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Determination of relationship between lichen diversity value and photosynthetic pigment content in Bursa province (Türkiye)

Şaban GÜVENÇ[1], Seyhan ORAN[2*], Şule ÖZTÜRK[3]
[1-3]*Bursa Uludağ University, Faculty of Arts and Sciences, Department of Biology, Bursa, Turkey*
**seyhana@uludag.edu.tr, [1]saban@uludag.edu.tr, [3]ozturks@uludag.edu.tr*

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Bursa ilinde (Türkiye) liken çeşitlilik değeri ve fotosentetik pigment içeriği arasındaki ilişkinin belirlenmesi

Abstract: In this study, 48 epiphytic lichen species on the trunk of oak trees from seven localities were reported in Bursa province. The correlation between Lichen Diversity Value (LDV) and photosynthetic pigment content at a location were evaluated for each locality. A negative correlation was found between LDV and photosynthetic pigment contents. The LDV was higher in rural areas, and decreased in areas affected by anthropogenic and agricultural activities. LDV values were increased from 21% to 47% from degraded areas exposed to environmental pollution and stress to undisturbed areas, whereas total photosynthetic pigment contents decreased from 39% to 19%, and Phaeophytinization ratio (PR) values from 36% to 30%.

Key words: Air pollution, epiphytic lichen, lichen diversity, *Parmelina tiliacea,* photosynthetic pigment

Özet: Bu çalışmada, Bursa ilinde yedi lokaliteden meşe ağaçlarının gövdelerinde bulunan 48 epifitik liken türü rapor edilmiştir. Aynı lokasyondaki Liken Çeşitlilik Değeri (LDV) ile fotosentetik pigment içeriği arasındaki korelasyon herbir lokasyon için değerlendirilmiştir. LDV değeri ve fotosentetik pigment içerikleri arasında negatif bir korelasyon bulunmuştur. LDV değerinin kırsal alanlarda yüksek olduğu, antropojenik ve tarımsal faaliyetlerden etkilenen alanlarda ise azaldığı tespit edilmiştir. LDV değerleri, çevre kirliliğine ve strese maruz kalan bozulmuş alanlardan bozulmamış alanlara doğru %21'den %47'ye yükselirken, toplam fotosentetik pigment içeriği %39'dan %19'a ve Feofitinizasyon oranı (PR) değerleri %36'dan %30'a düşmüştür.

Anahtar Kelimeler: Hava kirliliği, epifitik liken, liken çeşitliliği, *Parmelina tiliacea*, fotosentetik pigment

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1. Introduction

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 air quality in 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 algal and the fungal partners in the lichen thalli (Brodo et al., 2001). The epiphytic lichen diversity and community structures have been known to vary based on air pollution and environmental changes (Giordani, 2007; Cristofolini et al., 2008).

The decrease of lichen diversity in response to environmental conditions is widely used for a long time as an indicator of air pollution (Poličnik et al., 2008; Munzi et al., 2009,2014; Ozimec et al., 2016).

For assessing the effects of environmental stress in a short time is very important to monitor of the changes in physiological parameters in response to air pollution of lichens (Pisani et al., 2009; Sujetoviene and Galinyte, 2016). In several studies has been demonstrated a correlation between air pollution and photosynthetic pigment content of lichens (Riddel et al., 2012; Seed et al., 2013).

This study was aimed to determine the relationship between changes in photosynthetic pigment contents and lichen diversity values in localities.

2. Materials and Method

2.1. Study area

This study was conducted at seven localities in Bursa province. Bursa province is located between 39°30'-40°37'N and 28°06'-29°58'E in the southeast part of the Marmara region of Türkiye. It is usually dominated by a Mediterranean climate and is a transitional region between the Mediterranean and Black Sea climates (Öztürk, 2010). The mean annual temperature (1987-2012) in Bursa province (alt. 155 m) is 14.6 °C, the mean annual rainfall is 691 mm, at Mudanya district (alt. 13 m) in Bursa province is 16.7 °C and 614 mm, at Karacabey (alt. 15 m) is 14.7 °C and 585 mm, and at Orhaneli (alt. 484 m) 12.5°C, and 655 mm, respectively (TSMS, 2013).

2.2. Collection of lichen samples

Lichen samples were collected from total 21 oak trees at seven localities in Bursa province in the year of 2014-2015 (Figure 1, Table 1). Lichen samples on the trunk of three oak trees in each localities were collected using the methods specified by Asta and his colleagues (Nimis et al., 2002). The lichen diversity value (LDV) is the sum of the frequencies calculated for each aspect on a tree. The LDV of the locality was the arithmetic mean of the sums of frequencies for each sampling tree in a locality. For the analysis of pigment content and chlorophyll integrity, thalli of *Parmelina tiliacea* (Hoffm.) Hale was collected from each sampled localities. *Parmelina.tiliacea* was not found in

Table 1. Main characteristics of sampling sites

Locality	District - Site	Alt. (m)	Coordinates	Descriptions
1	Karacabey - Bayramdere	40	40°23′35″N 28°22′31″E	Trees in the picnic area
2	Karacabey - Kıranlar	206	40°16′09″N 28°19′00″E	Trees in the village cemetery
3	Karacabey - Örencik	340	40°18′10″N 28°17′21″E	Trees at the village cemetery in the Karadağ Mountain
4	Karacabey - Taşpınar	110	40°15′02″N 28°38′12″E	Trees in the slopes near to an asphalt roadside
5	Mudanya - Esence	140	40°20′22″N 28°40′42″E	Trees in the agricultural area
6	Osmangazi - Dürdane	328	40°20′02″N 29°05′30″E	Trees at the roadside cemetery on the Bursa - İstanbul highway
7	Orhaneli - Karaoğlan	744	39°50′43″N 28°59′15″E	Trees in the rural area at 70 km south-west of the city of Bursa

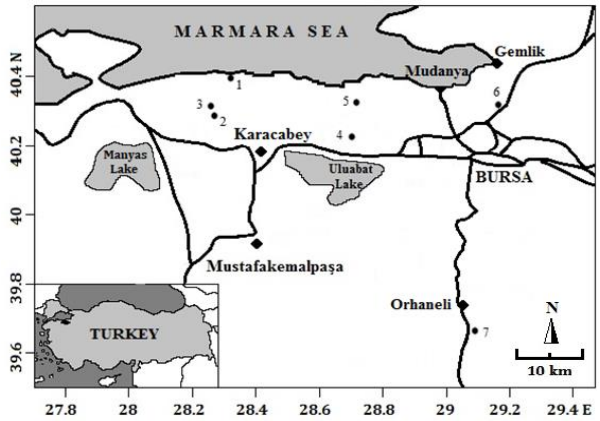

Figure 1. Map of study area and sampling sites (1-7)

the trees sampled for LDV value in the 4th and 5th localities. For photosynthetic pigment analysis, samples were taken from other trees in these localities.

2.3. Photosynthetic Pigment Analyses

20 mg of each lichen samples were used for the analysis of pigment content and chlorophyll integrity. Samples were first rinsed five times for 1 min each in $CaCO_3$-buffered acetone to remove lichen substances. After evaporation of acetone, 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 (Wellburn, 1994). The ratio of the absorbances at 435 and 415 nm, known as Phaeophytinization ratio (PR) (Ronen and Galun, 1984), was used to assess chlorophyll degradation to phaeophytin.

2.4. Statistical Analyses

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). The determination of grouping localities was obtained CANOCO ordination graph of groups according to the results of PCA analysis using the 5.4 software package (Ter Braak and Smilauer, 2002). A linear regression analysis was used to determined the degree of correlation expressed by multiple correlation coefficient (R^2) and probability (P) between the physiological parameters and lichen diversity values (LDV).

3. Results

A total of 48 epiphytic lichen species was recorded (Table 2). The most common species with the frequency of presence were *Lecidella elaeochroma* (Ach.) M. Choisy (40.95%), *Lecanora chlarotera* Nyl. (28.33%), *Parmelia sulcata* Taylor (25.48%), *Physcia adscendens* (Fr.) H.Olivier (23.81%), *Physconia grisea* (Lam.) Poelt (22.62%), *Xanthoria parietina* (L.) Th.Fr. (20.48%) and *P. tiliacea* (16.90%).

Parmelina tiliacea (Hoffm.) Hale is one of the most common lichens in the Mediterranean basin. It was used as an air pollution bioindicator in many regions (Núñez-Zapata et al., 2011). Photosynthetic pigment contents and chlorophyll degradation in thalli of *P. tiliacea* were summarized in Table 3. Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid) and PR values have statistically significant differences between the localities. Depending on environmental stress, the content of photosynthetic pigments, especially chlorophyll a is decreased while the conversion of chlorophyll a to pheophytin is increased. The high OD435/415 ratio (PR) indicates that the degradation of chlorophyll a is low.

The highest LDV (153.3) and the lowest PR values (0.95) were determined in locality 3. This area was less affected by environmental pollutants, and located in the mountainous area. The second highest LDV (128.7) was observed in locality 7. This locality are different in terms of elevation (744 m) than the other localities. On the contrary, the lowest LDV (59.7) and the second highest PR values (1.16) were calculated at locality 2. This locality was located near to the village which highly affected by various environmental pollutants. The LDV was higher in rural areas (localities 3, 5 and 7) and decreased in localities with traffic, settlements, and agricultural areas (1, 2, 4 and 6).

Seven localities evaluated in PCA analysis were divided into four groups according to their LDV value and photosynthetic pigment content (Figure 2).

The lowest LDV (59.3) and the highest PR values (1.21) were calculated at site I (Loc. 2, 4) where located near to the village which highly affected by various environmental pollutants. Site II (Loc. 1, 6, 7) is located in the area where the high of anthropogenic activities and traffic density. In this region, LDV and PR values were 78 and 1.08, respectively. Site III (Loc. 5) is located in a region where increased of agricultural activities. It has the second highest LDV and the second lowest PR values, but the species

Table 2. The frequency of epiphytic lichens found at the localities

	Localities									Localities							
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**%**	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**%**
Alyxoria varia	9	-	-	6	-	-	-	3.57	*Parmelia sulcata*	-	-	25	-	33	23	26	25.48
Amandinea punctata	-	-	25	1	23	16	-	15.48	*Parmelina carporrhizans*	-	-	3	-	-	-	2	1.19
Athallia holocarpa	-	-	3	-	1	-	8	2.86	*P. pastillifera*	-	-	-	-	-	-	8	1.90
Bacidia rosella	-	1	-	23	-	-	-	5.71	*P. tiliacea*	3	3	21	-	-	43	1	16.90
Bactrospora corticola	5	-	-	-	2	-	-	1.67	*Pertusaria albescens*	-	-	3	-	-	3	-	1.43
Buellia disciformis	-	-	22	-	-	-	2	5.71	*P. leioplaca*	-	3	-	-	-	4	-	1.67
B. griseovirens	4	-	3	-	-	-	1	1.90	*P. pertusa*	-	-	7	-	-	-	-	1.67
Candelariella vitellina	-	-	-	26	-	1	1	6.67	*Phaeophyscia orbicularis*	-	6	5	-	-	-	-	2.62
Catillaria nigroclavata	-	-	1	5	-	-	-	1.43	*Phlyctis argena*	6	10	-	-	-	2	-	4.29
Evernia prunastri	-	-	-	-	27	-	13	9.52	*Physcia adscendens*	11	8	16	17	41	-	7	23.81
Gyalecta truncigena	-	4	-	1	-	-	-	1.19	*P. aipolia*	-	8	-	-	1	-	5	3.33
Hyperphyscia adglutinata	-	-	-	51	8	-	13	17.14	*P. stellaris*	-	6	15	1	-	-	-	5.24
Hypogymnia farinacea	-	-	-	-	-	-	20	4.76	*Physconia distorta*	-	-	3	-	-	-	2	1.19
Lecanora carpinea	1	3	15	-	2	4	30	13.10	*P. enteroxantha*	-	-	11	-	-	11	18	9.52
L. chlarotera	19	-	37	-	7	15	41	28.33	*P. grisea*	-	48	6	41	-	-	-	22.62
L. hagenii	-	-	6	-	-	-	-	1.43	*Pleurosticta acetabulum*	-	-	3	-	-	-	49	12.38
L. rugosella	-	-	14	-	-	-	-	3.33	*Pseudevernia furfuracea*	-	-	1	-	-	-	20	5.00
Lecidella elaeochroma	24	-	28	6	34	32	48	40.95	*Ramalina fastigiata*	-	-	-	-	-	1	7	1.90
Lepraria incana	11	-	7	-	24	16	-	13.81	*R. fraxinea*	-	-	12	-	7	-	14	7.86
L. lobificans	24	-	-	-	-	-	-	5.71	*Rinodina exigua*	3	-	4	-	9	-	-	3.81
Melanelia subaurifera	-	-	-	-	-	5	-	1.19	*R. sophodes*	-	-	15	-	-	-	5	4.76
Melanelixia glabratula	-	-	5	-	-	-	2	1.67	*Scoliciosporum umbrinum*	2	-	3	-	49	-	-	12.86
Melanohalea elegantula	2	-	-	-	44	-	5	12.14	*Strigula affinis*	-	-	-	8	-	-	-	1.90
Opegrapha herbarum	31	-	-	3	3	-	-	8.81	*Xanthoria parietina*	-	41	2	27	1	-	15	20.48

Table 3. Mean±SD of photosynthetic pigment contents (mg g^{-1}) in thalli of *P. tiliacea*, and lichen diversity values (LDV) in the localities.

	Localities						
	1	**2**	**3**	**4**	**5**	**6**	**7**
LDV	64.7±4.0	59.7±8.3	153.3±4.0	76.0±11.3	106.7±19.0	75.3±14.2	128.7±31.7
Chlorophyll a	1.87±0.05	2.29±0.18	1.04±0.07	3.05±0.24	2.35±0.10	1.74±0.17	1.59±0.04
Chlorophyll b	0.59±0.06	0.65±0.05	0.35±0.05	0.84±0.09	0.72±0.03	0.52±0.08	0.49±0.01
Total Chlorophyll	2.46±0.04	2.94±0.23	1.39±0.10	3.89±0.32	3.06±0.13	2.26±0.25	2.08±0.05
Total Carotenoid	0.51±0.02	0.60±0.04	0.36±0.06	0.75±0.06	0.56±0.02	0.52±0.06	0.47±0.02
PR	1.01±0.11	1.16±0.01	0.95±0.08	1.25±0.13	1.06±0.01	1.13±0.05	1.10±0.01

PR: Phaeophytinization ratio

diversity is the lowest. The most number of species, the highest LDV (153.3) and the lowest PR values (0.95) were determined in Site IV (Loc. 3). This area was less affected by environmental pollutants, and located in the mountainous area (Table 4).

Consequently, there is a positive correlation (R^2: 0.895) between lichen diversity value and sites. On the other hand, a negative correlation (R^2: -0.642) was found between lichen diversity value and photosynthetic pigment contents (Table 5). LDV value was increased, while the PR value was decreased in sites.

4. Discussions

In this study, seven localities evaluated in PCA analysis were divided into four groups according to their LDV value and photosynthetic pigment content. The four groups are site I (Loc. 2, 4), site II (Loc. 1, 6, 7), site III (Loc. 5) and site IV (Loc. 3) (Figure 2).

According to alteration of naturality, 6 subclasses was determined in the province of Bursa (Güvenç, 2017). According to these subclasses, localities 3, 5, and 7 are found in high naturality zone. These localities are relatively remote areas from the settlement. Localities 4, 6 are found in medium naturality zone. These two localities are located along the roadside and are therefore under the influence of vehicle traffic. Localities 1 and 2 are found in low naturality zone. These two localities are close to the settlement areas and are under human influence. LDV and Shannon diversity values were increased from low to high naturality, whereas total carotenoid and PR values were decreased.

According to the our results, a negative correlation was found between LDV and photosynthetic pigment contents. Locality 7 has the highest LDV and the lowest total chlorophyll contents. Von Arb et al. (1990), were observed a very good correlation between the air pollutants (NO, NO_2, and O_3) and some physiological parameters of *P.*

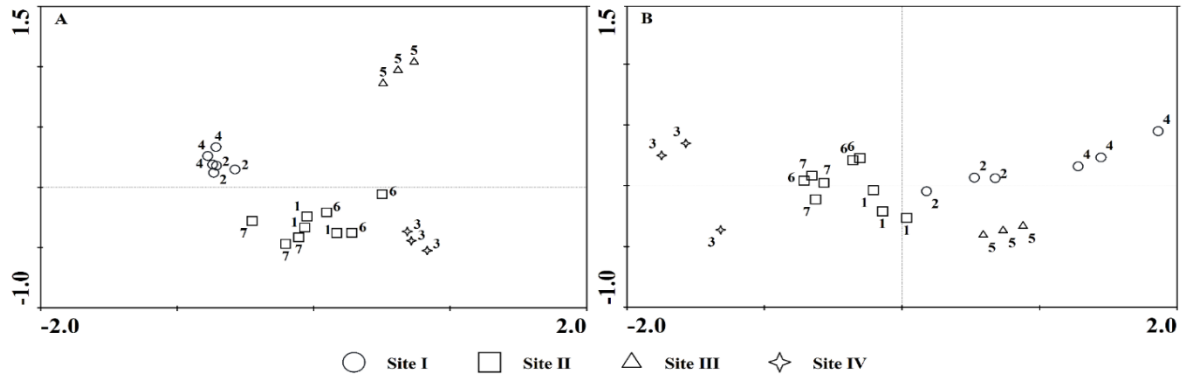

Figure 2. Ordination diagram of PCA analysis of localities (**A**: according to the lichen diversity values of localities, **B**: according to the photosynthetic pigment contents of localities)

Table 4. Mean±SD of photosynthetic pigment contents and lichen diversity values (LDV) in sites

	Site I	Site II	Site III	Site IV	F	Sig.
Localities	2, 4	1, 6, 7	5	3		
Number of sampling trees	6	9	3	3		
Number of species	24	31	20	33		
LDV	59.33±20.40	78.00±14.21	111.00±13.23	153.33±4.04	28.09	0.000
Chlorophyll a	2.67±0.46	1.73±0.15	2.35±0.10	1.04±0.07	28.91	0.000
Chlorophyll b	0.74±0.12	0.53±0.07	0.72±0.03	0.35±0.05	19.34	0.000
Total Chlorophyll (a+b)	3.42±0.58	2.26±0.21	3.06±0.13	1.39±0.10	27.58	0.000
Total Carotenoids	0.67±0.09	0.50±0.04	0.56±0.02	0.36±0.06	19.14	0.000
Total Chlorophylls/Carotenoids	5.05±0.16	4.52±0.27	5.49±0.03	3.86±0.47	24.71	0.000
Chlorophyll a/b	3.59±0.16	3.28±0.25	3.27±0.02	2.99±0.39	4.76	0.014
PR	1.21±0.05	1.08±0.08	1.06±0.01	0.95±0.08	10.34	0.000

PR: Phaeophytinization ratio

Table 5. Correlation between the photosynthetic pigments contents and lichen diversity value in the sites

	Pearson Correlation Sig. (1-tailed)							
	LDV	**Chlorophyll a**	**Chlorophyll b**	**Total Chlorophylls**	**Total carotenoids**	**Total Chlorophylls /Carotenoids**	**Chlorophyll a/b**	**PR**
Sites	0.895*	-0.691*	-0.614**	-0.679*	-0.752*	-0.428***	-0.633**	-0.771*
LDV		-0.524**	-0.465***	-0.514**	-0.565**	-0.380***	-0.550**	-0.642**

*: p<0.001 **:p<0.01 ***:p<0.05

sulcata in the northern part of Switzerland and its bordering area. Their results show that chlorophyll content increases with pollution.

Another similar result was obtained from *Usnea* sp. In parallel to the increase of air pollutants emitted by road traffic was increased the content of chlorophyl a+b in thalli of *Usnea* sp. and degradation of chlorophyll a (Carreras and Pignata, 2001). Their results demonstrate that chlorophylls would be effected by air pollutants emitted by traffic and as a compensatory mechanism the lichen would increase their synthesis. Our results are similar to those of Ra et al. (2005). They were indicated that the concentrations of total chlorophyll, chlorophyll a/b, carotenoids, and phaeophytinization ratio were generally higher in samples from the polluted areas than the clean area.

In present study, the highest total chlorophyll content (3.89), the highest PR value (1.25) and rather low LDV value (76.0) were determined at locality 4. This locality is located near the roadside in region which has a medium level alteration of naturality. We think that this locality is affected by air pollutants such as NOx, CO_2, CO and SO_2 emitted by vehicles on the road. Air pollutants emitted from the motor vehicles was as important as pollutants resulting from residential and industrial facilities (Mitreski et al., 2016). The concentrations of pollutants are higher in near the source and is decreasing with the distance from the source.

The common epiphytic species as *P. tiliacea* is very appropriate to determinate the correlation between photosynthetic pigment contents and lichen diversity value.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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