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BIOMONITORING OF CA, MG AND NA ACCUMULATION IN LICHENS AS EVIDENCE OF AIR POLLUTION STRESS IN ISPARTA, TURKEY

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ABSTRACT
This study is a part of project on biomonitoring the air quality of Isparta province (Turkey) through lichens, and has aimed to assess relation between the amounts of Ca, Mg and Na elements deposited in lichen thalli and the air quality in the area. Lichen samples of Physcia aipolia (Ehrh. ex Humb.) Fürnr. and Xanthoria parietina (L.) Th.Fr. collected from 18 localities in and around Gölcük Nature Park have been investigated in ICP-MS for Ca, Mg and Na. In addition, selected samples of both lichen species from the polluted and non-polluted sites for these three elements were observed by Scanning Electron Microscopy (SEM) micrographs and Energy Dispersion X-Ray Spectroscopy (EDS) analysis. The ratio for elemental contents in the lichen thalli resulted as Mg>Ca>Na for both species, with multiple times higher in X. parietina than P. aipolia. The concentrations of Ca and Mg in the samples were found reversely proportional to the altitude of the localities. Also, it has been observed that some thalline and apothecial samples of both lichens from intensely polluted localities contained crystal blocks of high Ca concentration, which are most likely calcium-oxalate (CaC_2O_4) forms. It was concluded that high amounts of Ca and Mg in lichen samples were associated with air pollution and may be demonstration of environmental stress in Isparta.
KEY WORDS: *Air quality, Ca-rich particles, Physcia aipolia, Xanthoria parietina, airborne elements*

INTRODUCTION

As many perennial terrestrial organisms, lichens are environmentally sensitive to sulphur dioxide (SO_2) and other contaminants such as heavy metals and radionuclides. Therefore, they are used as biomonitoring tools for the measurement of long-term atmospheric pollution. They have the ability to uptake and accumulate airborne contaminants with moisture in their bodies because, in their biological structures unlike plants, there is no protective outer cuticle layer on the thallus (Garty 2001, Wolterbeek et al. 2003). For this reason, the concentrations of elements in lichen thalli indicate the element levels in the atmosphere (Bari et al. 2001). One of the most common-used lichen biomonitoring methods is the analysis of thalli, which is a sensitive tool to detect the variety of atmospheric pollutants, such as heavy metals, PCBs (polychlorinated biphenyls), chlorinated hydrocarbons, PAHs and radioactive fallout (Wolterbeek et al. 2003). Lichens are quite useful organisms for spatial and temporal evaluation of the pollutant quantity in the environment (Loppi et al. 2003, Scerbo et al. 2003).

Furthermore, since lichens are sensitive to air pollution, their growth and health are closely related to the air pollutant rates in their environment. Biomonitoring of the adverse effects of environmental pollution on lichen thalli is also possible. Inductively coupled plasma mass spectrometry and scanning electron microscopy with energy-dispersive X-ray analyzes (ICP-MS/SEM-EDX) are a part of the analytical tools in biomonitoring studies (Ayrault et al. 2009, Cardell and Guerra 2017), and have been used in several studies (Bačkor and Fahselt 2004, Paoli et al. 2014, Marie et al. 2016) as well as in this study. SEM-EDX microanalysis is used as supporting evidence and the predictor of the morphological examination, quantification and localization of elements accumulated on lichen surfaces (Prithiviraj et al. 2011), commonly focused on trapment of particulate matter (PM) (Adamo et al. 2008b, Tretiach et al. 2011, Wang et al. 2013, Li et al. 2019), or some on the ultrastructural and physiological effects of pollution (Adamo et al. 2008a, Piovar et al. 2017).

Through SEM-EDS analyzes in the biomonitoring study by Adamo et al. (2008b) in Naples (Italy); crystalline and amorphous detrital components (quartz, calcite, volcanic glass, mica etc.), marine based salts (halite, gypsum, Mg-K sulfates, Mg-Ca carbonates) were observed in soil dust entrapped by lichens as PM_{10}. Paoli et al. (2014) defined Calcium as a good tracer for dust pollution, and lichens as good biomonitors in air pollution around the quarries and the cement plant. Also, influences of long-term dust pollution on lichen diversity and functional traits of lichens (growth form and main reproductive strategy) against long-term dust pollution were investigated by Degtjarenko et al. (2018). In the composition of species, they concluded that crustose species and sexual reproduction were most associated with higher pollution.

Pollution-induced crystal formations can be demonstrated by observing crystals of different shapes and contents in SEM-EDS analyzes. These crystals may be as in form of pruina, a white powdery surface layer consisting most commonly calcium oxalate crystals (Budel and Scheidegger 1996), on thalli or apothecial discs of some lichen species such as *Physcia aipolia*. The presence and increase of the amount of pruina in lichens is considered to be a response to environmental factors (Koch et al. 2019).

The main objective of the present study is to monitor the Ca, Mg and Na deposits in the air pollution around Gölcük Nature Park in Isparta province situated in North-Western Mediterranean Region of Turkey by means of lichens and to show the share of these elements. First, a study was performed evaluating the spatial distribution of the elements in the air with biomonitor lichen *Physcia aipolia* and obtained results as Ni> Cr> V> Fe> As> Al> Zn> Cu> Cd> Mn> Pb (Yavuz & Çobanoğlu 2019). In this second stage of the study, the evidence of environmental stress in Isparta has been explored by means of Ca, Mg and Na deposition in *Physcia aipolia* and *Xanthoria parietina* specimens via ICP-MS and SEM-EDS analyses. As mentioned in the detailed report by Demir (2010) in the centrum of Isparta, there are various workshops and some factories as industrial facilities, focused on sectors of forestry and carpentry (51 facilities), food and beverage (46), metal equipment (34), cement and marble (28), textile and leather (25), petro-chemistry and paint (19), cosmetics (4), and fertilizers (1). In addition, the city surrounded by mountains is under the influence of inversion. For this reason, biomonitoring works are substantial in the study area.

MATERIAL AND METHODS

Study Area and Sampling Methods

The study area is the surroundings of Gölcük Lake, Gölcük Nature Park, and city center of Isparta (Fig. 1). In this region of the country, urban area is usually surrounded by industrial zones along-with highways. The southern part of the city is mountainous while the western part is sporadically hilly and due to these geographical peculiarities, industrial zones are located in eastern and northern parts of the urban area. The northern and eastern parts of suburban area are surrounded by agricultural fields as well. There are several marble quarries in the eastern region the city, mainly in the rual area. In addition, the city settlement, due to surrounding mountains is under atmospheric inversion effect.

Isparta has a Semi-Arid Mediterranean climate with a mean annual rainfall of 506 mm and a mean annual temperature of 12 °C. The prevalent winds in the region blow from Southwestern (9 m/s), from Southern (8.1 m/s), from Southeastern (6 m/s) and from Western (1.6 m/s) directions (IMIM, 2010).

For the biomonitoring study, *Physcia aipolia* (Ehrh. ex Humb.) Furnr. and *Xanthoria parietina* (L.) Th.Fr, the two foliose lichen species were selected as biomonitoring organisms due to their frequent presence in the suburban regions. Lichen sampling was carried out in the period of April 2009-July 2010. Samples were collected from a total of 18 sampling locations distributed around suburban areas of Isparta (Fig. 1).

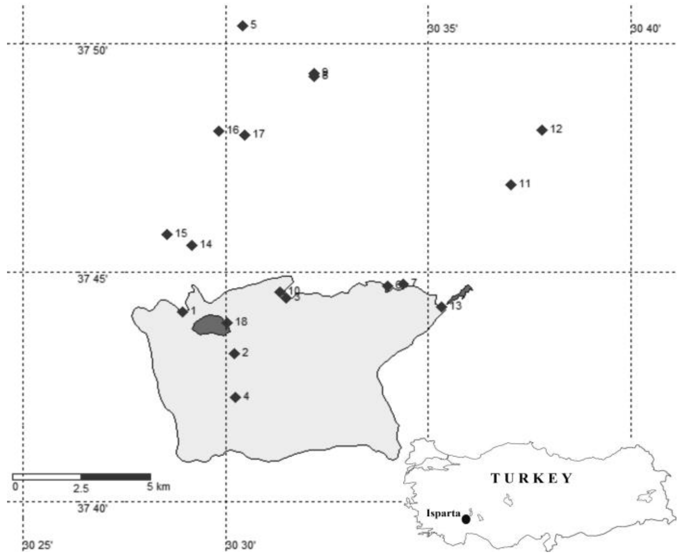

FIG. 1. The map of study area (the boundaries indicate Gölcük Nature Park)

TABLE 1. Geographical information of the sampling locations

No	Locality	GPS Coordinates	Altitude (m)	Date
1	Gölcük Nature Park, North-Western Part	37º44’07.70”N - 30º28’57.20”E	1454	23.05.2009
2	Gölcük Nature Park, South-Eastern Part	37º43’13.30”N - 30º30’13.70”E	1464	07.06.2009
3	Between Bezirgân and Hisartepe Hill	37º44’26.65”N - 30º31’30.33”E	1294	14.06.2009
4	Gölcük Nature Park, Southern Border	37º42’16.00”N - 30º30’15.50”E	1620	20.06.2009
5	Behind Campus, Koçtepe Village	37º50’23.00”N - 30º30’26.00”E	1118	04.04.2010
6	Sidre Hill	37º44’41.50”N - 30º34’00.00”E	1191	11.04.2009
7	Halife Sultan Cemetery	37º44’44.13”N - 30º34’23.82”E	1126	11.04.2009
8	Çünür Hill South Slope	37º49’15.80”N - 30º32’11.92”E	1070	11.04.2009
9	Çünür Hill North Slope	37º49’20,00”N - 30º32’11,30”E	1070	11.04.2009
10	Bezirgân Picnic Area	37º44’33.50”N - 30º31’20.50”E	1246	16.06.2009
11	Sav Village	37º46’54.40”N - 30º37’02.20”E	980	18.04.2010
12	Küçük Hacılar Village	37º48’05.60”N - 30º37’49.40”E	972	18.04.2010
13	Darı River Basin	37º44’14.40”N - 30º35’20.60”E	1105	18.04.2010
14	Gülbirlik Rose-oil Factory	37º45’34.80”N - 30º29’09.90”E	1215	24.04.2010
15	Gelincik Village	37º45’50.00”N - 30º28’33.70”E	1218	24.04.2010
16	Kayı Village West	37º48’05.30”N - 30º29’49.70”E	1106	24.04.2010
17	Kayı Village	37º47’59.70”N - 30º30’28.50”E	1069	24.04.2010
18	Gölcük Nature Park, North-Eastern Part	37º43’53.30”N - 30º30’02.50”E	1390	22.07.2010

Lichen samples were randomly collected in each site by exploring an area of 50 × 50 m. In each locality, 15 samples of lichen rosettes ≥ 2 cm in diameter were selected as samples of biomonitoring and were taken from bark of *Amygdalus* sp, *Pinus nigra, Populus nigra, Prunus domesticus, Quercus* sp, *Robinia pseudacacia*, and *Sorbus* sp. at a height of at least 120 cm above the ground in order to avoid terrestrial contamination. In the laboratory, lichen samples were air-dried and stored in polyethylene bags until chemical analysis. Geographic coordinates and altitude information of the localities were recorded by a *Garmin e-trex summit* device as given in Table 1.

Analytical Methods

Samples of the two lichen species from each sampling localities were prepared for ICP-MS to measure concentrations of Ca, Mg and Na. Some of the samples with higher contents were selected for further analyses by SEM and EDS.

Firstly, lichen samples were carefully cleaned with plastic tweezers to remove pieces of bark and extraneous materials. Then cleaned samples were stirred in deionized water for thirty seconds to remove the dust over surface of thalline samples. Washing lichen thalli is a regular part of standard protocols in order to avoid contamination over thalli. Clean samples were dried for 24 h at room temperature and then for 18 hours at 105 °C of incubation. The dried thalli of the lichen samples were subsequently ground and homogenized with an agate mortar and pestle. 200 mg of dry powdered sample was digested in a mixture of 10 mL 2:8:2 $HCl:HNO_3:H_2O_2$ at 180 psi pressure, 15 minutes between 0-95°C, 1 minute at 95°C and finally 15 minutes between 95-200°C in *CEM - Mars Xpress Microwave*. The digestion solution was finally diluted to 20 mL with ultra-pure water and analyzed for Ca, Mg, Na with ICP-MS (ACME Analytical Labs, Canada). Efficiency of the digestion procedure and the quality of analytical results were evaluated by analyzing a certified reference material, IAEA-336 Lichen (International Atomic Energy Agency, Vienna), with the same procedures adopted for the samples.

A correlation analysis was performed on the analytical data to evaluate the relationship between the pollutants and possible pollutant sources. Correlations between elements were tested by the Pearson's correlation test ($p<0.01$, 0.05). Two-tailed significance values ($n=15$, $\alpha=0.01$, $r_{crit}=0.6411$; $n=15$, $\alpha=0.05$, $r_{crit}=0.5140$) were used.

In addition to the ICP-MS element analysis of two lichen species, a SEM-EDS analysis was performed in order to observe and detect the elements or crystals over the thalli, morphologically. For the SEM study, 2 samples of *Physcia aipolia* and 2 samples of *Xanthoria parietina* were scanned in a Jeol (JSM 5910 LV) SEM (Scanning Electron Microscope) while the scans were investigated through an Oxford INCA EDS (Energy Dispersive X-Ray Spectroscopy). Both ICP-MS and SEM-EDS analyzes were performed in the Department of Environmental Engineering at Marmara University.

For each element analyzed, a contour map was drawn using Surfer® 15 software package. The kriging gridding method was used to create the contour maps representing the aerial distribution of elemental concentrations in Isparta. Graphical data through the element concentrations in the localities, and micrographs (SEM) of the lichen samples were also provided for the examined samples.

RESULTS AND DISCUSSION

The results of ICP-MS analyze for Ca, Mg, and Na elements in the samples of *P. aipolia* and *X. parietina* collected from localities in the study area are given in Table 2. Based on the concentrations in the lichen samples, areal distributions of these elements in the study area are also shown with maps in Figure 2.

TABLE 2. Average concentrations (µg/g) of Ca, Mg and Na elements measured in native lichen samples

Altitude (m)	Locality	Sample Species	Substrate	Ca	Mg	Na
1454	1	*Physcia aipolia*	*Pinus nigra*	1833.63	1579.41	1437.00
1454	1	*Xanthoria parietina*	*Pinus nigra*	3291.45	918.47	353.20
1464	2	*Physcia aipolia*	*Quercus* sp.	5176.09	1583.83	683.70
1294	3	*Physcia aipolia*	*Sorbus* sp.	2652.59	1948.57	353.10
1620	4	*Xanthoria parietina*	*Robinia pseudacacia*	2477.46	2035.33	961.60
1118	5	*Xanthoria parietina*	*Populus nigra*	2958.56	1547.91	318.60
1191	6	*Physcia aipolia*	*Populus alba*	3841.91	3563.35	1247.00
1191	6	*Physcia aipolia*	*Prunus domesticus*	3946.34	1590.65	859.20
1126	7	*Physcia aipolia*	*Pinus nigra*	1491.29	2119.88	1143.00
1126	7	*Xanthoria parietina*	*Pinus nigra*	1688.50	2260.81	1125.00
1070	8	*Physcia aipolia*	*Amygdalus* sp.	7342.46	1895.52	1118.00
1070	9	*Physcia aipolia*	*Amygdalus* sp.	3070.18	1524.15	636.80
1246	10	*Physcia aipolia*	*Amygdalus* sp.	3972.90	2099.99	837.20
980	11	*Xanthoria parietina*	*Quercus* sp.	8904.18	5686.55	1007.00
972	12	*Physcia aipolia*	*Amygdalus* sp.	10530.20	10610.48	631.60
972	12	*Xanthoria parietina*	*Amygdalus* sp.	12889.06	10654.69	747.90
1105	13	*Physcia aipolia*	*Robinia pseudacacia*	5753.99	4788.53	383.70
1215	14	*Physcia aipolia*	*Amygdalus* sp.	6205.28	1566.15	1359.00
1215	14	*Xanthoria parietina*	*Amygdalus* sp.	4999.78	2680.25	1709.00
1218	15	*Physcia aipolia*	*Populus nigra*	7212.73	7206.28	1101.00
1106	16	*Physcia aipolia*	*Prunus domesticus*	3905.79	2859.86	556.10
1069	17	*Xanthoria parietina*	*Populus nigra*	5784.28	3653.43	894.65
		Evernia prunastri	**IAEA**	1193.50	540.10	336.64
		Physcia aipolia	**Mean**	4,781.10	3,209.76	881.89
		Xanthoria parietina	**Mean**	6,335.55	4,376.36	939.79

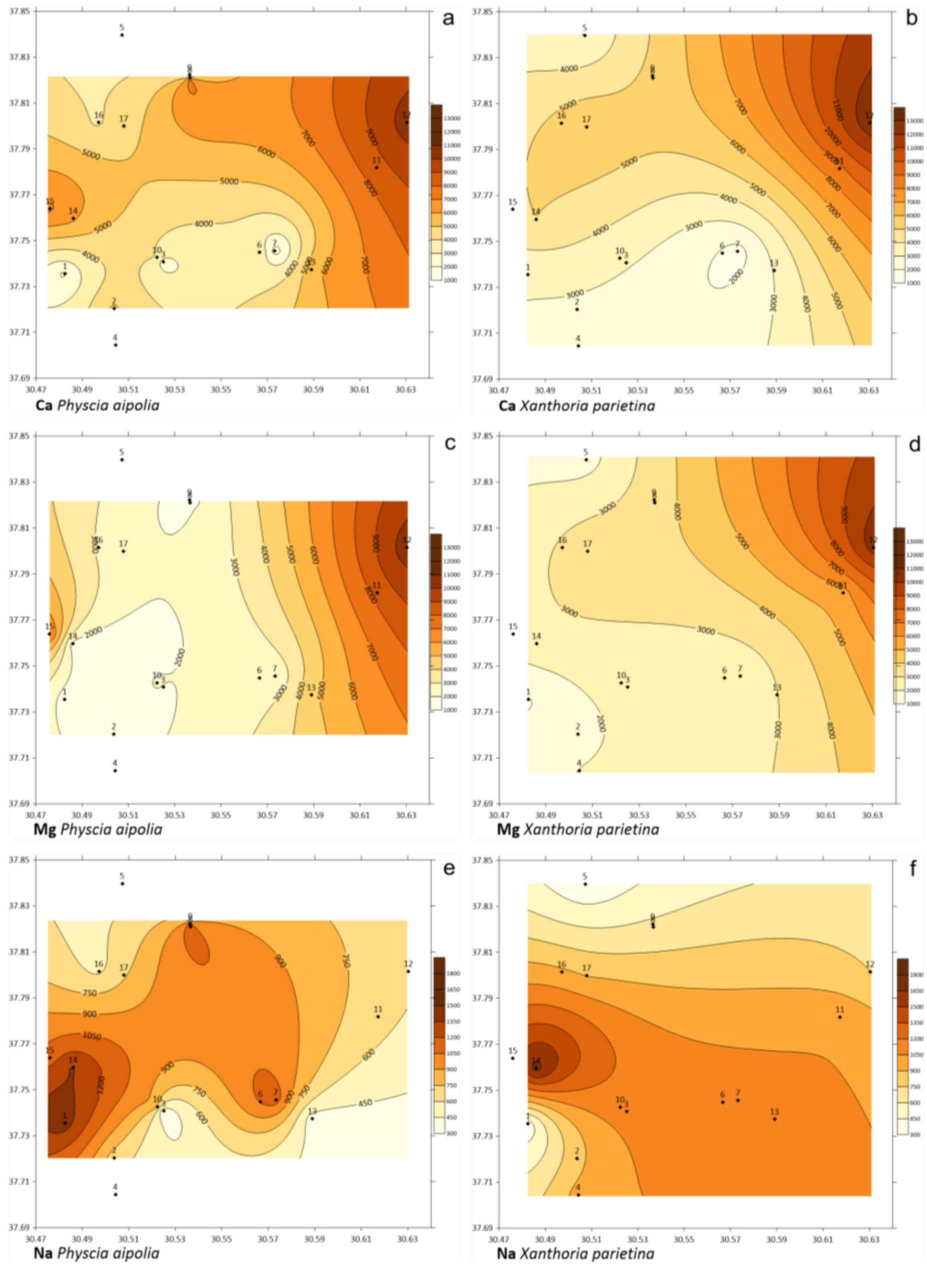

FIG. 2. Distribution of Ca, Mg and Na in the study area for *P. aipolia* (left) and *X. parietina* (right)

Ca: The aerial dispersion of Ca element in the study area was indicated in Figure 2 according to concentrations in *P. aipolia* (2a) and *X. parietina* (2b). In terms of Ca element, the richest samples of *P. aipolia* and *X. parietina* were found at locality 12; and the poorest samples of both species were found at locality 7. The maximum Ca concentration for *P. aipolia* is 10530.20 μg/g and that of *X. parietina* is 12889.06 μg/g, observed in path of Küçük Hacılar Village (L12), while the minimum Ca value in the case of *P. aipolia* is 1491.29 μg/g, and that of *X. parietina* is 1688.50 μg/g. collected at Halıkent Quarter, Halife Sultan Cemetery (L7). The mean value of Ca element measurements for *P. aipolia* is 4781.10 μg/g. while that of *X. parietina* is 5374.16 μg/g. Distribution map of Ca for *P. aipolia* fits with that of *X. parietina*. According to Table 2 and Figure 2, locality 12 has (or is near) an emission source of Ca element. The atmospheric concentration of Ca in *P. aipolia* and X. *parietina* samples have maximum degrees in the samples from locality 12, it slightly decreases in other samples directly proportional to the distance from localities 11 and 12, and increase in the altitude. Because the higher amount of Ca in the samples from these localities may presumably be related to the marble quarries in the area. Therefore, no sample from locality 12 with high Ca values known to be caused by the marble quarries were analyzed by SEM-EDS.

Mg: The mean value of Mg element measurements for *P. aipolia* is 3209.76 μg/g. while that of *X. parietina* is 3679.68 μg/g. The richest samples in terms of Mg element were found at locality 12; and the poorest samples of both species were found at localities 1 and 9. The maximum Mg concentration for *P. aipolia* is 10610.48 μg/g, and that of *X. parietina* is 10654.69 μg/g, observed in path of Küçük Hacılar Village. The minimum Mg value is found to be 1524.15 μg/g for *P. aipolia* in Çünür Hill North Slope and 918.47 μg/g for *X. Parietina,* collected at Gölcük Nature Park, North-Western Slopes of Crater (Fig. 2c, 2d). Distribution map of Mg for *P. aipolia* and *X. parietina* are similar to those given for Ca element. According to Figure 2 and 3, the samples from localities in the boundaries of Gölcük Nature Park have been less affected from the emission of Mg.

Na: In terms of Na element, the richest samples of *P. aipolia* was found at locality 1 and that of *X. parietina* at locality 14; while the poorest samples of *P. aipolia* at locality 3 and that of *X. parietina* at locality 5. The mean value of Na element measurements for *P. aipolia* is 881.89 μg/g. while that of *X. parietina* is 889.62 μg/g. The maximum Na concentration for *P. aipolia* is 1437.00 μg/g observed at Gölcük Nature Park, North-Western Slopes of Crater (L1) and that of *X. parietina* is 1709.00 μg/g, in Gülbirlik Rose-oil Factory (L14). The minimum Na value in the case of *P. aipolia* is 1491.29 μg/g, between Bezirgân and Hisartepe Hill (L3); while that of *X. parietina* is 1688.50 μg/g. collected behind Campus, Koçtepe Village (L5) (Fig. 2e, 2f).

Compared with the reference lichen (measured IAEA), the mean values for Ca element were 4-fold in *P. aipolia*, 5-fold higher in *X. parietina*; for Mg 6 times in *P. aipolia*, 8 times in *X. parietina*; and for Na element 2 times in *P. aipolia*, 3 times higher in *X. parietina* sample. The mean values of the 3 elements were higher in *X. parietina* than in *P. aipolia* samples. Eventually, the comparison of the mean values of the elements in the samples with those of the reference material showed a great difference. The ratio of magnitude for the elements Ca, Mg and Na was resulted as Mg>Ca>Na both in *P. aipolia* and in *X. parietina* (Table 2).

Localities with the numbers 1, 2, 3, 4, and 18 are in the borders of Gölcük Nature Park, relatively far from sources of urban/suburban pollution. Localities 6, 7 and 13 are in the suburban area at the border of Natural Park. The rest of 10 localities are surrounding the urban area. Considering the topographical properties of Isparta city center, it should be noted that the localities in South-Southwestern parts have higher altitudes than those in the North and East parts as visible in Figure 1 and Table 1. The prevalent winds in the region from SW (9 m/s) and from S (8.1 m/s) directions sweep airborne particles towards Northwestern, Northern and Northeastern parts of the city.

Locality 8 and locality 9 are twin localities on two opposite slopes of Çünür Hill, which is located next to the motorway starting at the city center connecting it to the campus of Süleyman Demirel University and to neighboring provinces like Burdur, Denizli and Afyonkarahisar. Çünür Hill is in the north of the industrial area as well. As shown in Table 2, both localities have the same altitude of 1070 m, but the only difference between them is the *façade*, facing to South (L8) and North (L9). Elemental concentrations of *P. aipolia* samples from L9 show lower degree than those of L8. Concentration of Ca in L8 is 2.40 times of that in L9 while Mg is 1.24 and Na is 1.76 times. L9 has also the minimum concentration of Mg for *P. aipolia* among the localities in the study area. The result indicates that the lichen samples from south slope of Çünür Hill have been affected more than those in the north, since the hill faces the urban area or any other source of emission. Due to the topographic peculiarities of the locations, the airborne elements may have been blown by the prevailing wind, in the study area.

The graphical data for evaluation of element concentrations varying with the localities were shown in Figure 3. Localities are arranged in the order of increasing altitude. In the graphics of elements there are two peaks, one around localities 12 and 11 (minimum altitude) while the other one around localities 14 and 15 (altitude > 1200 m). Having in consideration with the topography of the study area, that is, the urban region laying between 980-1200 m with an increase in altitude towards East to West and North to South, these data are compatible with the observation of pollution clouds or fog over the city atmosphere in winter season. Probably there are atmospheric inversions around 1200 m. Since there is not any previous data or study related either to the airborne element concentrations or to the atmospheric peculiarities in Isparta, we cannot cite to referential information.

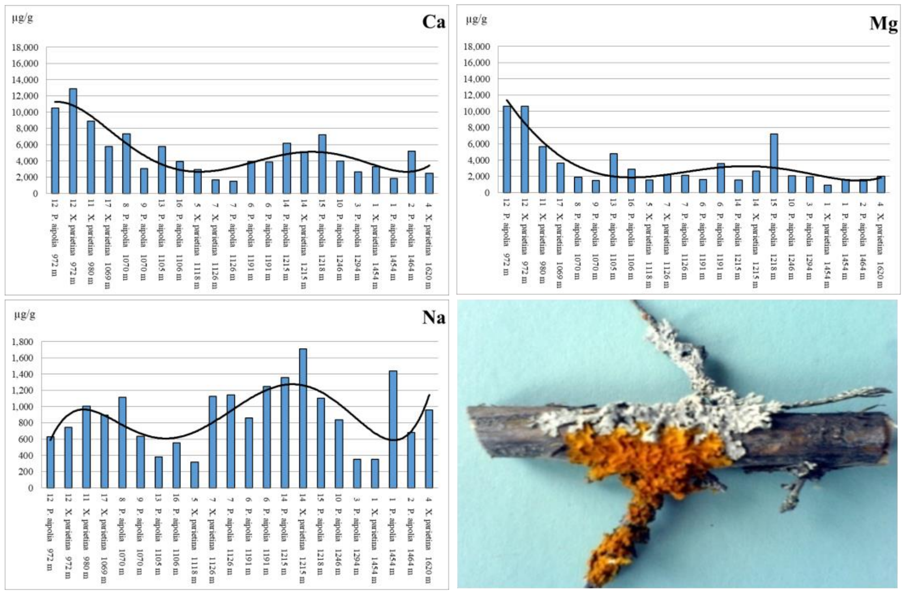

FIG. 3. Concentrations of Ca, Mg, Na elements in the sampling locations and image of lichens together

SEM - EDS Analysis

In addition to multi-element analyses, ultra-structural Scanning Electron Microscopy (SEM) micrographs of the lichen samples from 1 unpolluted and 2 polluted localities were analyzed by Energy Dispersive Spectroscopy Detector (EDS) for the elements of Ca, Mg and Na, supportively (Table 3).

SEM micrographs were provided for apothecial surfaces of the both lichen species collected from locality 5 (L5) considered unpolluted and relatively polluted localities L8 and L18. SEM01 (Fig. 4) is a *X. parietina* sample collected from L5, unpolluted area whereas SEM02 (Fig. 5) is a *X. parietina* sample collected from L18, relatively polluted area. SEM03 (Fig. 6) is a *P. aipolia* sample collected from L5, unpolluted area; while SEM04 (Fig. 7) is a *P. aipolia* sample collected from L8, another relatively polluted area. Concerning the SEM figures, in the left a general view is given while a closer image for enlargement of flakes was provided in the right.

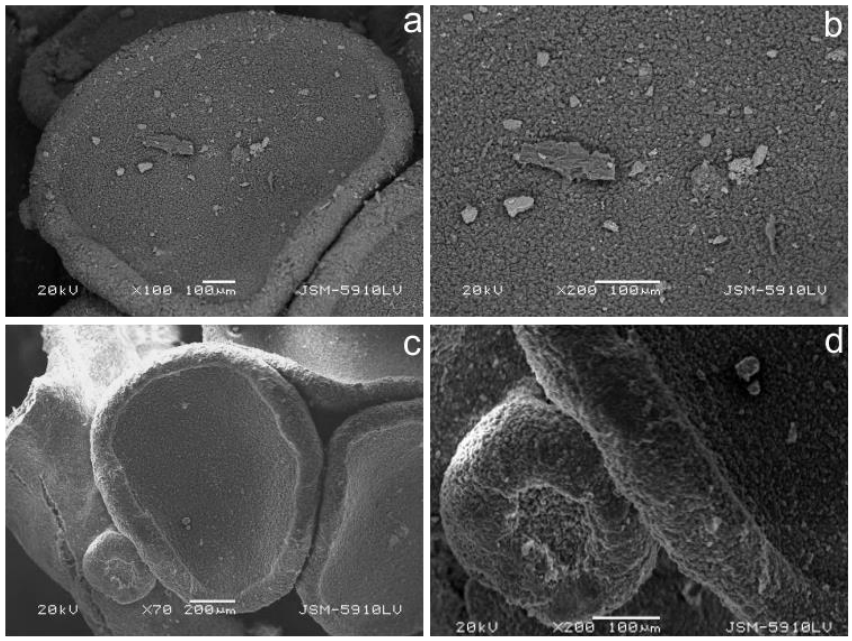

FIG. 4. SEM01-Micrographs of *X. parietina* apothecium from locality 5 (unpolluted site)

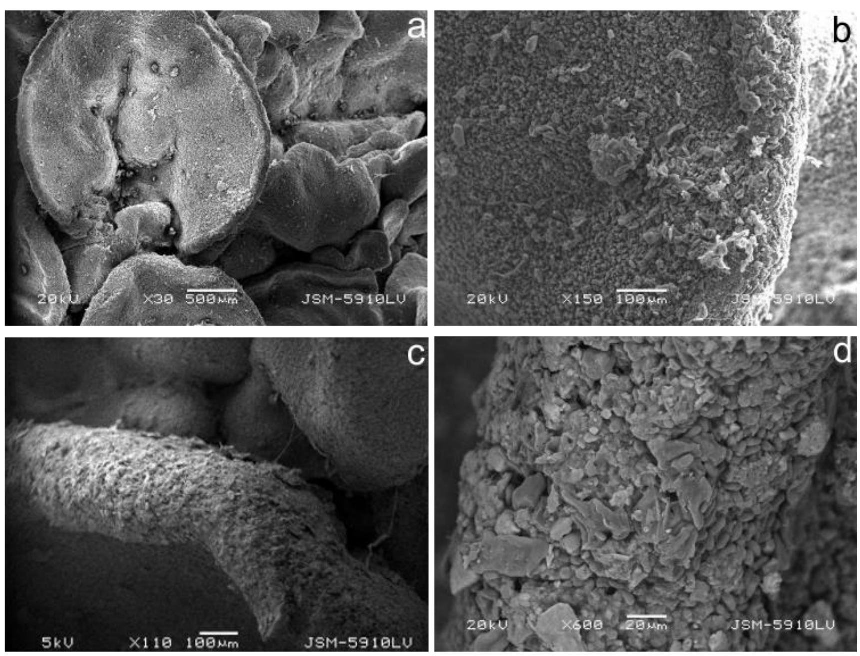

FIG. 5. SEM02-Micrographs of *X. parietina* apothecium from locality 18 (polluted site)

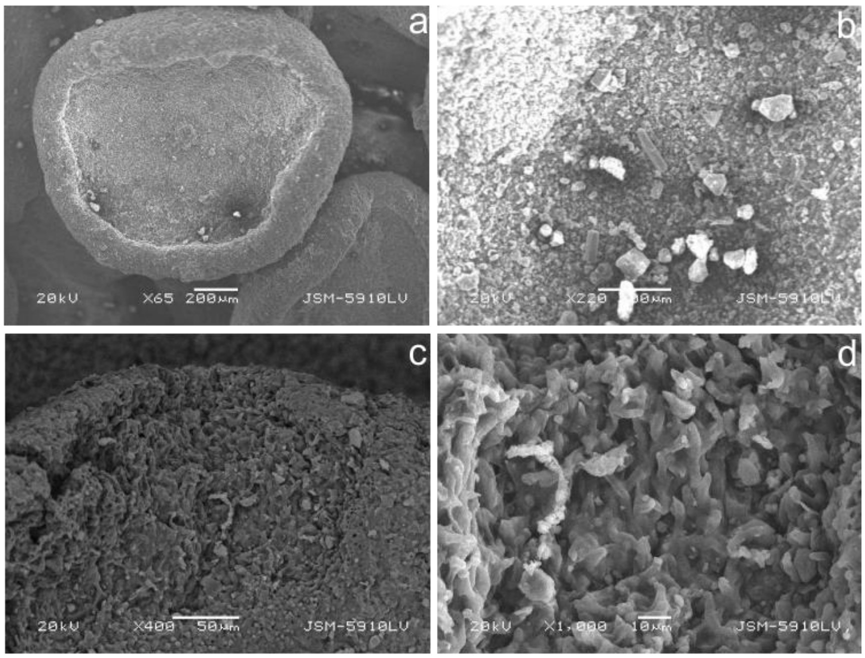

FIG. 6. SEM03-Micrographs of *P. aipolia* apothecium from locality 5 (unpolluted site)

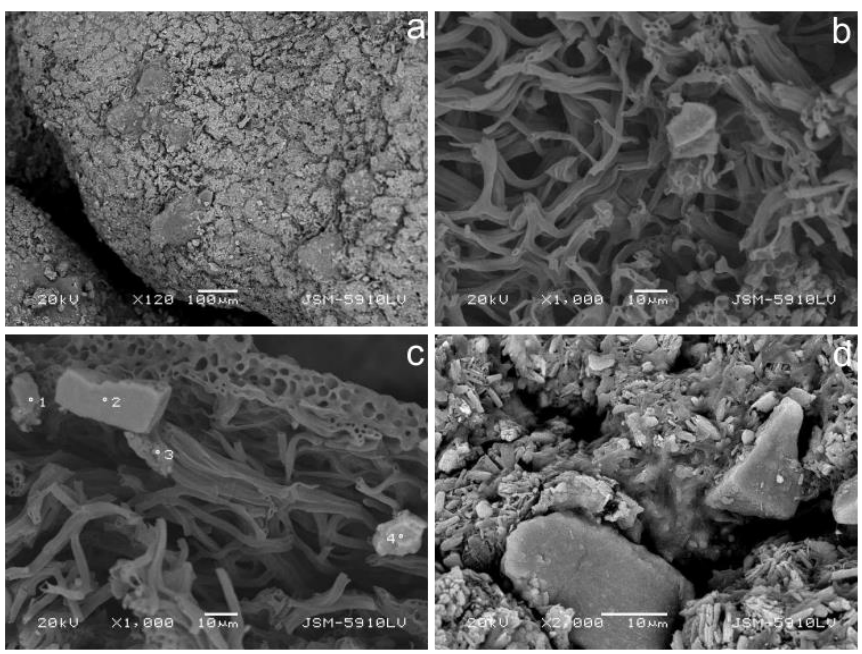

FIG. 7. SEM04-Micrographs of *P. aipolia* apothecium from locality 8 (polluted site)

The morphological deformations on apothecial disks of lichen samples are visible in the micrographs when SEM02 compared to SEM01, and SEM04 to SEM03. The results obtained from the EDS study of the SEM samples, including the flakes separately numbered with 1, 2, 3 and 4 on the image SEM04-c in Figure 7 were given in Table 3.

Since the samples are covered with Au and Pd films before the SEM investigation, the values for Au and Pd given under SEM Covering are neglectable. Moreover, Al, Fe and Si may be neglectable since they originate from the soil. However, these elements in soil dust and/or particulate matter in the air can be trapped by the lichen thalli. Thus, only airborne particles can be seen in the SEM micrographs as reflected in the EDS results.

TABLE 3. EDS results of the SEM samples

Spectrum (%Weight)	Site No.	Thalline Elements				Organic Elements		Soil Elements			SEM Covering	
		Ca	**Mg**	**Na**	**K**	C	O	Al	Fe	Si	Au	Pd
SEM01	L5	2.79		3.61	2.61	5.59	50.46	10.33		24.61		
SEM02	L18	18.97	0.74		0.68	14.56	51.30	1.85	0.79	6.98	4.13	
SEM03	L5	1.57	0.56		0.91	45.08	39.53	1.84	0.89	5.67	3.94	
SEM04-c1	L8			2.29	6.68	2.43	46.88	9.60	0.84	26.51	5.31	
SEM04-c2	L8	63.13					22.3				14.57	
SEM04-c3	L8						68.51				19.84	11.65
SEM04-c4	L8					14.30	3.73		73.21		9.20	
Maximum		63.13	0.74	3.61	6.68	45.08	68.51	10.33	73.21	26.51	19.84	11.65
Minimum		1.57	0.56	2.29	0.68	2.43	3.73	1.84	0.79	5.67	3.94	11.65

In the present study, the ICP-MS values of the elements correspond to the EDS analysis results of the same samples. We assume that the results of ICP-MS element analysis of two lichen species represent atmospheric levels of Ca, Mg and Na in the study area. The amounts of these 3 elements (in decreasing order of Mg, Ca, Na) in the both biomonitoring lichens were found several times higher than in the reference material. Accordingly, the results of EDS analysis of the samples taken from some localities that have reached high levels in this region or are very low also overlap and complement each other. In addition, it was found that higher Ca and Mg concentrations, comparing the samples at the same location, were correlated with the high concentrations of Al, Fe and Pb elements reported in our active monitoring study, conducted for the first time in Isparta city (Yavuz, Çobanoğlu 2019). Although it is not easy to interpret the sources of air pollutants through the results obtained for the contents of the particles in lichen samples, it is clear that there is some air pollution in the area.

With SEM study, we intended to provide visual support to the ingredients of the particles from the atmosphere trapped in the thalli of native lichen samples. The SEM images exhibited several particles on the surface of apothecia in various size and shapes, as well as deformations of thalline surfaces with rough appearance. The deformities seen in the samples are injuries probably caused by air pollutants such as particulate matter or dust in air as mentioned in Degtjarenko et al. (2018). The particles concentrated on both the edges and the discs of apothecia, with the sizes vary up to dimensions exceeding 30 µm. Qualitative elemental analysis of the lichen samples by EDS, revealed the presence and levels of Ca, Mg, Na, Al, Fe, Si, K, C and O in the airborne particles (Table 3).

The EDS analysis of SEM01 and SEM04 samples, compared to SEM02 and SEM03, indicated soil elements such as Si, Al and Fe were higher. These elements on the lichen thalli can be caused by atmospheric deposition of soil dust particles (Chenan 2015). On the other hand, the micrograph of SEM04-c2 (Fig. 7) visualized a clearly large Ca-oxalate crystal. In other words, the deposition of Ca on the apothecia of *P. aipolia* was found to be in the form of irregular-angular shaped crystals. However, SEM04-c3 and c4 did not have any of the thalline elements, could have organic, metal or plastic content. In particular, it is noteworthy that the concentration of C in the SEM03 sample from unpolluted site (L5) is significantly higher than the others.

In SEM04-c2 image of *P. aipolia* and SEM02 image of *X. parietina* samples, both from the relatively polluted localities L8 and L18, crystal blocks with high concentration of Ca are prominent, most likely Ca-oxalate (CaC_2O_4) crystals. As stated in Modenesi (1993) study, while the distribution of these crystals on the healthy or lightly stressed thallus cortex is moderate and stable, however on the one under the stress of pollution, they form a dense layer and even the cortex layer undergoes corrosion. As seen in the SEM micrographs in our study (Figs. 5, 7), it is estimated that particle formation with high Ca levels on lichen thalli is related to air pollution stress in the region.

In some studies, it was emphasized that the metal content of lichen thallus was higher than in the environment. In the EDX-microanalysis study by Bačkor & Fahselt (2004) on metal uptake in lichens (*Lecidea lithophila* and *Rhizocarpon oederi)* grown on very old copper mine spoil heaps in Slovakia, showed that significantly more Ca than in the substrate, but O, Na and Mg were found to be at the same levels in apothecia of both species. Nevertheless, it was unclear if the higher Ca concentrations on apothecial surfaces were accumulated from the air or from the mineral substrate. However, in the present study, to avoid contamination from soil or rock substrate, epiphytic lichens picked on bark 120 cm above from the ground were tested. Therefore, it is accepted that the results show the reaction of lichens to airborne pollutants.

In the lichen samples of *X. parietina* collected around the quarries and cement factory by Paoli et al. (2014), Ca, Ti, Fe, V, Al and Ni were significantly higher than in the surrounding environment. In the same study, Ca was defined as a good tracer for dust pollution, and lichens as the good biomonitors of air pollution around the quarries and the cement plant. Furthermore, the Ca in aerosols could deposit on thalli of epiphytic lichens, via dust storms as mentioned by Garty et al. (2002). They examined formation of calcium-containing structures on the surface of *Ramalina lacera* in response to air pollution; their results showed the presence of whewellite, weddellite crystals and gypsum as powders from thalli taken from polluted sites.

Some lichen species have pruina, a white powdery surface layer consisting most commonly Ca-oxalate (CaC_2O_4) crystals on thalli or apothecial discs (Budel and Scheidegger 1996). However, the shape, size and rate of formation of these species-specific crystals are estimated to be related to habitat. Pollution-induced formations can be demonstrated by crystals with different shape and content than normal observed in SEM-EDS analysis. In Giordano et al. (2005) study, Ca-oxalate crystals were observed in the samples of *Pseudevernia furfuracea* exposed to air pollution for 4 months. Lichens in rural areas or in areas remote from atmospheric pollution, may also contain Ca-oxalate on thallus cortex since oxidative stress, high light intensity, herbicides, SO_2, or other atmospheric contaminants may cause the thalline cortex become more pruinose as in the form of Ca-oxalate (Modenesi, 1993). Wadsten and Moberg (1985) identified, by diffraction and SEM in various lichen species, two crystalline phases for Ca-oxalate: the monoclinic monohydrate whewellite ($CaC_2O_4 \cdot H_2O$) and the tetragonal dihydrate weddellite ($CaC_2O_4 \cdot 2H_2O$). The predominant morphology of Ca-oxalate crystals in lichens from polluted environments is bipyramidal dodecahedron according to Modenesi (1993).

The presence and amount of pruina in lichens is thought to be physical protection against excessive light or other extreme environmental conditions and a way of response to environmental factors (Koch et al. 2019). *P. aipolia*, one of the two lichen species in this study, has apothecial discs with sometimes weakly white-pruinose character. However, the higher Ca concentrations not only in *P. aipolia* but in both species indicate that these ratios were air borne rather than slight-pruinose character of *P. aipolia*. In fact, the elemental data revealed that the *X. parietina* thallus in the L1, L7, L12 and L18 localities accumulated higher concentrations of Ca.

In the biomonitoring study with bag technique in Naples (Italy), the main minerals in PM_{10}; Al, Ca, Cu, Fe, K, Mg and Mn elements were reported to be significantly associated with the accumulation of soil dust in lichens (Adamo et al. 2008b). Through SEM-EDS analyzes, crystalline and amorphous detrital components (quartz, calcite, volcanic glass, mica etc.), marine based salts (halite, gypsum, Mg-K sulfates, Mg-Ca carbonates) were observed. Na and Mg in PM_{10} are shown as indicators of marine aerosols. It is also stated that crystallization can be caused by interactions between sea salt and calcite or between atmospheric acids and calcite. In a previous study by Yenisoy-Karakaş and Tuncel (2004), in İzmir and environs, Aegean Sea is reported as the emission source of Na element. In this study, since the nearest sea is more than a hundred kilometers away, the sea-water cannot have a spray-effect on thalli. Probably the emission source of Na and Mg in the present study is the agricultural fertilizers used in the fields surrounding Isparta. In addition, in the case of *P. aipolia* from L8, where the K-rich particle (SEM04c1) is seen by EDS, the source of K appears to be the application of fertilizer to the adjacent agricultural areas.

CONCLUSIONS

This is the first biomonitoring study on elements like Ca, Mg and Na in lichens of Isparta. Thus, this study puts forth the base data for any future biomonitoring studies in the region. The airborne element deposition of Ca, Mg and Na in the urban vicinity of Isparta province was monitored by using *Physcia aipolia* and *Xanthoria parietina* as the biomonitoring organisms.

In terms of comparing the species in this study, it was concluded that, it was not important to determine which of the two lichen species as a pollution monitoring organism, since there is no significant distinction in the analysis results. The results indicate that, the degree of elemental deposition is severe in some localities of the suburban area. However, element accumulation rates were not very different between two lichen species. Regarding the contamination levels in the suburban area, this study indicates the necessity of a regulation on the operation of marble quarries and another regulation on uses of fertilizers in the agriculture, in order to decrease the concentration of airborne elements in the atmosphere.

Our study highlights the importance of tracking the levels of pollution in biomonitoring studies, through direct measurements using vital lichen samples.

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