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Physiological responses of epiphytic lichens to air quality of Nilüfer district in Bursa City

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Abstract

In this study, Uludag Fir (*Abies nordmanniana* subsp. *bornmulleriana* Mattf.) branches lined with lichens from the region determined in Kirazlıyayla locality in Uludağ National Park, a rural area far from atmosferic pollutants, were placed in an area more exposed to pollutants in Nilüfer as the central district of Bursa province. Photosynthetic pigment contents of epiphytic lichen species (*Hypogymnia physodes* (L.) Nyl., *Parmelia sulcata* Taylor and *Pseudevernia furfuracea* (L.) Zopf) were measured in three-month intervals. Then, one-year pigment exchange rates and exposure to seasonal pollutants were statistically evaluated. Chlorophyll degradation increased in winter months and decreased in summer months for *H. physodes* samples compared to control. It was decreased in winter and increased in summer for *P. furfuracea* specimens. In *P. sulcata*, no significant difference was found between the values measured in the control and transplanted samples. Chlorophyll degradation was significantly increased for *P. furfuracea* and decreased for *P. sulcata* compared to control specimens with prolonged transplantation time. The amount of chlorophyll a was decreased significantly for *H. physodes* and *P. sulcata* compared to the control samples due to the prolongation of the transplantation period, while the change in *P. furfuracea* was not significant. Chlorophyll b and carotenoid content were significantly decreased in all three lichen species due to the prolongation of the transplantation period.

Keywords: Bursa, chlorophyll content, photosynthetic pigment, epiphytic lichens

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Bursa şehrindeki Nilüfer ilçesinde epifitik likenlerin hava kalitesine karşı verdiği fizyolojik tepkiler

Özet

Bu çalışmada, Atmosferik kirleticilerden uzak kırsal bir alan olan Uludağ Milli Parkı'ndaki Kirazlıyayla mevkiinde belirlenen bölgeden likenlerle kaplı Uludağ Göknarı (*Abies nordmanniana* subsp. *bornmulleriana* Mattf.) dalları, Bursa'nın merkez ilçesi Nilüfer'de kirleticilere daha açık bir alana yerleştirilmiştir. Epifitik liken türlerinin (*Hypogymnia physodes* (L.) Nyl., *Parmelia sulcata* Taylor ve *Pseudevernia furfuracea* (L.) Zopf) fotosentetik pigment içerikleri üçer aylık aralıklarla ölçülmüştür. Daha sonra bir yıllık pigment değişim oranları ve mevsimsel kirleticilere maruz kalma istatistiksel olarak değerlendirilmiştir. Klorofil bozulması, kontrole kıyasla *H. physodes* numunelerinde kış aylarında artmış, yaz aylarında ise azalmıştır. *P. furfuracea* örnekleri için kışın azalırken yazın artmıştır. *P. sulcata*'da, kontrol ve nakledilen numunelerde ölçülen değerler arasında anlamlı bir fark bulunmamıştır. Klorofil bozunması, uzun transplantasyon süresi olan kontrol örneklerine kıyasla *P. furfuracea* için önemli ölçüde artmış ve *P. sulcata* için ise azalmıştır. Klorofil a miktarı, *H. physodes* ve *P. sulcata* için transplantasyon süresinin uzaması nedeniyle kontrol örneklerine göre önemli ölçüde azalırken, *P. furfuracea*'daki değişim önemli değildir. Transplantasyon süresinin uzaması nedeniyle her üç liken türünde de klorofil b ve karotenoid içeriği önemli ölçüde azalmıştır.

Anahtar kelimeler: Bursa, klorofil içeriği, fotosentetik pigment, epifitik likenler

1. Introduction

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Lichens are long-term and obligatory symbiotic associations established between a heterotrophic fungus partner whose metabolic activities depend on the water content suitable for use in the environment and cannot produce its own food, and one or more autotrophs that can produce their own food through photosynthesis [1]. Almost half of the 30,000 Ascomycetes and Basidiomycetes fungi, which spread around the world, are located as mycobionts in the lichen thalli [2]. About 40 different types of green algae and blue-green bacteria are included in the structure of lichens as photobionts. The green algae species belonging to the genus *Trentepohlia* and *Trebouxia* and the blue-green algae belonging to the *Nostoc* genus are the most common photobionts in lichens [3].

There is a physiological division of labor between the photobiont that forms the thallus and the mycobiont. The most important contribution of the fungus is that it creates a habitat for photobiont and provides moisture. The fungal tissues and fungal pigments within the structure of lichen act as a protection shield for photobiont against light. If this were not the case, photobionts would not have been able to survive freely on rock surface, dry bark and other lichen substrates. Because excessive light can damage the photobionts photobionts in the lichen thalli. In addition when fungi, which do not contain chlorophyll and obtain their nutrients by their haustoria, are in the structure of lichens, they can meet the carbohydrates they need by their photosynthesizing partner photobiont. The type of carbohydrates produced by photobiont varies according to the type of photobiont. In lichens containing blue-green bacteria, the carbohydrate produced by photobiont and transferred to mycobiont is glucose, whereas in lichens containing green algae, it is sugar alcohols such as erythritol, sorbitol or ribitol [1, 2]. Such as these alcohols, more than 1.000 lichen substances are produced in lichens. These substances act as antiviral, antibiotics, antitumor, allergenic, plant growth inhibitors, antiherbivores and enzyme inhibitors [4].

As a result of metabolic activities in lichens, primary and secondary metabolites are produced. Primary metabolites are essential products such as carbohydrates, fats and proteins and can be produced by both mycobiont and photobiont. All of the secondary metabolites are of fungal origin and their number is around 854. The vast majority of secondary metabolites are produced exclusively by lichen-forming fungi, while the remainder is found in free-living fungi and higher plants [5]. Secondary metabolites have many benefits to lichens. For example, secondary metabolites in the cortex may exhibit different properties in absorbing light and protect the thallus from UV radiation. Thus, suitable light conditions are provided for the layer in which the partner performing photosynthesis is located [6]. In addition, some secondary metabolites protect thalli from biotic factors, as in the example of vulpinic acid produced in *Letharia vulpina* (L.) Hue [7].

In evolutionary terms, the association of fungi and photosynthesizing partners is very successful and is represented worldwide by over 20,000 different species of lichen, rich in size, shape and color. Besides benefiting from the metabolic products of lichens, lichens are important members of the ecosystem. Lichens, known as the first members to settle on the rock surface, play a great role in the preparation of the soil, which is an important habitat for both plant and animal organisms [2]. Since lichens carry out photosynthesis, they help purify the air by fixing atmospheric carbon dioxide and, in turn, with the release of oxygen [1]. In addition, cyanolichens, which contain blue-green bacteria in common photobionts in the tallus, are ecologically important as they convert free nitrogen in the atmosphere to bound nitrogen [7]. The epiphytic lichens growing on the tree, which provides the water mineral substances required for its growth from the atmosphere, are sensitive to air pollution. Lichens, due to their poikilohydric nature, are also very sensitive to natural and anthropogenic disturbances [8] They are used as bioindicator because they respond very quickly to changes in the environment. Therefore, lichens species richness is highest in the forest area and decreases towards urban areas [9]. It has been shown that the epiphytic lichen diversity on *Q. pubescens* in Bursa changes with the distance from the city center and the center [10]. Due to these features, epiphytic lichens can also be used as biological monitoring material in order to monitor ecological continuity in areas such as residential areas, rural and old forest areas.

For growth and development organisms whose nutrients are tightly bound to the atmosphere can be used as bioindicators to assess the effects of many variables in the atmosphere. Lichens are capable of reacting to changes in atmospheric factors due to their dependence on the atmosphere in providing water and nutrients. Due to these properties, they are very suitable bioindicators for the evaluation of atmosphere quality. Lichens are long-lived and slow growing. In addition, they have different anatomical features from higher plants [11 7]. The most important of these features; as in higher plants, the absence of a protective semipermeable cuticle layer and stomata, contact with air takes place directly with the entire surface of the thallus. Therefore, lichens have extremely favorable substances accumulation properties [2].

The content of photosynthetic pigment of transplanted epiphytic lichens into the urban environment and residential areas were found significantly reduced compared to the control region [12, 13]. Increases in chlorophyll degradation was particularly measured in residential zones, major transport routes and in the vicinity of oil refinery [14].

We thought that when lichens accumulate substances that cause air pollution, it will induce the changes in the amount of photosynthetic pigments in the lichen content. Therefore, this research was planned as a transplantation study to evaluate the effect of air pollution on photosynthetic pigment contents of some lichen speciess. It was decided to the lichen samples were transfer from natural area to urban area with poor air quality. Abies tree branches covered with three lichen species were transplanted to the Nilüfer district of Bursa province, where urbanization and traffic density are high. When the air quality of Nilüfer District in Bursa City is evaluated within the scope of the limit values recommended by our country, EU, USA and World Health Organization (WHO), it is generally clean, although there are daily increases throughout the year [15]. But daily air pollution is the third priority problem in Nilüfer district. Among the factors that cause air pollution in Nilüfer district are the use of coal for heating, increasing urbanization and heavy traffic and pollution caused by industries. Nilüfer is the second among the districts with many heavy industry plants in Bursa and there are two industrial zones (Küçüksanayi zone and Nilüfer Organized Industrial Zone (NOSAB)) [16].

In this study, it was carried out to determine the changes in the amount of photosynthetic pigments that may occur due to the accumulation of air pollutants in the lichen samples, periodic measurements were made from the transplanted groups. In addition pheophytinization rates, which presents chlorophyll degradation, were calculated. The differences between the data of transplanted and control groups were determined statistically. The results are interpreted considering the seasonal pollution values of the region.

2. Materials and methods

An isolated area less affected by air pollution and anthropogenic factors in the Kirazlıyayla region of Uludağ National Park was selected as the control area (40°06′27″N - 29°06′18″E, alt.: 1703 m, date: 03.08.2016). Branches of *Abies* trees with on three epiphytic lichen thalli (*H. physodes*, *P. sulcata* and *P. furfuracea*) from the control area were taken and transplated to a region with high urbanisation and traffic density (İhsaniye neighborhood, FSM Boulevard, 40°13′23″N - 28°58′46″ E, alt.:158 m, date: on 03.08.2016) in Nilüfer district which is one of the central districts of Bursa City. After collection, the branches were transferred to the place where they will be transported in plastic bags on the same day. For photosynthetic pigment analysis, the samples were transported to the laboratory in plastic bags the same day and kept in the refrigerator overnight. In order to determine the photosynthetic pigment content of lichens in their natural environment, measurements were made on the control group samples on day after transplantation (on 04.08.2016). Photosynthetic pigment measurements were made periodically at 3, 6, 9 and 12 months after transplantation and the obtained values were compared with the control values. Photosynthetic pigment measurements were made in triplicate.

After the lichen samples were weighed as 20 mg, they were put into 15 ml test tubes. During the extraction, acetone saturated with $CaCO_3$ was used to remove substances that would reduce photosynthetic pigments. The lichen thalli were washed 5 times for 1 minute each with 3 ml [17]. Then, 5 ml of pure Dimethylsulfoxide (DMSO) was added to the tubes and extraction was carried out by keeping it in an oven at 65 °C for 40 minutes to minimize chlorophyll degradation by the chlorophylase enzyme [18].

The samples taken out of the oven were left to cool in the dark at room temperature to prevent photosynthetic pigments from being broken down by light, that is, photolysis. Then, 5 ml of pure Dimethylsulfoxide (DMSO) was added to the tubes containing lichen extract and the solution was diluted. Light absorbances in the 400-750 nm wavelength range were determined with the Beckman Coulter DU 730 brand spectrophotometer calibrated with DMSO. Absorbance values at 665, 649, 480 nm wavelengths were used to calculate the chlorophyll a, chlorophyll b and total carotenoid content of the extract. Chlorophyll a, chlorophyll b and total carotenoid concentrations were calculated using the dimethylsulfoxide equations given below by Wellburn [19].

The absorbance values at 415 and 435 nm wavelengths were used to determine the conversion ratio of chlorophyll a to pheophyte (OD435/OD415 = Pheophytinization ratio). 435 nm wavelength is the wavelength of chlorophyll a, and 415 nm wavelength is the wavelengths where pheophyte a, which is the degradation product of pigments, shows high absorption. Therefore, the high ratio of OD435 / OD415 indicates that chlorophyll degradation is less. One-Way Analysis of Variance (ANOVA) was used to determine whether there was a difference in the

photosynthetic pigment contents of species samples in localities. To determine correlation between airborne pollutant gases and particles and photosynthetic pigment contents of epiphytic lichen species during transplantation period in Nilüfer district were used to Pearson's correlation analysis (2-tailed). All statistical analyzes were made using the IBM SPSS Statistics 22. In all tests, the significance level was taken as $p \leq 0.05$, except $p < 0.01$ was also given in Pearson's correlation analysis.

3. Results

The 3 month average data of pollutant particles and gases in the exposed air of epiphytic lichen thallus during transplantation period in Nilüfer District are given in Table 1. The transplantation period of lichen specimens corresponds seasonally to Autumn, Winter, Spring and Summer. Therefore, the amount of particles and gases in the air in Nilüfer District is high at the beginning of the transplantation period and the following months, and it is low towards the end of the period. This result explains the negative relationship between the transplantation period and the particles and gases in the air. Only ozone (O_3) is positively correlated with the transplantation period. Other gases and particles (except PM_{10}) are negatively correlated.

Table 1. The 3-month average data of pollutant particles and gases in the exposed air of epiphytic lichen thallus during transplantation period in Nilüfer District [20], and change of particles and gases in the air from the beginning of the transplantation period to the end date

Airborne pollutant particles and gases	**Transplantation periods**				**Pearson correlation**
	01 August 2016 – 31 October 2016	**01 November 2016 – 31 January 2017**	**01 February 2017 – 30 April 2017**	**01 May 2017 – 31 July 31 2017**	
	Data (Mean±SD)				
PM_{10}	44.7±16.9	37.7±8.1	43.6±3.6	37.8±1.5	-.179
$PM_{2.5}$	29.4±14.5	28.3±5.2	24.9±3.8	14.1±8.7	-.539**
SO_2	9.1±4.9	12.0±1.5	7.6±1.0	3.5±0.6	-.607**
NO	22.8±3.7	23.9±15.1	11.3±4.7	2.1±1.1	-.528**
NO_2	45.7±15.9	50.1±8.2	41.0±5.9	27.7±4.0	-.578**
CO	661.9±369.9	761.9±117.6	549.4±90.9	361.4±27.5	-.529**
O_3	42.9±23.6	27.1±8.3	43.8±9.9	76.5±5.4	.600**
CH_4	1307.5±72.8	1287.8±39.1	1257.9±84.1	1120.0±21.6	-.724**
NCH_4	32.2±12.9	28.1±8.9	21.2±4.9	14.3±1.4	-.665**

Photosynthetic pigment values measured from control and transplanted samples of *H. physodes*, *P. sulcata* and *P. furfuracea* are given in Table 2. According to results pheophytinization rate, which presents chlorophyll degradation, increased in winter months and decreased in summer months for *H. physodes* samples compared to control. On the contrary, it decreased in winter and increased in summer for *P. furfuracea* specimens. In *P. sulcata*, no significant difference was found between the values measured in the control and transplanted samples. For *H. physodes*, the amount of chlorophyll a slightly increased in the winter months, while it decreased in the summer months compared to the control samples. The amounts of chlorophyll b and carotenoid decreased gradually during the transplantation period compared to control samples. Similar results to the changes in chlorophyll a, chlorophyll b and carotenoid contents of *H. physodes* were also obtained for *P. sulcata* and *P. furfuracea* (Table 2).

A graph in Figure 1 was made in order to examine the changes in the measured amount of photosynthetic pigments of three epiphytic lichen species during separate transplantation periods. As seen from the graph, chlorophyll degradation is significantly increased (r^2: 0.654, $p<0.05$) for *P. furfuracea* compared to control specimens with prolonged transplantation time and decreased (r^2: 0.635, $p<0.05$) for *P. sulcata* (Figure 1A). The amount of chlorophyll a decreased significantly (r^2: 0.885, $p<0.05$) for *H. physodes* and (r^2: 0.723, $p<0.01$) *P. sulcata* compared to the control samples due to the prolongation of the transplantation period, while the change in *P. furfuracea* was not significant (Figure 1B). Chlorophyll b and carotenoid content were significantly decreased in all three lichen species compared to the control samples due to the prolongation of the transplantation period (Figure 1C and D).

Table 2. The results of the photosynthetic pigment analyzes of control and transplanted samples of *Hypogymnia physodes, Parmelia sulcata* and *Pseudevernia furfuracea* (mg/g)

Photosynthetic pigments	**Measurement times**					**ONE WAY ANOVA**	
	Control	**Transplantation times**					
	0	1st	2nd	3rd	4th		
	04 August 2016	04 November 2016	03 February 2017	05 May 2017	04 August 2017		
	Data (Mean±SD)					**F**	**Sig.**
	Hypogymnia physodes						
Pheophytinization	1.16±0.11	1.24±0.08	1.22±0.07	1.17±0.10	1.09±0.05	3.077	.034
Chlorophyll a	1.22±0.54	1.26±0.36	1.08±0.27	0.88±0.39	0.60±0.23	3.226	.029
Chlorophyll b	0.40±0.18	0.39±0.11	0.36±0.08	0.33±0.11	0.24±0.08	1.756	.169
Carotenoid	0.31±0.09	0.29±0.06	0.23±0.04	0.22±0.06	0.16±0.05	5.820	.002
Chlorophyll a/b	3.02±0.31	3.10±0.39	2.97±0.39	2.54±0.37	2.47±0.37	3.709	.017
Carotenoid/total Chlorophyll	0.20±0.03	0.18±0.02	0.16±0.02	0.19±0.03	0.19±0.02	1.445	.249
	Parmelia sulcata						
Pheophytinization	0.92±0.09	0.89±0.09	0.84±0.02	0.74±0.13	0.82±0.14	2.707	.053
Chlorophyll a	1.38±0.24	1.46±0.57	1.49±0.22	0.81±0.42	0.54±0.22	8.657	.000
Chlorophyll b	0.54±0.13	0.45±0.16	0.47±0.06	0.28±0.10	0.16±0.05	11.757	.000
Carotenoid	0.34±0.06	0.31±0.09	0.32±0.05	0.19±0.08	0.11±0.06	11.659	.000
Chlorophyll a/b	2.64±0.65	3.19±0.12	3.16±0.12	2.73±0.52	3.23±0.39	2.658	.056
Carotenoid/total Chlorophyll	0.18±0.05	0.17±0.02	0.17±0.02	0.18±0.02	0.15±0.03	.702	.598
	Pseudevernia furfuracea						
Pheophytinization	1.01±0.19	0.99±0.05	0.99±0.12	1.16±0.09	1.14±0.05	3.166	.031
Chlorophyll a	1.02±0.35	1.19±0.35	1.15±0.29	1.01±0.32	0.71±0.13	2.335	.083
Chlorophyll b	0.38±0.12	0.35±0.11	0.33±0.11	0.33±0.11	0.21±0.03	2.198	.098
Carotenoid	0.27±0.09	0.31±0.07	0.27±0.09	0.24±0.04	0.17±0.03	3.360	.025
Chlorophyll a/b	2.78±0.71	3.47±0.10	3.56±0.53	3.19±0.26	3.34±0.40	2.729	.052
Carotenoid/total Chlorophyll	0.20±0.03	0.20±0.01	0.18±0.04	0.19±0.04	0.18±0.01	.705	.596

Chlorophyll degradation increased in autumn and winter months and decreased in spring and summer months in *H. physodes* samples compared to control. On the contrary, it decreased in autumn and winter and increased in spring and summer for *P. furfuracea*. In *P. sulcata*, no significant difference was found between the values measured in the control and transplanted samples There is a positive correlation between transplantation time and chlorophyll degradation in *P. furfuracea* and is a negative in *H. physodes*. Whereas in *P. sulcata* there is no relationship between transplantation time and chlorophyll degradation. Chlorophyll a, chlorophyll b and carotenoid contents were significantly decreased with transplantation time in all three lichen species. In *H. physodes*, there is a significant positive correlation only between CH_4 and chlorophyll degradation. In *H. physodes*, there is a significant positive correlation only between CH_4 and chlorophyll degradation, whereas in *P. furfuracea* there is a negative correlation between SO_2, NO, and NCH_4 from gases and chlorophyll degradation. Chlorophyll a, chlorophyll b and carotenoid contents in *P. sulcata* were a positive relationship with airborne pollutant particles ($PM_{2.5}$) and gases (SO_2, NO, NO_2, CO, CH_4 and NCH_4), and was a negative relationship with O_3. Similarly, Ozone (O_3) was decreased the chlorophyll a, chlorophyll b and carotenoid content of *P. furfuracea* (Table 3).

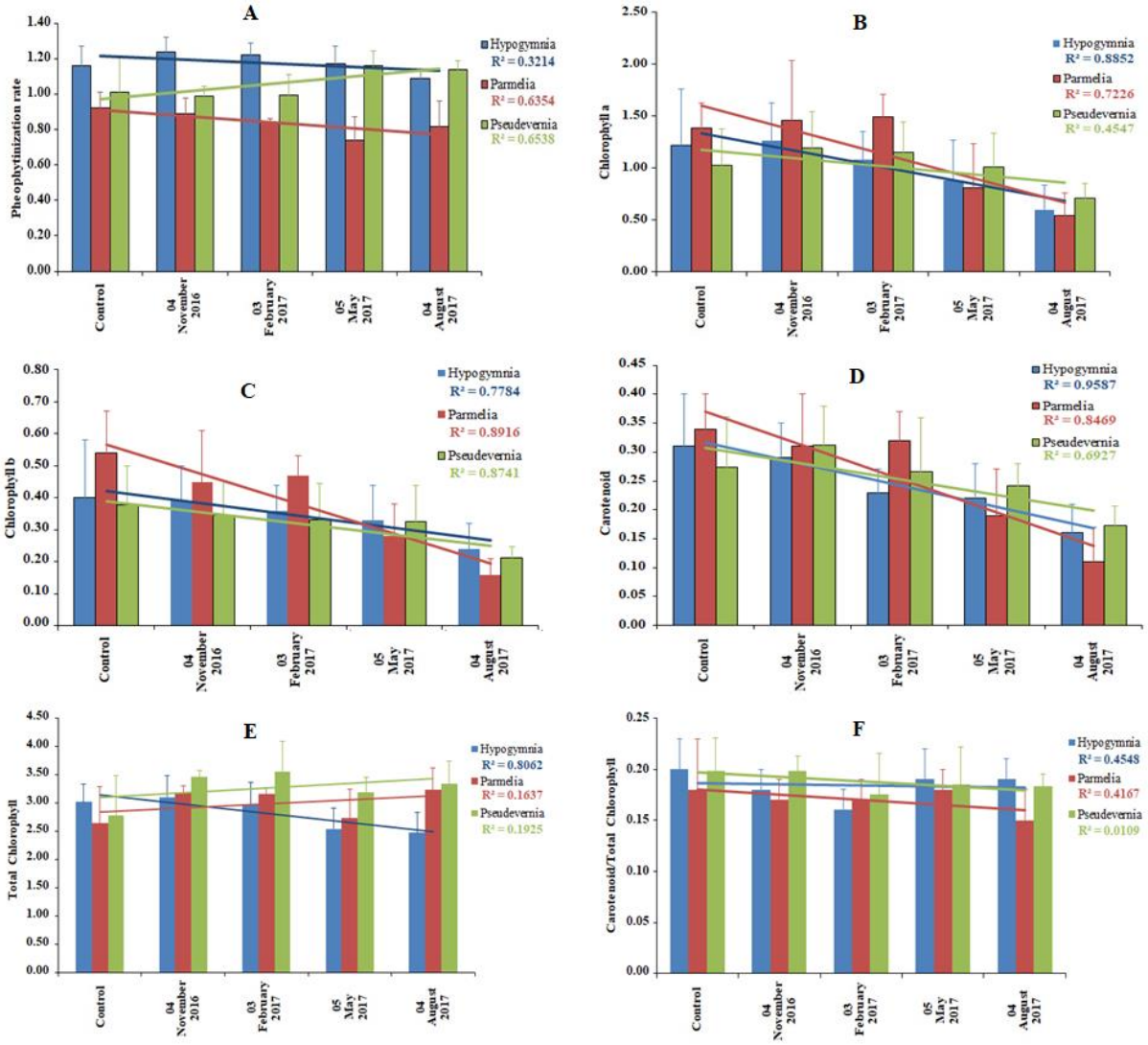

Figure 1. Graph showing the change in the measured amount of photosynthetic pigments of the three epiphytic lichen species at the seperate transplantation periods

4. Conclusions and discussion

Bursa City is in the position of a city where there is not much air circulation due to the large number of industrial establishments and the city settlement being located on the outskirts of Uludağ Mountain. Despite the widespread use of natural gas in winter, pollution reaches high levels especially in winter months due to the preference of poor quality heating materials in some regions and heavy vehicle traffic. Another reason for the perceptible level of air pollution in the city center, where the population is dense, is that the settlement has reached close to two major industrial zones as a result of the increase in population and the intensification of construction activities with urban transformation projects.

The air quality of Nilüfer district is relatively good. The results obtained in this study also confirm this situation. In three transplanted lichen samples, after the measurements made at the beginning of the one-year period in the first control group, it was determined that the photosynthetic pigment analyzes performed on the lichen thalli in three-month periods changed with the air pollution parameters obtained from the air quality station data. In the measurements made at the end of the first trimester, an increase in the amount of chlorophyll was observed in three lichen species. This result is in parallel with the results of the research conducted in Luisiana [21].

Table 3. Correlation between airborne pollutant particles and gases photosynthetic pigment contents of epiphytic lichen species during transplantation period in Nilüfer district (**: p<0.01, *: p<0.05 (2-tailed)

Species	Airborne pollutant particles and gases	Pearson Correlation (N=24)					
		Pheophytinization ratio	Chlorophyll a	Chlorophyll b	Carotenoid	Chlorophyll a/b	Carotenoid/total Chlorophyll
Hypogymnia physodes	PM_{10}	-.080	-.194	-.303	-.151	-.101	.352
	$PM_{2.5}$	.257	.164	.077	.181	.104	.050
	SO_2	.339	.216	.112	.192	.255	-.096
	NO	.195	.088	-.053	.093	.178	.097
	NO_2	.305	.213	.103	.210	.226	-.019
	CO	.223	.131	.012	.112	.169	.005
	O_3	-.375	-.311	-.223	-.291	-.271	.129
	CH_4	.469*	.358	.264	.402	.340	-.050
	NCH_4	.292	.212	.062	.247	.261	.081
	Transplantation times	-.627**	-.642**	-.496*	-.698**	-.586**	.199
Parmelia sulcata	PM_{10}	.114	.070	.109	.072	-.171	.105
	$PM_{2.5}$	.082	.441*	.495*	.473*	-.121	.219
	SO_2	.148	.559**	.617**	.592**	-.060	.201
	NO	.216	.439*	.490*	.439*	-.047	.144
	NO_2	.172	.519**	.580**	.536**	-.099	.181
	CO	.197	.491*	.543**	.497*	-.060	.098
	O_3	-.145	-.586**	-.655**	-.609**	.141	-.151
	CH_4	.117	.504*	.568**	.560**	-.119	.280
	NCH_4	.244	.501*	.549**	.509*	-.020	.163
	Transplantation times	-.320	-.709**	-.741**	-.751**	-.100	-.133
Pseudevernia furfuracea	PM_{10}	-.056	.057	.000	.055	.214	-.008
	$PM_{2.5}$	-.291	.298	.242	.305	.128	-.056
	SO_2	-.476*	.352	.259	.332	.271	-.074
	NO	-.409*	.302	.170	.279	.398	-.093
	NO_2	-.403	.400	.301	.394	.282	-.053
	CO	-.391	.349	.242	.342	.320	-.066
	O_3	.348	-.506*	-.424*	-.481*	-.241	.088
	CH_4	-.398	.344	.279	.358	.212	.006
	NCH_4	-.448*	.376	.248	.386	.345	-.045
	Transplantation times	.621**	-.548**	-.432*	-.646**	-.233	-.141

This situation was associated with the climatic conditions of the control zone different from the climatic conditions of the transplanted location. In the second and third measurements, a significant decrease was observed in the values of chlorophyll pigment content and chlorophyll pigment ratios depending on the increase in air pollution parameters, seasonal conditions and exposure to pollutants. The increase in atmospheric pollution values of stations close to the study locations and the decrease in chlorophyll content of lichen species are in line with the literature information [22]. On the other hand, it has been determined that the change in photosynthetic pigment content of *P.*

furfuracea examined in this study due to environmental stress is much more pronounced than that of *H. physodes* and *P. sulcata*.

A higher degradation was observed in chlorophyll contents as a result of *P. furfuracea* having a branched morphological structure and thus adhering to the substrate at one point and being exposed to pollutants due to the high number of isidia on the thallus surface due to its high contact surface with the atmosphere. This result is parallel to the source information [23]. The thallus diameter of *P. sulcata* in leafy form, which is affected by atmospheric pollutants in the second degree, is small and the thallus texture is thin. The most important feature of *P. sulcata* is that it has cortex cracks called pseudocyphella on the thallus surface. Pseudocyphellae are known to assist in gas exchange of thallus tissue, similar to the stomata of higher plants. For this reason, the rate of degradation in chlorophyll pigment contents was higher in *P. sulcata* after *P. furfuracea*. Since the *H. physodes* thalli in leafy form is firmly attached to the substrate with its lower surface, this type of chlorophyll degradation has been found to be the least, since only the upper surface comes into contact with air. This result shows that *P. furfuracea* can be a better indicator species in the assessment of environmental stress and is compatible with the source information [24, 25, 26].

Fruticose lichens grow in a drooping or upright position, holding only one point on the tree trunk and branches, without substrate exposure. For this reason, the surface areas that come into contact with air are much more than those of leafy and crustaceans. In the source information, it is stated that branched lichens are more suitable than other morphological groups in determining the air quality with lichens [2]. As a result, lichens can adapt to environmental conditions by showing morphological, anatomical and physiological responses to air quality and stress conditions. Lichens in the same morphological group do not respond similarly to stress factors. The structure of the thallus tissue is also of great importance. Species that have structures that facilitate gas exchange in their tallus are more susceptible to pollutants than those that do not have these structures. It is seen that examining the chlorophyll content of epiphytic lichens in branchy form is a useful and practical way to evaluate air quality.

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