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Original Research Article

Comparison of Photosynthetic Pigment Contents in Lichen Samples were Collected from Different Localities in Bursa

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ABSTRACT

In this study, contents of photosynthetic pigment in foliose *Hypogymnia physodes* (L.) Nyl. and fruticose *Pseudevernia furfuracea* (L.) Zopf were compared. Lichen species were collected from five localities at Osmangazi and İznik districts in Bursa. Chlorophyll-a, chlorophyll-b, total carotenoid contents (mg/g), chlorophyll-a/chlorophyll-b ratio, total carotenoid/total chlorophyll ratio and OD435/OD415 ratio in the lichen extracts were differently determined between localities. These changes were found statistically significant ($p<0.001$). The highest chlorophyll-a contents were measured in Osmangazi-Soğukpınar (2.79±0.17 m/g), İznik-İhsaniye (2.37±0.24 mg/g), the lowest contents of chlorophyll-a were measured in İznik-Sağırhisar (2.02±0.21 mg/g), İznik-Nüzhetiye (2.22±0.19 mg/g). These results have been observed that Soğukpınar and İhsaniye localities were less affected by anthropogenic effects due to their presence in the rural areas. Whereas, the localities of Sağırhisar and Nüzhetiye were affected by agricultural activities. Only, the difference between total carotenoids/total chlorophyll ratio was not statistically significant. In addition, it has been found between the lichen species. The changes in the content of photosynthetic pigments of *Pseudevernia furfuracea* were much more than *Hypogymnia physodes*. These results showed that negative atmospheric conditions are more effective at fruticose lichens than at foliose lichens.

Keywords: Atmospheric pollution, Bursa, Content of Photosenthetic pigment, Epiphytic, Lichen

INTRODUCTION

Lichens are connected to the water used metabolic activity, heterotrophic fungi partner with one or more autotrophs photosynthetic partners are between long-term obligatory symbiotic union (Nash III 2008). Lichens are although considered as a small ecosystem sample act as a real organism at some features (Honegger 1998, Nash III 2008).

Lichens can be found all over the world, showing tolerance to extreme environmental conditions and different biotope (Kershaw 1985). They have to the many adaptive mechanism allows live in these conditions. Lichens have been used to bioindication works for long time because they do not have a regulate the intake of gas molecules such as stomata cell and cuticle or wax protective layer in vascular plants as (Giordano *et al.* 2005).

Lichens are the best indicators of the negativity that occur due to different reasons in their living area (Pinho *et al.* 2004). Epiphytic lichens can be used as an indicator in environmental stress due diversity, community structures and bio-accumulator properties (Garty *et al.* 2001, Paoli *et al.* 2011). Because some lichens are sensitive to trace elements are accumulated in the thallus and they give physiological responses (Conti *et al.* 2001).

Phytotoxic gases emitted from motor vehicles, epiphytic lichens in diversity reduction and community structures leads to important changes especially in roadside (Giordani *et al.* 2002, Loppi *et al.* 2002).

In studies have shown that the impact of SO_2 physiological processes, such as lichen photosynthesis, respiration, membrane functions that pigment concentrations and enzyme activity (Nash and Gries 1991, Häffner *et al.* 2001).

In several studies has been demonstrated air pollution and lichens photosynthetic pigment in content changes between that there is a correlation (Carreras and Pignata 2001, Ra *et al.* 2005, Riddell *et al.* 2012, Seed *et al.* 2013).

The photosynthetic capacity and respiration in lichens are also significantly affected by SO_2. These metabolic process have been identified this damage is linked to the interaction time with SO_2 and concentration of SO_2 (Häffner *et al.* 2001).

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Closely related to the healthy development of lichen thallus with monitoring the resulting changes in the physiological parameters of the effects of air pollution, which is of great importance to provide reliable information in a short time for assessing the effects of stress (Munzi *et al.* 2009).

In this study, the photosynthetic pigment contents of epiphytic foliose lichen (*Hypogymnia physodes* (L.) Nyl.) and furticose lichen (*Pseudevernia furfuracea* (L.) Zopf) which grown from five localities at Osmangazi and İznik districts in Bursa were compared in rural and urban localities. Atmospheric pollution characteristics of the localities and the level of impact of different morphological species were evaluated with the obtained results.

MATERIALS and METHODS

In this study, the lichen samples which grown on *Quercus* sp. were collected from a total of 5 different localities that Osmangazi and İznik districts in Bursa (Table 1).

Table 1. Study localities in Bursa province.

District	Village	Habitat	Altitude (m)	Coordinates	Date
Osmangazi	Soğukpınar	Oak and beech forest	1110	40º03' N 29º07' E	23.04.2016
Osmangazi	Hüseyinalan	Village inside	969	40º07'05" N 29º01'09" E	23.04.2016
İznik	Sağırhisar	Pine and oak forest	773	40º31'46" N 29º51'49" E	05.06.2016
İznik	İhsaniye	Oak forest	767	40º27'33" N 29º48'35" E	05.06.2016
İznik	Nüzhetiye	Oak forest in roadside	290	40º23'10" N 29º43'31" E	05.06.2016

Firstly, 20 mg of lichen samples were weighed. Then, the lichen samples were rinsed five times for 1 minute, each in 3 ml acetone saturated with $CaCO_3$ (calcium carbonate) to remove lichen substances and it was placed into a 15 ml test tube (Barnes *et al.*1992).

For the preparation of lichen extracts were used 5 ml pure DMSO (Dimethylsulfoxide) for extraction to minimize degradation of chlorophyll by chloropyllase enzyme. Tubes with DMSO and lichen material were incubated at 65 °C for 40 min in dark and then allowed to cool down to room temperature (Ronen and Galun 1984). Later in the lichen sample tubes with 5 ml of DMSO was added and the solution was diluted.

The absorbances at 400-750 nm were determined label of Beckman Coulter DU 730 spectrophotometer which was calibrated with DMSO. Concentrations of chlorophyll-a, chlorophyll-b and total carotenoids in the extracts were measured with spectrophotometer at 665, 649, 480, 435 and 415 nm. 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 quotient (Ronen and Galun, 1984), was used to assess chlorophyll degradation to phaeophytin. Therefore, the high ratio OD435/OD415 indicates that low degradation of the chlorophyll. The statistical analyzes were performed using SPSS version 22 software package. The level of significance was taken as $p \leq 0.05$ all tests. In terms of the content of photosynthetic pigments to test whether differences between localities was used One-Way Analysis of Variance (ANOVA).

RESULTS and DISCUSSION

Thallus of lichen chlorophyll a, chlorophyll b, total carotenoid content (mg/g) and chlorophyll a/b ratio, total carotenoid/total chlorophyll ratio and OD435/OD415 ratio is different between localities and these changes are significantly statistical ($p<0.001$) it has been found.

The highest chlorophyll-a contents of *Hypogymnia physodes* were measured in Osmangazi-Soğukpınar (2.79±0.17 mg/g), İznik-İhsaniye (1.64±0.76 mg/g), the lowest contents of chlorophyll-a were measured in İznik-

Sağırhisar (0.92±0.16 mg/g), İznik-Nüzhetiye (1.16±0.11 mg/g). In Osmangazi-Hüseyinalan, chlorophyll-a content was found as 1.22±0.22 mg/g (Table 2).

The highest chlorophyll-a contents of *Pseudevernia furfuracea* were measured in Osmangazi-Soğukpınar (1.95±0.22 mg/g), İznik-İhsaniye (2.37±0.24 mg/g), the lowest contents of chlorophyll-a were measured in İznik-Sağırhisar (2.02±0.21 mg/g), İznik-Nüzhetiye (2.22±0.19 mg/g). In Osmangazi-Hüseyinalan, chlorophyll-a content was found as 2.25±0.44 mg/g (Table 3).

In this study, it was observed that the contents of chlorophyll a were different from those of lichen samples. The chlorophyll a content of *Pseudevernia furfuracea* was found to be higher than that of *Hypogymnia physodes*.

Table 2. The photosynthetic pigments results of *Hypogymnia physodes.*

Localities		Soğukpınar	Hüseyinalan	İhsaniye	Nüzhetiye	Sağırhisar
Photosynthetic pigment contents	**Chlorophyll a**	1.95±0.22	1.22±0.22	1.64±0.76	1.16±0.11	0.92±0.16
	Chlorophyll b	0.27±0.06	0.37±0.09	0.38±0.24	0.29±0.01	0.36±0.06
	Total carotenoids	0.26±0.06	0.26±0.05	0.40±0.14	0.29±0.01	0.25±0.03
	Chlorophyll a/b	0.13±0.04	0.30±0.07	0.23±0.04	0.25±0.06	0.39±0.03
	Total carotenoids/Total chlorophyll	0.11±0.04	0.16±0.01	0.19±0.04	0.22±0.03	0.19±0.04
	OD435/OD415	1.21±0.08	1.06±0.06	1.13±0.01	1.14±0.03	1.13±0.02

Table 3. The photosynthetic pigments results of *Pseudevernia furfuracea*.

Localities		Soğukpınar	Hüseyinalan	İhsaniye	Nüzhetiye	Sağırhisar
Photosynthetic pigment contents	**Chlorophyll a**	2.79±0.17	2.25±0.44	2.37±0.24	2.02±0.21	2.22±0.19
	Chlorophyll b	0.34±0.04	0.44±0.06	0.38±0.07	0.56±0.04	0.57±0.05
	Total carotenoids	0.31±0.04	0.43±0.01	0.33±0.04	0.44±0.03	0.45±0.04
	Chlorophyll a/b	0.12±0.04	0.19±0.07	0.16±0.04	0.27±0.06	0.25±0.03
	Total carotenoids/Total chlorophyll	0.09±0.04	0.15±0.01	0.12±0.04	0.17±0.03	0.16±0.04
	OD435/OD415	1.16±0.23	1.22±0.34	1.21±0.23	1.27±0.31	1.29±0.50

These results have been observed that Soğukpınar and İhsaniye localities were less affected by anthropogenic effects due to their presence in the rural areas. Chlorophyll values of lichen samples taken from the localities of Sağırhisar and Nüzhetiye indicate that these regions are not suitable conditions for lichens. The photosynthetic pigment contents of lichens in these two localities are lower than the other localities studied.

Bursa- Kültürpark air quality station is located close to Soğukpınar and Hüseyinalan localities (Figure 1). The data of this air quality station are parallel to the changes in chlorophyll contents of lichen samples. Dilovası air quality station is located close to Nüzhetiye and Sağırhisar (Figure 2). There are many industrial facilities in the Dilovası district (Anonymous 2). Consistent with these values, the chlorophyll degradation of lichen samples to Nüzhetiye and Sağırhisar localities was found to be higher than chlorophyll degradation of lichen samples to İhsaniye and Soğukpınar localities. Dilovası is located to the north of Nüzhetiye and Sağırhisar localities and the effective winds blowing from the north in the region cause the pollutants move to southwards.

Although the use of nitrogenous fertilizers agricultural applications are appropriate for nitrophile species when it comes to a combination of anthropogenic effects, lichen diversity may be change in these localities.

The chlorophyll content of *Pseudevernia furfuracea* was found to be higher than that of *Hypogymnia physodes* as shown in Table 2 and 3. These two species seem to have different metabolic properties. Therefore, it seems that species respond differently to environmental factors.

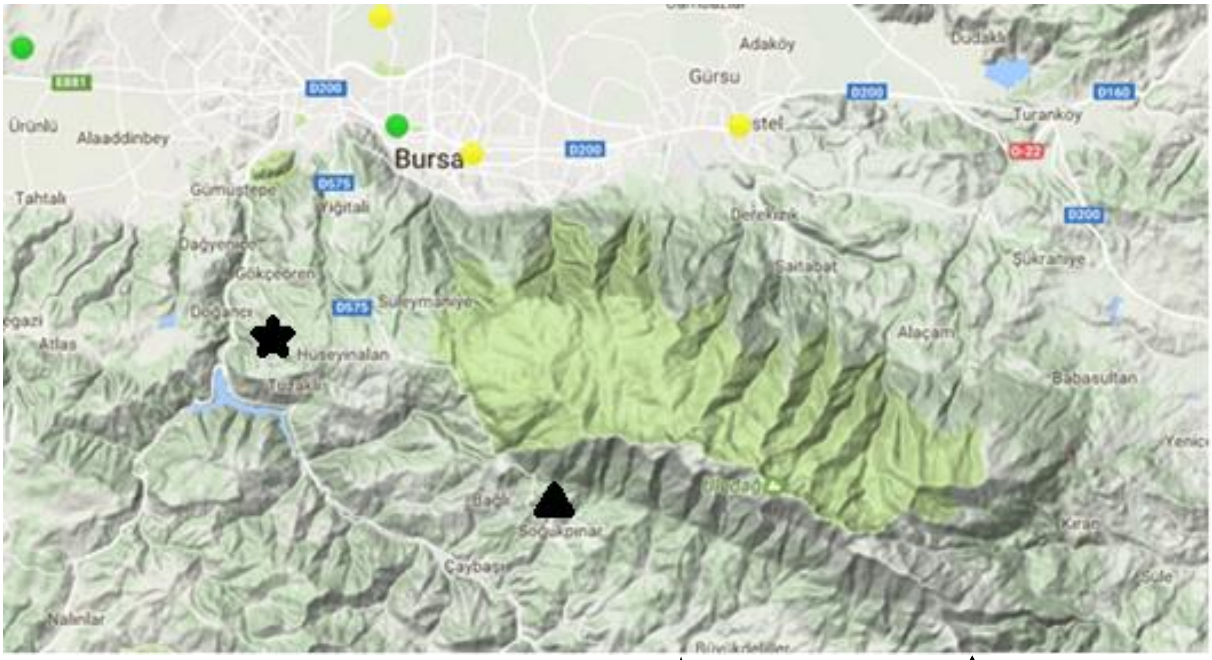

Figure 1. Study area in Bursa city center and air quality stations (Anonymous 1).

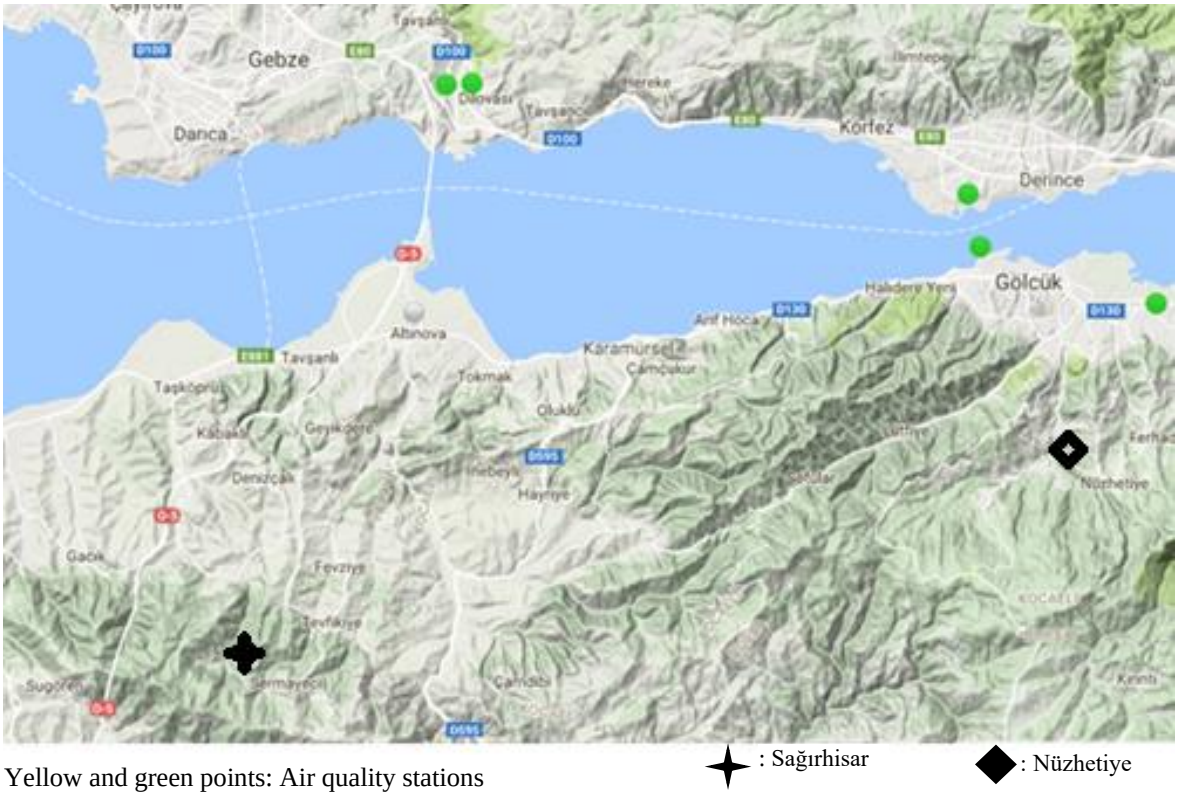

Figure 2. Study area in İznik and air quality stations (Anonymous 1).

The changes in the content of photosynthetic pigments of *Pseudevernia furfuracea* were much more than *Hypogymnia physodes*. These results showed that negative atmospheric conditions are more effective at fruticose lichens than at foliose lichens. In the literature information, it is stated that when the air quality is determined by lichens, the fruticose lichens are more suitable than the other morphological groups (Brodo *et al*. 2001). The reason for this is that the surface are of fruticose lichens in contact with air more than of foliose and crustose lichens.

In addition, the fruticose lichens only hold on from one point on the trunk and branches of tree. So, fruticose lichens not effected by substrate.

According to this information, a higher degradation of the chlorophyll contents of *Pseudevernia furfuracea* was observed. Because of the foliose lichen *Hypogymnia physodes* is more adherent to substrate, only the upper surface is in contact with the air and less of the affected surface is found low chlorophyll degradation in this species.

The atmospheric pollution values of stations near the study localities are parallel to the chlorophyll content of lichen species. The values of SO_2, CO_2, NO, NO_x, CO and O_3 of Dilovası air quality station are higher than those of Kültürpark air quality station (Table 4).

Table 4. National Air Quality monitoring network data (Anonymous 3).

Air quality Stations	PM10/2.5	SO_2	NO	NO_2	NO_x	CO	O_3
Bursa (Kültürpark)	29	5	15	23	43	-	49
Kocaeli (Dilovası)	73	15	21	48	49	540	41

Photosynthetic pigment of algae, chlorophyll content in the lichen thallus is significantly effected from air pollutants. The chlorophyll content of lichen samples can provide information about the pollution levels of localities without morphological changes on lichen thallus.

As a result, in evaluating air quality, the examination of the chlorophyll content of epiphytic lichens in the fruticose form can be used as a useful and practical way.

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