J. BIOL. ENVIRON. SCI.,
2019, 13(38), 65-69

Original Research Article

Changes of Photosynthetic Pigment Content in Lichens Collected from Urban and Rural Localities in Bursa Province

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Received: 08.05.2019; Accepted: 21.06.2019; Published Online: 31.10.2019

ABSTRACT

In this study, contents of photosynthetic pigment in foliose *Melanelixia subaurifera, Parmelia sulcata* and fruticose *Evernia prunastri, Ramalina farinacea* were compared. Lichen species were collected from five localities in Bursa. Chlorophyll a, chlorophyll b, total chlorophlly, total carotenoid contents (mg/g), chlorophyll a/b ratio, total carotenoid/total chlorophyll ratio and phaeophytinization (OD435/OD415) ratio in the lichen extracts were differently determined between localities. These changes were found statistically significant ($p \leq 0.05$). The chlorophyll degradation rate at the localities in urban environments is determined to be higher than in the natural environment. It was observed that the air quality exposure ratio was related to the lichen morphology and thus the fruticose lichen species were more sensitive than foliose.

Keywords: Bursa, Chlorophyll degradation rate, Epiphytic lichen, Photosynthetic pigment content

INTRODUCTION

Lichens are valuable biomonitors for determination of atmospheric pollution and meet all the characteristics of the ideal biomonitor organism (Beeby 2001). Lichens are symbiotic organisms composed by an autotrophic photobiont, a green alga and/or a cyanobacterium, and an heterotrophic mycobiont, usually an ascomycetes. Lichen thalli, unlike higher plants, do not have cuticle or stomata and are strictly dependent on the atmosphere for their metabolism, thus being ideal organisms to be used as "accumulators" of airpollution (Loppi 2014).

Unlike lichens growing on soil or rock which have the possibility to interact with the substratum in addition to air, but epiphytic lichens that grow on tree barks are exposed directly to atmosphere and receive nutrients only from air or precipitation. Therefore, epiphytic lichens are frequently used as bioindicators of air quality (Majumder *et al.* 2013).

The many adaptive mechanisms of lichens allow showing tolerance to extreme environmental conditions and different biotope all over the world (Giordiano *et al.* 2005).
The responses of lichens against pollutants may differ between morphological groups. These properties of lichens may be used to distinguish acumulator, sensitive and tolerant types.

Lichens can accumulate metal to levels above their physiological requirements (Bačkor and Loppi 2009). Different lichen species in the same locality contain varying amounts of metal. The metal accumulation amount of foliose lichens is higher than that of fruticose lichens (Garty 2001).

According to literature, there is a direct correlation between epiphytic lichen diversity and air quality. In areas with low air quality such as the urban area or roadside, species diversity and cover value of lichens are a significantly decreased (Hultengren *et al.* 2004, Llop *et al.* 2012, Paoli *et al.* 2011).

Lichens undergo significant changes when they are transported from relatively clean areas to more polluted areas. Chlorophyll content, fluorescence reduction and higher cell membrane damages in transplantations are seen as harmful effects of air pollution in the urban environment (Sujetoviene and Galinyte 2016, Majumder *et al.* 2013).

In this study, the photosynthetic pigment contents of epiphytic foliose lichens (*Melanelixia subaurifera, Parmelia sulcata*) and fruticose lichens (*Evernia prunastri, Ramalina farinacea*) which collected from five different localities in Bursa were compared. The differences in photosynthetic pigment content of lichens were investigated depending on the environment (urban or rural) and morphological growth forms (foliose or fruticose).

MATERIALS AND METHODS

In this study, two fruticose (*Evernia prunastri, Ramalina farinacea*) and two foliose (*Melanelixia subaurifera, Parmelia sulcata*) lichen species were collected on oak trees in five different localities in Bursa province (Figure 1).

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Sampling localities;

1. BURSA: Osmangazi; Hüseyinalan, oak-pine forest in roadside, 874 m, 40°07ˈ50" N 29°01ˈ11" E, 28.10.2018.
2. BURSA: Kestel; Babasultan, oak forest, 650 m, 40°06ˈ16" N 29°22ˈ34" E, 01.11.2018.
3. BURSA: İznik; Gürmüzlü, oak forest, 482 m, 40°30ˈ8" N 29°44ˈ23" E, 24.12.2018.
4. BURSA: Nilüfer; Görükle, Uludağ University, mixed forest of oak and pine around the Faculty of Arts and Sciences, 108 m, 40°13ˈ33" N 28°51ˈ55" E, 27.12.2018.
5. BURSA: Yenişehir; Mecidiye, oak area, 539 m, 40°21ˈ35" N 29°41ˈ4" E, 27.01.2019.

Figure 1. Map of study area. Among the sampling localities, Görükle and Mecidiye are urban, Hüseyinalan and Babasultan are semi-rural and Gürmüzlü is rural.

Determination of photosynthetic pigment contents

Firstly, lichen samples were cleaned. A total of 20 mg of both the middle and marginal parts of the tallus of each lichen species was used at each station for the experiment. Then, each samples were washed five times for 1 minute with 3 ml acetone saturated with $CaCO_3$ to remove lichen substances and they were placed into a 15 ml test tube. 10 ml pure dimethylsulfoxide (DMSO) was used to prepare lichen extracts and therefore to minimize degradation of chlorophyll by chloropyllase enzyme. Test tubes were incubated at 65 °C for 60 min in dark and then allowed to cool down to room temperature (Barnes *et al.* 1992).

The absorbances at 665, 649, 480, 435 and 415 nm were measured with spectrophotometer (Beckman Coulter DU 730) which was calibrated with DMSO. Concentrations of chlorophyll a, chlorophyll b and total carotenoids were calculated using the equations of Wellburn (1994). The experiment was repeated two times.

The chlorophyll degradation was assessed through the ratio of the absorbances at 435 and 415 nm, also known as phaeophytization quotient (Ronen and Galun, 1984).

Statistical Analyses

The statistical analyzes were performed using GraphPad Prism 8. The level of significance was taken as $p \leq 0.05$ all tests. One-Way Analysis of Variance (ANOVA) was used to test whether variations in the content of photosynthetic pirments between localities.

RESULTS AND DISCUSSION

In this study, chlorophyll a, chlorophyll b, total chlorophyll, total carotenoid content (mg/g) and chlorophyll a/b ratio, total carotenoid/total chlorophyll ratio and OD435/OD415 ratio of lichen samples were determined to be different between the localities and these changes has been found statistically ($p<0.05$) significant. The measurement values obtained in the study are given in Table 1.

The amount of photosynthetic pigment varies in each station according to the species. In this study, the highest photosynthetic pigment content was found to be in *Evernia prunastri* in Görükle which is the urban area, and the lowest photosynthetic pigment content in *Parmelia sulcata* in Gürmüzlü which is the rural area. When all stations are examined, amoung the four species the highest total amount of chorophyll was found to be in *Evernia prunastri* (Figure 2).

Table 1. Mean ± SD values of the photosynthetic pigment contents (mg/gr) of examines species.

		Localities					One-Way ANOVA	
		1	2	3	4	5	F	P value (summary)
Evernia prunastri	Chlorophyll a	3.64±0.72	4.58±0.81	5.94±0.68	5.78±1.10	3.32±0.49	4.65	0.062 (ns)
	Chlorophyll b	1.15±0.23	1.22±0.68	1.78±0.32	1.96±0.28	1.10±0.03	2.25	0.199 (ns)
	Total chlorophyll	4.79±0.95	5.80±1.49	7.72±1.01	7.74±1.38	4.42±0.52	3.93	0.083 (ns)
	Total carotenoids	1.18±0.24	1.37±0.21	1.89±0.20	1.82±0.25	1.18±0.16	5.13	0.051 (ns)
	Chlorophyll a/b	3.15±0.01	4.23±1.68	3.36±0.23	2.94±0.13	3.02±0.37	0.89	0.530 (ns)
	Total carotenoids/Total chlorophyll	0.50±0.35	0.24±0.03	0.24±0.01	0.23±0.01	0.26±0.01	1.03	0.473 (ns)
	OD435/OD415	1.33±0.03	1.28±0.01	1.26±0.04	1.36±0.01	1.27±0.05	3.15	0.120 (ns)
Melanelixia subaurifera	Chlorophyll a	2.24±.0.32	4.58±0.81	2.65±0.20	3.71±0.04	3.50±0.34	10.28	0.012 (*)
	Chlorophyll b	0.88±0.28	1.22±0.68	1.08±0.20	1.39±0.01	1.41±0.01	11.70	0.009 (**)
	Total chlorophyll	3.12±0.35	5.80±1.49	3.73±0.64	5.10±0.35	4.91±0.35	12.05	0.008 (**)
	Total carotenoids	0.98±0.09	1.37±0.21	1.02±0.11	1.34±0.35	1.42±0.14	10.65	0.012 (*)
	Chlorophyll a/b	2.54±0.28	4.23±1.68	2.46±0.04	2.66±0.04	2.48±0.21	0.57	0.695 (ns)
	Total carotenoids/Total chlorophyll	0.31±0.01	0.24±0.03	0.27±0.02	0.26±0.01	0.28±0.01	4.44	0.067 (ns)
	OD435/OD415	1.22±0.02	1.28±0.01	1.09±0.09	1.27±0.04	1.22±0.01	3.26	0.114 (ns)
Parmelia sulcata	Chlorophyll a	3.13±1.50	3.71±1.91	2.19±0.63	5.39±0.28	2.57±0.39	2.40	0.181 (ns)
	Chlorophyll b	1.48±0.71	1.60±0.76	0.71±0.06	1.55±0.19	0.90±0.18	1.48	0.334 (ns)
	Total chlorophyll	4.61±2.21	5.31±2.66	2.91±0.69	6.94±0.47	3.48±0.56	1.94	0.242 (ns)
	Total carotenoids	1.13±0.44	1.24±0.53	0.82±0.10	1.62±0.08	0.90±0.13	2.01	0.231 (ns)
	Chlorophyll a/b	2.11±0.01	2.28±0.11	3.04±0.61	3.48±0.24	2.86±0.13	6.86	0.029 (*)
	Total carotenoids/Total chlorophyll	0.25±0.03	0.24±0.01	0.28±0.03	0.23±0.01	0.26±0.00	1.71	0.284 (ns)
	OD435/OD415	0.88±0.06	0.93±0.01	0.85±0.09	1.19±0.01	0.95±0.01	14.06	0.006 (**)
Ramalina farinacea	Chlorophyll a	3.36±0.43	2.76±0.03	2.89±0.30	2.99±0.58	2.91±0.44	0.65	0.650 (ns)
	Chlorophyll b	1.17±0.68	1.20±0.42	0.90±0.20	1.00±0.23	0.85±0.17	0.84	0.554 (ns)
	Total chlorophyll	4.53±0.50	3.96±0.44	3.79±0.49	3.99±0.81	3.76±0.61	0.57	0.699 (ns)
	Total carotenoids	1.02±0.14	0.87±0.05	0.98±0.18	0.95±0.18	0.94±0.15	0.26	0.888 (ns)
	Chlorophyll a/b	2.86±0.19	2.43±0.82.	3.25±0.39	3.00±0.10	3.44±0.17	1.67	0.291 (ns)
	Total carotenoids/Total chlorophyll	0.22±0.01	0.22±0.01	0.26±0.01	0.24±0.00	0.25±0.00	6.22	0.035 (*)
	OD435/OD415	1.37±0.03	1.29±0.04	1.25±0.03	1.38±0.01	1.35±0.04	6.20	0.035 (*)

(AN0VA results: ns not significant; * p≤0.05; ** p≤0.01; *** p≤0.001)

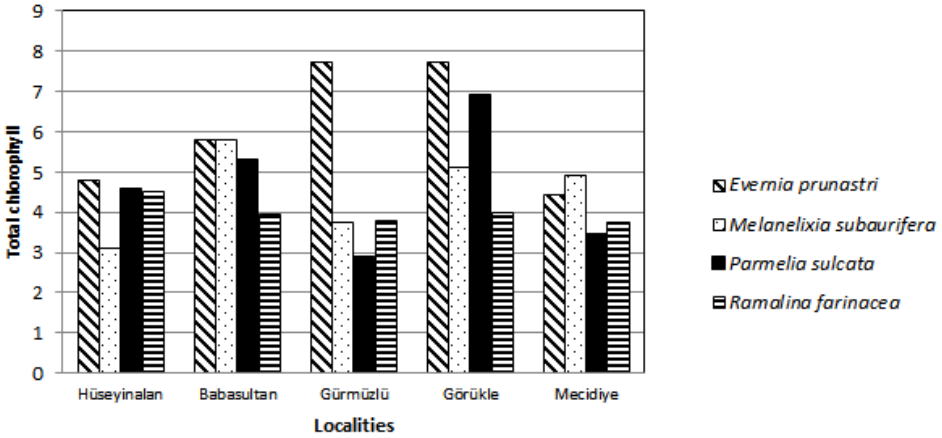

Figure 2. Comparison of total chlorophyll mean according to species and localities.

In studies evaluating the air quality, it was emphasized that air pollution negatively affects on physiological parameters in lichen thallus (Paoli and Loppi 2008, Pirintsos *et al.* 2011, Kalinowska *et al.* 2015, Loppi 2014, Vannini *et al.* 2015). In our study, the best example reflecting the change in physiological parameters in the samples from different localities is *Parmelia sulcata*. It is one of the better indicator species of environmental quality (Güvenç and Bilgin 2018). In this study, Chlorophyll a and Chlorophyll b values measured for *E. prunastri* and *P. sulcata* collected from Hüseyinalan were found to be higher than those collected in 2016 (Güvenç *et al.* 2018).

According to the results of this study, the highest chlorophyll degradation rate among the localities is Görükle, an important living area with an intense human population and the lowest rate of chlorophyll degradation is Gürmüzlü located in a rural area (Figure 3).

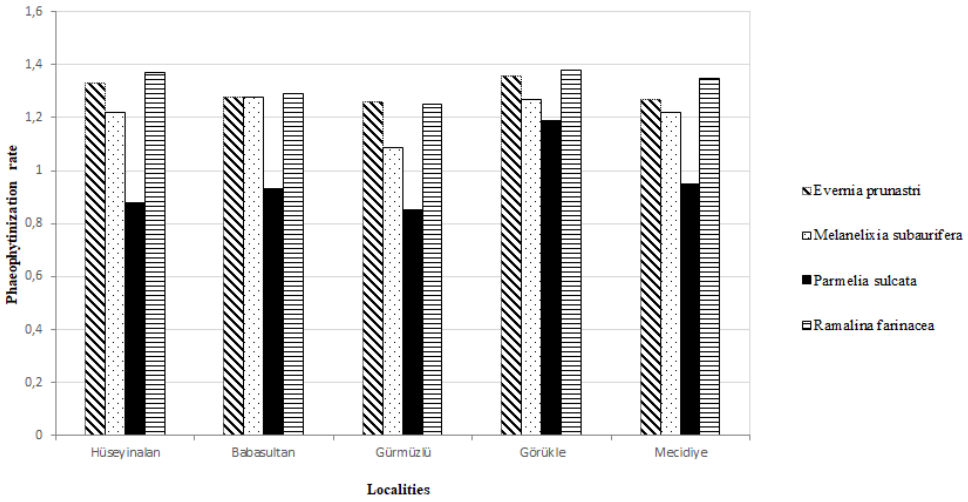

Figure 3. Comparison of chlorophyll degradation rate according to species and localities.

The degree to which the lichen environment is affected by the air quality varies according to the morphological growth forms. In various studies, it is stated that fruticose lichens are more sensitive to environmental conditions and foliose lichens are moderately sensitive or tolerant (Kershaw 1985, Munzi *et al.* 2010, Seed *et al.* 2013, Will-Wolf *et al.* 2017). For instance, in a study conducted by Karakaş *et al.*, fruticose (*Pseudevernia furfuracea*) and a foliose (*Hypogymnia physodes*) lichen collected from 5 different localities in Bursa province were examined the content of photosynthetic pigments. The rate of chlorophyll degradation was found to be the highest in *Pseudevernia furfuracea* which is a fruticose growth form (Karakaş *et al.* 2017).

In the present study, the highest and lowest chlorophyll degradation rate was found to be near to each other in two fruticose species *(Evernia prunastri, Ramalina farinacea)* and is associated with the lichen growth form. The chlorophyll degradation rate in the foliose species (*Melanelixia subaurifera*, *Parmelia sulcata*) is less than in the fruticose species. These results are in line with the results of the study conducted by Karakaş *et al.* (2017).

Chlorophyll degradation is the most valid criterion for determining air quality (Munzi *et al.* 2009, Sen *et al.* 2014, Karakaş *et al.* 2017, Vannini *et al.* 2018). Accordingly, it is possible to give a result about the air quality from the chlorophyll degradation values of the localities where the analysis is made. The values of chlorophyll degradation at Hüseyinalan, Babasultan and Mecidiye stations are lower than Görükle station with the highest chlorophyll degradation and more than Gürmüzlü station with the lowest chlorophyll degradation (Figure 3). These three districts are preferred as residential areas due to their nearness to Bursa city center. At the end of such activities, it is a fact that the natural characteristics of the related stations will change over time and create negative effects on biodiversity. This leads to a reduction of sensitive species and may allow the tolerant species to survive for some time.

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