic area. About 25% of the tourists visiting Uludağ National Park prefer Kirazlıyayla and 30% of them favor Sarıalan during summer seasons. Approximately 80% of the visitors go to the plateau by their own private vehicles and 20% of them use cable cars (BEBKA 2017).

The number of tourists and local visitors at Uludağ National Park is increasing annually. In 1986, approximately 446.093 tourists visited Uludağ. Out of these, 56.7% came by road and 43.2% used cable cars. In 1988, the total numbers of visitors were 507.652 (Doğaner 1991). Recently, Uludağ National Park has approximately 800.000 visitors per year and about 600.000 visitors get to the national park by road and 200.000 of them by cable cars. 50% of these visitors visit the national park during the summer season, 35% of them prefer winter season and 15% of them favor spring (Ministry of Agriculture and Forestry in Republic of Turkey 2019a). The number of visitors during the first nine months in 2018 has been reached 1.484.000 person (Ministry of Agriculture and Forestry in Republic of Turkey 2019b).

### Collection of lichen samples

In order to determine the effect of anthropogenic activities on the physiology of epiphytic lichens, lichen samples were collected on *Abies nordmanniana* (Steven) Spach subsp. *börnmuelleriana* (Mattf.) Coode & Cullen from Kirazlıyayla and Sarıalan picnic areas in Uludağ National Park (Fig. 1).



**Figure 1.** Map of the study area.

The collection of lichen samples for photosynthetic pigment analysis was carried out in two stages. In the first stage, the sampling was done in May at the beginning of summer when human activities were little, and the second stage was performed in September at the end of the summer period when anthropogenic activity was intense (Table 1). To determine the photosynthetic pigment contents of epiphytic lichens, foliose lichens as *Hypogymnia physodes* (L.) Nyl. and *Parmelia sulcata* Taylor, and fruticose lichens as *Evernia prunastri* (L.) Ach. and *Pseudevernia furfuracea* (L.) Zopf were collected from Kirazlıyayla and Sarıalan.

**Table 1.** Geographical and ecological characteristics of the sampling area.

Site	Uludağ National Park			
	Kirazlıyayla		Sarıalan	
Collection time				
The beginning of summer	04.05.2017		08.05.2017	
The end of summer	26.10.2017		26.10.2017	
Sampling area	Control area	Picnic area	Control area	Picnic area
Latitude (N)	40°06'38"	40°06'45"	40°07'30"	40°08'06"
Longitude (E)	29°05'23"	29°05'24"	29°06'47"	29°06'33"
Altitude (Alt)	1555	1532	1735	1631
Relative humidity (%)	40	39	41	38
Light (Lux)	2121±955.51	2148±211.46	890±597.98	5434±2240.85

On the other hand, *E. prunastri* was collected only from Kirazlıyayla picnic area because it was absent on the trees in the central parts of Sarıalan picnic area. In addition, an area with a distance of at least 500 meters from the edge of the picnic area was selected as the control area and samples were also collected from that area. Three different thallus samples were taken for each lichen species in each of the sampling areas. The relative humidity (%) and light (Lux) at the sampling locality were measured using with an environmental tester (PCE-EM 886). Relative humidity and light were measured at four times from the sites.

### Photosynthetic pigments and chlorophyll degradation

20 mg of each cleaned lichen samples were used in order to measure the content of photosynthetic pigments and evaluate chlorophyll degradation to phaeophytin. Samples were first rinsed five times for 5 min. in CaCO<sub>3</sub>-buffered acetone solution to remove lichen substances. After the evaporation of acetone in the dark, the samples were extracted in the dark for 40 min. at 65°C in 5 ml of dimethyl sulfoxide (DMSO) and then allowed to cool down at

room temperature (Barnes *et al.* 1992). The extracts were filtered with Whatman no 3 filter paper and were diluted with 5 ml of DMSO. Three samples for each lichen species were extracted. Absorbances at 665, 649, 480, 435 and 415 nm were determined spectrophotometrically (Beckman Coulter DU 730). Concentrations of chlorophyll a, chlorophyll b and total carotenoids were calculated using Wellburn equations (1994). The absorbance ratio at 435 and 415 nm (OD435/415), known as phaeophytinization rate (Ronen and Galun 1984), was used to assess chlorophyll degradation in response to phaeophytin.

### Statistical analyses

The differences in pigment contents of the species in picnic and control areas of Kirazlıyayla or Sarıalan were analyzed using a one-way analysis of variance (ANOVA) with LSD test (IBM SPSS Statistics v.23). Correlation between the photosynthetic pigments and climatic conditions in the sampling area was assessed using Pearson's correlation coefficient (2-tailed).

## RESULTS

Chlorophyll a content was found to be higher in all of the species collected from Kirazlıyayla and Sarıalan picnic areas as compared to the control areas. The chlorophyll content of the samples collected at the end of summer from the picnic areas is higher than that of collected at the beginning of summer.

Chlorophyll a content in *E. prunastri* collected from Kirazlıyayla was found higher in picnic areas compared to control areas both in the beginning and end of summer. Similar results were obtained for *H. physodes*, *P. sulcata* and *P. furfuracea*. Chlorophyll b, carotenoid contents, and OD435/415 ratio of *E. prunastri* were significantly different between the control and picnic area. Chlorophyll a and carotenoid contents of *E. prunastri* were significant at  $P < 0.05$ , and chlorophyll b and OD435/415 ratio were significant at  $P < 0.001$ . In particular, the changes in the OD435/415 ratio was more significant between both control and picnic areas and between the beginning and end of summer. The difference in the chlorophyll a content in the thalli of *H. physodes* and *P. sulcata* was significant at  $P < 0.05$  level. Except *P. furfuracea*, carotenoid content was found higher in all the species collected from Kirazlıyayla picnic area as compared to the control area. The carotenoid content of *P. furfuracea* from the control and picnic areas were not statistically significant. The OD435/415 value which is an important indicator in determining the effect of anthropogenic activities on photosynthetic pigment content of epiphytic lichens and the conversion rate of chlorophyll a to phaeophytin, was found to be higher in picnic areas compared to the control area. The OD435/415 value of *E. prunastri* and *P. furfuracea* collected from Kirazlıyayla was higher in picnic area as compared to the control area both in the beginning and end of summer (Table 2).

Chlorophyll a contents of the epiphytic lichen samples collected in both in the beginning and end of summer from Sarıalan picnic areas were higher as compared to the control areas. Whereas differences in chlorophyll a content of *H. physodes* from control and picnic areas were not significant. Chlorophyll a and b contents of *P. sulcata* from Sarıalan were significant. The photosynthetic pigment contents of *P. furfuracea* from Sarıalan varied significantly between both control and picnic areas and between the beginning and end of summer. Among fruticose lichen species, the most significant difference was observed in chlorophyll b contents of *P. furfuracea* ( $P < 0.001$ ) samples which were collected from the control and picnic areas. At the end of summer, change in the carotenoid content of *P. furfuracea* gathered from Sarıalan picnic area was quite noticeable especially as compared to the control area. The chlorophyll a/b ratio of *P. furfuracea* collected from Sarıalan picnic areas, gradually decreased towards the end of summer as compared to the control areas. The OD435/415 value of *H. physodes* and *P. furfuracea* collected from Sarıalan was higher in picnic area as compared to the control area both in the beginning and end of summer. The change OD435/415 ratio in samples of *H. physodes* and *P. furfuracea* was significant at  $P < 0.001$  level (Table 3).

**Table 2.** The results of ANOVA and mean  $\pm$  standard deviations of photosynthetic pigment contents in epiphytic lichen species collected from Kirazliyayla camping and daily picnic area (n=3).

Species	Sampling area	Sampling time	Chlorophyll a	Chlorophyll b	Carotenoid	Chlorophyll a/b	OD 435/415	
<i>E. prunastris</i>	Control	Beginning of summer	0.29 $\pm$ 0.06 <sup>a</sup>	0.09 $\pm$ 0.08 <sup>a</sup>	0.10 $\pm$ 0.01 <sup>a</sup>	4.84 $\pm$ 3.10 <sup>a</sup>	1.07 $\pm$ 0.02 <sup>a</sup>	
		End of summer	0.45 $\pm$ 0.01 <sup>a</sup>	0.23 $\pm$ 0.03 <sup>b</sup>	0.14 $\pm$ 0.01 <sup>a</sup>	1.96 $\pm$ 0.21 <sup>a</sup>	1.08 $\pm$ 0.02 <sup>a</sup>	
	Picnic	Beginning of summer	0.69 $\pm$ 0.18 <sup>b</sup>	0.15 $\pm$ 0.05 <sup>ab</sup>	0.24 $\pm$ 0.07 <sup>b</sup>	4.51 $\pm$ 0.52 <sup>a</sup>	1.35 $\pm$ 0.05 <sup>b</sup>	
		End of summer	0.92 $\pm$ 0.16 <sup>b</sup>	0.39 $\pm$ 0.02 <sup>c</sup>	0.30 $\pm$ 0.05 <sup>b</sup>	2.34 $\pm$ 0.29 <sup>a</sup>	1.28 $\pm$ 0.02 <sup>c</sup>	
	<b>ANOVA (F:)</b>			<b>14.58**</b>	<b>20.42***</b>	<b>14.90**</b>	<b>2.61</b>	<b>69.13***</b>
	<i>H. physodes</i>	Control	Beginning of summer	0.64 $\pm$ 0.26 <sup>a</sup>	0.22 $\pm$ 0.10 <sup>a</sup>	0.21 $\pm$ 0.07 <sup>a</sup>	2.97 $\pm$ 0.21 <sup>a</sup>	1.18 $\pm$ 0.07 <sup>a</sup>
End of summer			0.38 $\pm$ 0.04 <sup>a</sup>	0.21 $\pm$ 0.06 <sup>a</sup>	0.13 $\pm$ 0.03 <sup>a</sup>	1.94 $\pm$ 0.70 <sup>b</sup>	1.07 $\pm$ 0.02 <sup>ab</sup>	
Picnic		Beginning of summer	1.19 $\pm$ 0.48 <sup>b</sup>	0.39 $\pm$ 0.10 <sup>b</sup>	0.38 $\pm$ 0.10 <sup>b</sup>	3.05 $\pm$ 0.52 <sup>a</sup>	1.23 $\pm$ 0.12 <sup>ac</sup>	
		End of summer	1.20 $\pm$ 0.21 <sup>b</sup>	0.42 $\pm$ 0.03 <sup>b</sup>	0.39 $\pm$ 0.05 <sup>b</sup>	2.87 $\pm$ 0.33 <sup>a</sup>	1.22 $\pm$ 0.03 <sup>ac</sup>	
<b>ANOVA (F:)</b>			<b>5.86*</b>	<b>5.0*</b>	<b>11.09</b>	<b>3.52</b>	<b>3.24</b>	
<i>P. sulcata</i>		Control	Beginning of summer	0.71 $\pm$ 0.08 <sup>a</sup>	0.25 $\pm$ 0.02 <sup>a</sup>	0.29 $\pm$ 0.03 <sup>a</sup>	2.80 $\pm$ 0.09 <sup>a</sup>	0.88 $\pm$ 0.03 <sup>a</sup>
	End of summer		0.80 $\pm$ 0.07 <sup>a</sup>	0.41 $\pm$ 0.10 <sup>a</sup>	0.26 $\pm$ 0.03 <sup>a</sup>	1.98 $\pm$ 0.29 <sup>b</sup>	0.91 $\pm$ 0.01 <sup>a</sup>	
	Picnic	Beginning of summer	1.03 $\pm$ 0.13 <sup>b</sup>	0.36 $\pm$ 0.08 <sup>a</sup>	0.36 $\pm$ 0.04 <sup>b</sup>	2.96 $\pm$ 0.60 <sup>a</sup>	0.95 $\pm$ 0.08 <sup>a</sup>	
		End of summer	0.80 $\pm$ 0.16 <sup>a</sup>	0.39 $\pm$ 0.15 <sup>a</sup>	0.24 $\pm$ 0.03 <sup>a</sup>	2.18 $\pm$ 0.49 <sup>ab</sup>	0.94 $\pm$ 0.05 <sup>a</sup>	
	<b>ANOVA (F:)</b>			<b>4.35*</b>	<b>1.55</b>	<b>7.01</b>	<b>3.86</b>	<b>1.27</b>
	<i>P. furfuracea</i>	Control	Beginning of summer	0.67 $\pm$ 0.10 <sup>a</sup>	0.17 $\pm$ 0.01 <sup>a</sup>	0.24 $\pm$ 0.04 <sup>a</sup>	3.93 $\pm$ 0.53 <sup>a</sup>	0.95 $\pm$ 0.02 <sup>a</sup>
End of summer			1.15 $\pm$ 0.05 <sup>ab</sup>	0.46 $\pm$ 0.10 <sup>b</sup>	0.37 $\pm$ 0.03 <sup>ab</sup>	2.62 $\pm$ 0.72 <sup>bc</sup>	0.95 $\pm$ 0.04 <sup>a</sup>	
Picnic		Beginning of summer	1.16 $\pm$ 0.55 <sup>ab</sup>	0.34 $\pm$ 0.19 <sup>ab</sup>	0.39 $\pm$ 0.11 <sup>b</sup>	3.53 $\pm$ 0.39 <sup>ab</sup>	1.09 $\pm$ 0.11 <sup>b</sup>	
		End of summer	1.32 $\pm$ 0.33 <sup>b</sup>	0.54 $\pm$ 0.14 <sup>b</sup>	0.39 $\pm$ 0.10 <sup>b</sup>	2.46 $\pm$ 0.01 <sup>c</sup>	1.10 $\pm$ 0.06 <sup>b</sup>	
<b>ANOVA (F:)</b>			<b>2.23</b>	<b>4.68*</b>	<b>2.77</b>	<b>6.27*</b>	<b>4.80*</b>	

\* P<0.05

\*\* P<0.01

\*\*\* P<0.001

**Table 3.** The results of ANOVA and mean  $\pm$  standard deviations of photosynthetic pigment contents in epiphytic lichen species collected from Sarialan camping and daily picnic area (n=3).

Species	Sampling area	Sampling time	Chlorophyll a	Chlorophyll b	Carotenoid	Chlorophyll a/b	OD 435/415	
<i>H. physodes</i>	Control	Beginning of summer	0.63 $\pm$ 0.26 <sup>a</sup>	0.20 $\pm$ 0.09 <sup>a</sup>	0.22 $\pm$ 0.07 <sup>a</sup>	3.08 $\pm$ 0.26 <sup>a</sup>	1.07 $\pm$ 0.04 <sup>a</sup>	
		End of summer	1.07 $\pm$ 0.15 <sup>b</sup>	0.36 $\pm$ 0.01 <sup>b</sup>	0.31 $\pm$ 0.03 <sup>ab</sup>	2.93 $\pm$ 0.34 <sup>ac</sup>	1.25 $\pm$ 0.03 <sup>b</sup>	
	Picnic	Beginning of summer	0.94 $\pm$ 0.11 <sup>ab</sup>	0.27 $\pm$ 0.04 <sup>ab</sup>	0.31 $\pm$ 0.04 <sup>ab</sup>	3.46 $\pm$ 0.16 <sup>ab</sup>	1.18 $\pm$ 0.04 <sup>c</sup>	
		End of summer	1.13 $\pm$ 0.26 <sup>b</sup>	0.41 $\pm$ 0.12 <sup>b</sup>	0.34 $\pm$ 0.08 <sup>b</sup>	2.79 $\pm$ 0.20 <sup>ac</sup>	1.22 $\pm$ 0.01 <sup>bc</sup>	
	<b>ANOVA (F:)</b>			<b>3.64</b>	<b>4.46*</b>	<b>2.36</b>	<b>4.13*</b>	<b>19.81***</b>
	<i>P. sulcata</i>	Control	Beginning of summer	0.49 $\pm$ 0.15 <sup>a</sup>	0.21 $\pm$ 0.10 <sup>a</sup>	0.29 $\pm$ 0.10 <sup>a</sup>	2.55 $\pm$ 0.84 <sup>a</sup>	0.89 $\pm$ 0.03 <sup>a</sup>
End of summer			0.73 $\pm$ 0.02 <sup>b</sup>	0.34 $\pm$ 0.06 <sup>bc</sup>	0.23 $\pm$ 0.02 <sup>ab</sup>	2.20 $\pm$ 0.44 <sup>a</sup>	0.86 $\pm$ 0.08 <sup>a</sup>	
Picnic		Beginning of summer	0.84 $\pm$ 0.04 <sup>bc</sup>	0.29 $\pm$ 0.05 <sup>ab</sup>	0.37 $\pm$ 0.03 <sup>a</sup>	2.92 $\pm$ 0.36 <sup>a</sup>	0.84 $\pm$ 0.05 <sup>a</sup>	
		End of summer	1.02 $\pm$ 0.19 <sup>c</sup>	0.44 $\pm$ 0.02 <sup>c</sup>	0.30 $\pm$ 0.03 <sup>ab</sup>	2.35 $\pm$ 0.54 <sup>a</sup>	0.90 $\pm$ 0.00 <sup>a</sup>	
<b>ANOVA (F:)</b>			<b>10.20**</b>	<b>6.53*</b>	<b>2.86</b>	<b>0.87</b>	<b>1.01</b>	
<i>P. furfuracea</i>		Control	Beginning of summer	0.34 $\pm$ 0.08 <sup>a</sup>	0.09 $\pm$ 0.03 <sup>a</sup>	0.15 $\pm$ 0.04 <sup>a</sup>	4.18 $\pm$ 0.59 <sup>a</sup>	0.86 $\pm$ 0.04 <sup>a</sup>
	End of summer		1.07 $\pm$ 0.01 <sup>b</sup>	0.35 $\pm$ 0.02 <sup>b</sup>	0.34 $\pm$ 0.01 <sup>b</sup>	3.01 $\pm$ 0.14 <sup>bc</sup>	1.00 $\pm$ 0.05 <sup>b</sup>	
	Picnic	Beginning of summer	0.88 $\pm$ 0.14 <sup>b</sup>	0.24 $\pm$ 0.04 <sup>c</sup>	0.32 $\pm$ 0.04 <sup>ab</sup>	3.56 $\pm$ 0.16 <sup>ab</sup>	1.05 $\pm$ 0.02 <sup>b</sup>	
		End of summer	1.96 $\pm$ 0.52 <sup>c</sup>	0.68 $\pm$ 0.10 <sup>d</sup>	0.56 $\pm$ 0.17 <sup>c</sup>	2.87 $\pm$ 0.36 <sup>c</sup>	1.13 $\pm$ 0.00 <sup>c</sup>	
	<b>ANOVA (F:)</b>			<b>18.37**</b>	<b>64.31***</b>	<b>11.10**</b>	<b>8.29*</b>	<b>36.73***</b>

\* P<0.05

\*\* P<0.01

\*\*\* P<0.001

Chlorophyll a, chlorophyll b, carotenoid contents, and OD435/415 ratio of the samples collected from Kirazlıyayla picnic areas showed significant differences between the examined species. Contrarily, the differences in chlorophyll a/b and OD435/415 ratio of the species collected from Sarıalan were significant between the examined species. In the comparison between control and picnic areas, the change in chlorophyll a, chlorophyll b and carotenoid contents of the samples collected from Kirazlıyayla and Sarıalan were significant. Chlorophyll a, chlorophyll b and a/b ratio varied between the beginning and end of summer. The best species showing the effects of anthropogenic activities on photosynthetic pigments of epiphytic lichens was *E. prunastri* in Kirazlıyayla picnic area and was *P. furfuracea* in Sarıalan picnic area (Table 4).

## DISCUSSION

There is a gradual increase in daily visitors at Kirazlıyayla and Sarıalan picnic areas during summer seasons. Therefore, the first samples were collected at the beginning of May and the last samples were collected at the end of September following the summer months when an intense anthropogenic activity was experienced. Since Kirazlıyayla can be reached only by vehicles therefore during summer season anthropogenic activity remains less in Kirazlıyayla compared to Sarıalan. Although there is easy access to Sarıalan picnic area by vehicles, the cable car which is operational throughout the year is also an option to get to the picnic site. Therefore, major anthropogenic activities are observed at Sarıalan throughout the year.

Change in chlorophyll a content and OD435/415 ratio was one of the most important indicators in determining the effect of environmental pollution on photosynthetic pigment content of epiphytic lichens. The contents of photosynthetic pigment of epiphytic lichens in the urban and residential sites were decreased as compared to the rural site (Sujetoviene and Galinyte 2016).

**Table 4.** LSD test results of photosynthetic pigment contents between the species, sampling area and time.

Region	Comparisons	N	df	Chlorophyll a	Chlorophyll b	Carotenoid	Chlorophyll a/b	OD 435/415
<b>Kirazlyayla</b>	Between the species	12	3	4.36**	3.27*	5.11**	1.74	23.18***
	Between control and picnic areas	24	1	20.71***	8.81**	21.01***	0.11	11.94***
	Between the beginning and end of summer	24	1	0.6	13.3***	0	22.38***	0.17
<b>Sarialan</b>	Between the species	12	2	1.41	0.1	0.67	9.06***	43.83***
	Between control and picnic areas	18	1	10.05**	7.51*	11.11**	0	1.81
	Between the beginning and end of summer	18	1	15.73***	32.71***	4.02	10.33**	2.44



A positive correlation was found between lichen diversity, OD435/OD415, chlorophyll a content and total chlorophyll, in the response of *E. prunastri* to urban environmental conditions after the decrease in air pollution (Lackovicova *et al.* 2013).

The OD435/415 value in the current study was found to be higher in picnic areas compared to the control area. In a study performed under natural environments without using the transplantation technique, the concentrations of total chlorophyll, chlorophyll a/b, carotenoid, and OD435/415 ratio were found to be generally higher in samples collected from the polluted sites as compared to clean sites (Ra *et al.* 2005). Chlorophyll a, b and carotenoid contents of *E. prunastri*, *P. sulcata*, and *P. furfuracea* collected from the Huseyinalan village which is 17 km away from Bursa city center and is located on the way to Bursa-Uludağ National Park, were less than as compared to the samples collected from Kirazlıyayla and Sarialan. Whereas lowest values were found in the same samples collected from the Uludağ University campus area (Güvenç *et al.* 2018). Unlike clean areas not only chlorophyll a, b contents, and OD435/415 ratio in transplanted lichen samples decreased in the urban environment (Manrique *et al.* 1989, Paoli *et al.* 2011) but it also showed lower photosynthetic efficiency (Sujetoviene and Galinyte 2016).

Chlorophyll a showed a positive correlation with chlorophyll b, carotenoid, and OD435/415, and showed a negative correlation with relative humidity and altitude. Chlorophyll b has a positive relationship with carotenoid, chlorophyll a/b, OD435/415, and altitude. Carotenoid showed a positive correlation with chlorophyll b and OD435/415 and was negatively associated with relative humidity and altitude. OD435/415 ratio was positively correlated with the other photosynthetic pigments, whereas it was only negatively correlated to the altitude among other environmental variables. Increase in the amount of light in the environment decreases the relative humidity. While the amount of light is not effective on photosynthetic pigments, it has a negative effect on relative humidity, chlorophyll a, and carotenoid. As the altitude increased chlorophyll a, carotenoid and OD435/415 ratio decreased. Contrarily, chlorophyll b content increased (Table 5).

**Table 5.** Pearson correlation between the photosynthetic pigments and climatic conditions in the sampling area.

	Chl a	Chl b	Carot	Chl a/b	OD435/415	RH	Light
Chl b	0.831 **	1					
Carot	0.901 **	0.726 **	1				
Chl a/b	-0.035	0.526 **	-0.020	1			
OD435/415	0.499 **	0.290 *	0.299 *	0.075	1		
RH	-0.403 **	0.263	-0.391 **	-0.026	-0.257	1	
Light	0.179	0.093	0.230	0.047	0.095	-0.737 **	1
Alt	-0.496 **	0.536 **	-0.324 *	-0.293 *	-0.293 *	-0.366 **	-0.063

\* P<0.05

\*\* P<0.01

RH: Relative humidity

A negative relationship between OD435/415 ratio and altitude was shown in *P. sulcata* samples collected from the Bursa province. Especially, chlorophyll a and carotenoid had a significant negative correlation with environmental variables (Güvenç and Bilgin 2018). Epiphytic lichen diversity is strongly affected by the altered microclimate, resulting from changes in the forest structure and repercussions on forest ecosystem due to immense anthropogenic activities (Aragon *et al.* 2010, Garrido-Benavent *et al.* 2015, Svoboda *et al.* 2010). Total species richness increases as a result of the growing number of eutrophication tolerant species due to the increase in the intensity of land usage (Pinho *et al.* 2012). The growth form of epiphytic lichen was a reliable indicator for evaluating and comparing the responses of lichens to climate, human disturbance, and stand structure-related conditions in forest ecosystems (Giordani *et al.* 2012).

Light and temperature has a positive correlation with species richness, while relative humidity is negatively related to richness (Lopez *et al.* 2016). Different lichen species give different physiological responses to light stress. Chlorophyll a content decreases in high light while chlorophyll b is not affected (Balarinova *et al.* 2014). Chlorophyll a and chlorophyll b content was found lower in *Cetraria islandica* populations collected from highly irradiated habitats of central Europe compared to the shady habitats by Hajek *et al.* (2001). Lichens in light-exposed habitats have generally higher chlorophylls contents than the lichens in shady habitats (Piccotto and Tretiach 2010).

The influence of sun irradiance and water availability on lichen photosynthetic pigments during Mediterranean summer season has been demonstrated by measuring the high chlorophyll deterioration (low OD435/415) and low pigment concentration in thalli of *E. prunastri* from south rather than the examples from North (Paoli *et al.* 2010). Due to the increased light intensity in the environment after deforestation, lichens such as *Lobaria pulmonaria* that are adapted to damp and shady environments were immensely damaged by high light intensity (Gauslaa and Solhaug 2000).

The effects of high temperatures on *E. prunastri* lichen were investigated by analyzing the photosynthetic pigment contents and chlorophyll degradation. *E. prunastri* could tolerate a temperature of 40 °C for 24 h but especially its chlorophyll b content decreased when exposed for longer periods. The photosynthetic pigment content at a temperature of 80 °C was adversely affected due to the inhibition of chlorophyll synthesis and decrease in chlorophyll degradation to phaeophytin (Pisani *et al.* 2007).

## CONCLUSIONS

Since Kirazliyayla picnic area is accessible only by a car, the anthropogenic activities in Kirazliyayla is lower than that of Sarialan in summer. Although the Sarialan picnic area is easily accessible by car, great anthropogenic activities are observed in Sarialan as it is also accessible by cable car throughout the year. In this study, the physiological responses of the epiphytic lichens to the anthropogenic activities that it were intensively exposed in summer in the Kirazliyayla and Sarialan picnic areas in Uludağ National Park were measured. Change in chlorophyll a content and OD435/415 ratio was one of the most important indicators in determining the effect of environmental pollution on photosynthetic pigment content of epiphytic lichens. Chlorophyll a content and OD435/415 ratio of epiphytic lichens collected from Kirazliyayla and Sarialan were higher in picnic area as compared to the control area both in the beginning and end of summer. The best species showing the effects of anthropogenic activities on photosynthetic pigments of epiphytic lichens was *E. prunastri* in Kirazliyayla picnic area and was *P. furfuracea* in Sarialan picnic area.

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