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Original Research Article

Which is the Better as an Indicator of Environmental Quality: *Parmelia sulcata* Taylor or *Parmelina tiliacea* (Hoffm.) Hale

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ABSTRACT

In this study, the changes in photosynthetic pigment contents of epiphytic foliose lichens *Parmelia sulcata* and *Parmelina tiliacea* collected on the trunk of oak trees in each one of four different localities in Bursa Province were investigated. Almost all of the photosynthetic parameters of *P. sulcata* in all the localities were measured higher than in *P. tiliacea*. Chlorophyll and carotenoid contents, and phaeophytinization rate measured in thalli of *P. sulcata* showed significant differences between the localities. Only chlorophyll b/a ratio and phaeophytinization rate in thalli of *P. tiliacea* was not significant between the localities. Other parameters showed significant differences. The highest photosynthetic pigment contents and ratio for each lichen species was measured in samples collected from 3th locality. The lowest chlorophyll a content and phaeophytinization rate for each lichen species were measured in samples collected from 2nd locality. Compared to variations in the chlorophyll a, total chlorophyll, and total carotenois content for each lichen species between the localities were found statistically significant at level P<0.01. *P. sulcata* was the better as an indicator of environmental quality than *P. tiliacea*.

Keywords: Epiphytic lichen, Photosynthetic pigment, *Parmelia sulcata*, *Parmelina tiliacea*, *Quercus cerris*, Bursa

INTRODUCTION

Lichens are highly sensitive organisms to the environmental stress and especially to the atmospheric pollutants (Nimis *et al.* 2000, Conti and Cecchetti 2001, Giordano *et al.* 2005). The pollutants as NOx, CO_2, CO and SO_2 released by vehicle traffic is one of the main sources of air pollution in the urban areas and roadside (Gilbert *et al.* 2003). Phytotoxic gases emitted from motor vehicles is reduced the diversity and changed the composition of epiphytic lichens in the urban environments and roadside (Giordani *et al.* 2002, Loppi *et al.* 2002).

Lichens are good indicators of habitat changes, providing an integrated measure of all disturbances present in their environment (Pinho *et al.* 2004). The diversity of epiphytic lichens, the composition of lichen communities, the accumulation of trace elements and the response of physiological parameters in sensitive species can be used as indicators of environmental stress (Garty *et al.* 2001, Paoli *et al.* 2011).

Several papers report a correlation between changes in photosynthetic pigment content of lichens and atmospheric pollution (Carreras and Pignata 2001, Ra *et al.* 2005, Riddell *et al.* 2012, Seed *et al.* 2013). Physiological parameters reflect the health status of lichen thalli, offer rapid information on the effects of stress and allow monitoring of the biological effects of air pollution (Munzi *et al.* 2009).

In this study, photosynthetic pigment contents of epiphytic foliose lichens *Parmelia sulcata* and *Parmelina tiliacea* collected on the trunk of oak trees in each one of four different localities in Bursa Province were compared.

MATERIALS AND METHODS

Collection of lichen samples

Two foliose lichens, *Parmelia sulcata* and *Parmelina tiliacea* were collected on the trunk of oak trees in each one of four different localities in Bursa Province between 8 August and 23 October, 2014 (Fig. 1). Bursa is usually dominated by a Mediterranean climate and is a transitional region between Mediterranean climate and the Black Sea climate (Öztürk 2010). The mean annual temperature (1987-2012) at Mudanya district (alt. 13 m) in Bursa province is 16.7 °C, the mean annual rainfall is 614 mm (as climate of 3th locality), at Karacabey (alt. 15 m) is 14.7 °C, and 585 mm (as 2nd locality), at Büyükorhan (alt. 1000 m) 10.3 °C, and 758 mm (as 4th locality). The

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mean annual temperature (1987-2012) at Bandırma in Balıkesir province (alt. 63 m) is 14.2 °C, and 681 mm (as climate of 1st locality) (TSMS 2013). *Parmelia sulcata* and *Parmelina tiliacea* are two common epiphytic lichen species on oak trees in the province of Bursa (Doğru and Güvenç 2016, Gül and Güvenç 2016). These species grows on acid to subneutral bark of trees in sites with low eutrophication and SO_2 levels (Nimis and Martellos 2018).

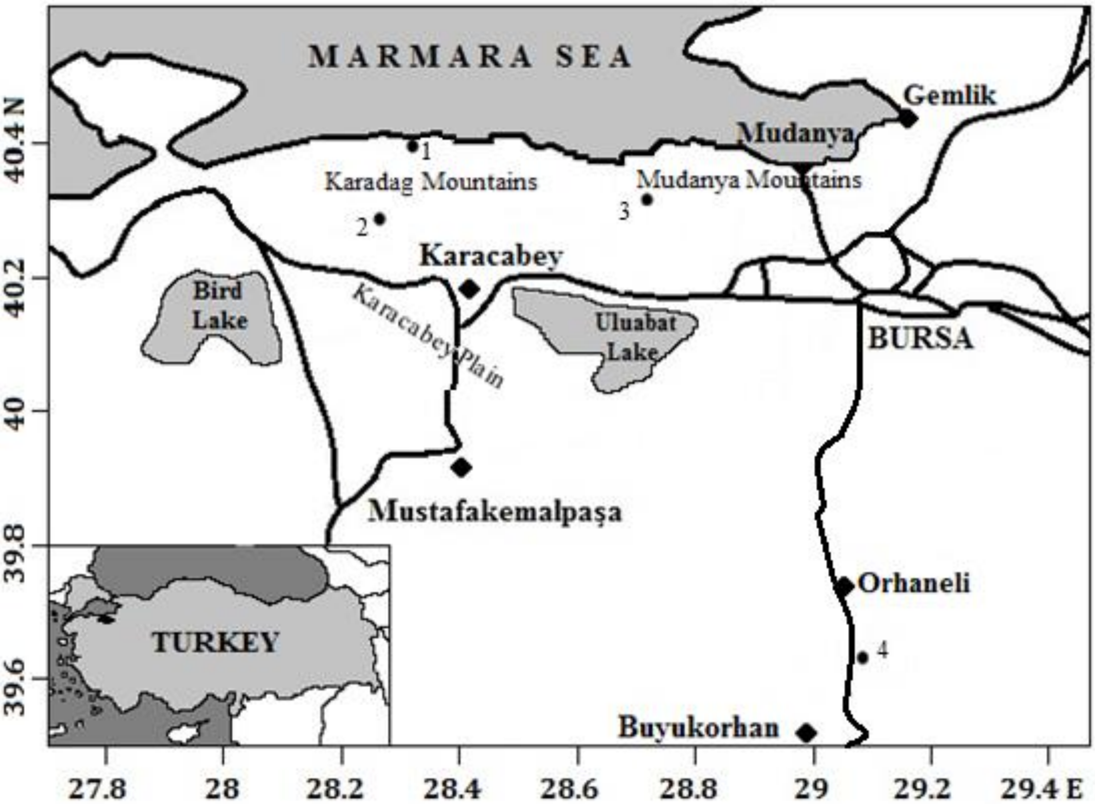

Figure 1. Map of the study area and sampling sites.

The lichen sampling sites:

1. Bursa: Karacabey; Bayramdere, Picnic area, on *Quercus cerris*, 40°23′35″ N - 28°22′31″ E, alt. 40m, 14.08.2014.
2. Bursa: Karacabey: Örencik; village cemetery, on *Quercus cerris*, 40°18′10″ N - 28°17′21″E, alt. 340m, 07.08.2014.
3. Burs: Mudanya; Esence, Çayönü-Esence road, Söğütpınar crossroads, on *Quercus cerris*, 40°20′22″ N - 28°40′42″ E, alt. 140m, 23.10.2014.
4. Bursa: Orhaneli; Karaoğlan; Orhaneli-Harmancık road, Karaoğlan crossroads, on *Quercus cerris*, 39°50′43″ N - 28°59′15″ E, alt. 744m, 29.09.2014

Determination of photosynthetic pigment content and chlorophyll degradation to phaeophytin

Firstly, lichen samples were cleaned to measure the content of photosynthetic pigments and evaluate chlorophyll degradation to phaeophytin. 20 mg of each lichen samples were used for the analysis of pigment content and chlorophyll integrity. Samples were first rinsed five times for 5 min each in $CaCO_3$-buffered acetone to remove lichen substances. After 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 to room temperature (Barnes *et al.* 1992). The extracts were filtered with Whatman no 3 filter paper and were diluted with addition to 5 mL of DMSO. Extraction was repeated three times for each sample. The 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 the equations of Wellburn (1994). The ratio of the absorbances at 435 and 415 nm, known as phaeophytinization rate (Ronen and Galun 1984), was used to assess chlorophyll degradation to phaeophytin.

Statistical analyses

Standard statistical analyses were conducted using IBM SPSS Statistics 23. The level of significance was taken as p≤0,05 all tests. In terms of the content of photosynthetic pigments to test whether differences between localities was used One-Way Analysis Of Variance (ANOVA). Comparison of the photosynthetic pigment contents of *P. sulcata* and *P. tiliacea* were assessed using Independent Samples T-Test. Correlations between the photosynthetic pigment contents in thallus of *P. sulcata* and *P. tiliacea* in the localities was assessed using Pearson's correlation coefficient.

RESULTS and DISCUSSION

The physiological parameters measured for each lichen species were given in Table 1. Almost all of the photosynthetic parameters of *P. sulcata* in all the localities were measured higher than in *P. tiliacea*. Chlorophyll and carotenoid contents, and phaeophytinization rate measured in thalli of *P. sulcata* showed significant differences between the localities. Only chlorophyll b/a ratio and phaeophytinization rate in thalli of *P. tiliacea* was not significant between the localities. Other parameters showed significant differences. Photosynthetic pigment contents were showed differences between different regions and even between different species from the same region (Karakaş *et al.* 2017).

Table 1. Mean ± Standard deviation (SD) values of the photosynthetic pigments (mg/gr) measured in thallus of *P. sulcata* and *P. tiliacea* collected from four localities (df:3, n:12).

		Localities					
		1	**2**	**3**	**4**		
	Altitude (meter)	**40**	**340**	**140**	**744**	**ONE-WAY ANOVA**	
	Temperature (°C)	**14.2**	**14.7**	**16.7**	**10.3**		
	Rainfall (mm^3)	**681**	**585**	**614**	**758**	**F**	**Sig.**
Parmlia sulcata	Chlorophyll a	2.12±0.13a	1.65±0.03b	2.58±0.05c	2.63±0.09cd	85.78	**0.000**
	Chlorophyll b	0.55±0.02a	0.51±0.01a	0.83±0.01b	0.76±0.03c	160.41	**0.000**
	Total chlorophyll (a+b)	2.67±0.15a	2.16±0.03b	3.41±0.06c	3.39±0.12c	109.61	**0.000**
	Total carotenoid	0.56±0.03a	0.47±0.01b	0.66±0.02c	0.71±0.03c	60.25	**0.000**
	Chlorophyll b/a ratio	0.26±0.01a	0.31±0.01bc	0.32±0.01b	0.29±0.01c	31.98	**0.000**
	Total chlorophyll / Carotenoid ratio	4.79±0.11a	4.63±0.06a	5.20±0.19b	4.79±0.04a	13.60	**0.002**
	Phaeophytinization rate	1.07±0.01a	0.89±0.02bc	0.96±0.01c	0.89±0.07bc	14.11	**0.001**
Parmelina tiliacea	Chlorophyll a	1.87±0.05a	1.04±0.07b	2.23±0.23c	1.59±0.04a	47.79	**0.000**
	Chlorophyll b	0.59±0.06a	0.35±0.05b	0.68±0.06ac	0.49±0.01ad	25.34	**0.000**
	Total chlorophyll (a+b)	2.46±0.0a	1.39±0.10b	2.91±0.29c	2.08±0.05a	49.49	**0.000**
	Total carotenoid	0.51±0.02a	0.36±0.06b	0.53±0.05a	0.47±0.02a	10.39	**0.004**
	Chlorophyll b / a ratio	0.32±0.04a	0.34±0.04a	0.31±0.01a	0.31±0.01a	0.83	0.512
	Total chlorophyll / Carotenoid ratio	4.85±0.15a	3.86±0.47b	5.46±0.06ac	4.40±0.11abd	21.41	**0.000**
	Phaeophytinization rate	1.01±0.11a	0.95±0.08a	1.05±0.01a	1.10±0.01a	2.39	0.145

The highest photosynthetic pigment contents and ratio for each lichen species was measured in samples collected from 3. locality. This locality is located at the agricultural area near to asphalt village road. On the contrary, the lowest chlorophyll a content and phaeophytinization rate for each lichen species were measured in samples collected from 2. locality. This locality is located at the village cemetery surrounded by walls in the south of Karadag Mountain in Karacabey district.

Similarly, the total chlorophyll and carotenoid contents in the pollution-tolerant epiphytic lichens *P. sulcata* from uncontaminated areas were found to be higher in samples from contaminated sites (Ra *et al.* 2005). Chlorophyll a and chlorophyll b content in *Evernia prunastri* was found to be significantly lower at the urban and residential sites in comparison with the control site (Sujetoviene and Galinyte 2016). The lowest chlorophyll content was measured in *Ramalina lacera* collected close to the highway and industrial areas, and the highest values were measured in the samples from rural areas (Garty *et al.* 2001).

Photosynthetic pigments (chlorophyll a, chlorophyll b, chlorophyll a+b and total carotenoids), their ratios (chlorophyll a/b, carotenoids/chlorophylls) and phaeophytinization quotient (OD435/OD415) in transplanted samples of *E. prunastri* were not significantly change in comparison with the control samples. A small decrease in total chlorophylls occurring with chlorophyll degradation was measured in residential zones with infrastructure and major transport routes, and in the vicinity of an oil refinery (Lackovicova *et al.* 2013).

Unlike, in a study occured on *P. sulcata* collected from the vicinity of 17 air pollution monitoring stations in the northern part of Switzerland and its bordering area, the lowest chlorophyll content was measured in samples from the rural station, and the highest chlorophyll content was found from the urban stations. In chlorophyll content contains was detected a gradual decreased from urban to rural sites (von Arb *et al.* 1990).

The photosynthetic pigment contents (excluding chlorophyll b/a ratio and total chlorophyll/carotenoid ratio) measured for each lichen species collected from the same localities showed a significant differences between the species (Fig. 2).

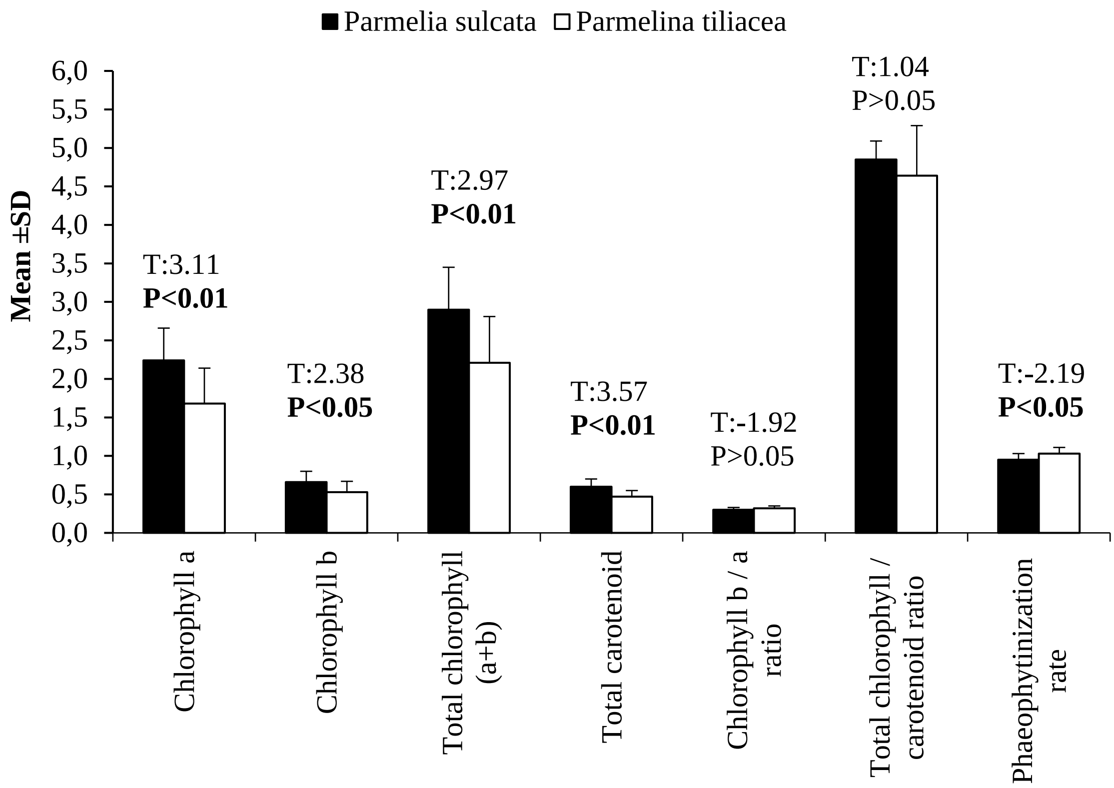

Figure 2. Comparison of the mean ± SD values using independent samples t-test of the photosynthetic pigment contents in thallus of *P. sulcata* and *P. tiliacea*.

Compared to variations in the chlorophyll a, total chlorophyll, and total carotenois content for each lichen species between the localities were found statistically significant at level $P<0.01$. Similarly, variations in chlorophyll b and phaeophytinization rate in thalli of *P. sulcata* and *P. tiliacea* were statistically significant at level

P<0.05. *P. sulcata* was more tolerant to air pollution than *P. tiliacea*. *P. sulcata* was an indicator of the moderately polluted zone, whereas *P. tiliacea* was the slightly polluted zone (Dymytrova 2009). *P. sulcata* that considered to be one of the lichen species tolerant to the effects of atmospheric pollutants in the city environment was used as an indicator of environment conditions (Stamenković *et al.* 2010).

Table 2. Pearson's correlation of the variations in the photosynthetic pigment contents of *P. sulcata* and *P. tiliacea* between the localities. Sig. (two-tailed), n=12

Parmelia sulcata	Localities	Altitude	Temperature	Rainfall
Altitude	0.793**			
Temperature	- 0.495	- 0.858**		
Rainfall	0.436	0.590*	- 0.856**	
Chlorophyll a	0.681*	0.289	- 0.264	0.600*
Chlorophyll b	0.781**	0.273	- 0.048	0.296
Total chlorophyll (a+b)	0.719**	0.290	- 0.212	0.532
Total carotenoid	0.758**	0.442	- 0.409	0.679*
Chlorophyll b/a ratio	0.466	0.118	0.387	- 0.552
Total chlorophyll/carotenoid ratio	0.268	- 0.314	0.469	- 0.117
Phaeophytinization rate	- 0.645*	- 0.711**	0.336	0.042
Parmelina tiliacea				
Chlorophyll a	0.091	- 0.418	0.344	0.154
Chlorophyll b	0.014	- 0.478	0.396	0.094
Total chlorophyll (a+b)	0.074	- 0.435	0.358	0.141
Total carotenoid	0.099	- 0.295	0.154	0.313
Chlorophyll b/a ratio	- 0.281	- 0.105	0.135	- 0.325
Total chlorophyll/carotenoid ratio	0.045	- 0.480	0.453	0.013
Phaeophytinization rate	0.487	0.304	- 0.332	0.534

P<0.01**, P<0.05*

Variations in the photosynthetic pigment contents of *P. sulcata* were positively correlated with the localities and only phaeophytinization rate was negatively (Table 2). The photosynthetic pigment contents of *P. tiliacea* were not associated with the localities. These results seem to indicate that the photosynthetic pigment contents of *P. sulcata* was the better as an indicator of environmental quality than *P. tiliacea*.

Photosynthetic pigments were also influenced by climatic parameters (Paoli *et al.* 2010). Concentrations of chlorophyll a, chlorophyll b, and carotenoids in thalli of *E. prunastri* and *Pseudevernia furfuracea* were found decreased with the decrease in altitude and along with the hottest and driest months of the year (Pirintsos *et al.* 2011). Chlorophyll a and carotenoid contents in *P. sulcata* in our study were positively correlated with rainfall. the localities Phaeophytinization rate in *P. sulcata* was negatively correlated with altitude.

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